**A Study on the association between plasma glucose, glycosylated hemoglobin and macro metals Calcium and Magnesium in Diabetes Mellitus**

**Abstract**

To find out the association between Fasting plasma glucose, glycosylated Hemoglobin to macro metals calcium and magnesium in a randomly selected 50 patients ( both diabetic and non diabetic) attending the MHC. Comparison of both men and women (men= 26, women=24) in the age group of 23 to 72 years were enrolled, Fasting plasma glucose, glycoHb, calcium and magnesium were measured using the fasting blood samples. The parameters were then subjected to statistical analysis to find out the relation between any two analytes. Results of paired t tests with a p of <0.01 was considered significant and p<0.001 were considered highly significant to conclude an association between the analytes studied. This study has proved beyond doubt that calcium and magnesium are indeed involved in diabetes mellitus both in its regulation and glucose metabolism.

As the study was carried out during a short period of 1 Month, we were unable to indicate control subjects but purely worked out to find the association between FPG, Glycosylated hemoglobin, calcium and magnesium.

The study recommends that along with glucose and Glycosylated hemoglobin, calcium and magnesium assays too should be carried out as additional parameters in the evolution of diabetes mellitus.Based on this study we strongly recommend that further research should be carried out to establish the role of calcium and magnesium especially red cell calcium and magnesium and diabetes mellitus.

**Key words**

Fasting Plasma Glucose, Glycosylated Hemoglobin, Calcium, Magnesium

**Introduction**

Diabetes Mellitus comprises a heterogeneous group of diseases, which causes the patients, if untreated to have abnormally high plasma glucose levels. Four main types of diabetes Mellitus have been defined:

* Type I Insulin dependent Diabetes Mellitus (IDDM)
* Type II or Non Insulin dependent Diabetes Mellitus (NIDDM).
* Gestational Diabetes.
* Diabetes related to other conditions e.g. pancreatic diseases.

The forms of Diabetes other than type I or type II are rare. As the name indicates, at the time IDDM is diagnosed, the patients are totally dependent on exogenous insulin therapy, because all the insulin producing beta cells within the pancreas are totally destroyed by auto immune diseases.

Majority of type I diabetes are diagnosed before the age and its incidence increases over age. The prevalence of type II is much higher than type I.The incidence of childhood diabetes is highest in the world and is rising during the past few decades. The number of patients with type II diabetes is also on the increase.

The common organ complications that may develop due to poor control of both terms of diabetes mellitus include retinopathy, nephropathy, neuropathy, ischemic heart diseases and hypertension.

Large follow up studies have shown that good glucose control may prevent or delay the manifestations of complications despite the long duration of disease. Earlier daily or occasional plasma glucose determinations were the only way to get information on glucose control. These measurements still constitute the basis for day to day management of diabetes. Occasional plasma glucose measurements can however produce quite variable results depending, for instance on the type and timing of insulin injections food, drugs and physical exercises.(1)

**Glycohemaglobin (GHb)**

Glycohemaglobin is a blood test that checks the amount of sugar (glucose) bound to hemoglobin. Normally, only a small percentage of hemoglobin in the blood (4% to 6%) has glucose bound to it.The GlycohemaglobinA1c test checks the long term control of blood glucose levels in people with diabetes. Most doctors think the Glycohemaglobin A1c level is the best way to check how well a person is controlling his or her diabetes.(2)The Glycohemaglobin test is one blood sample every 3 to 4 months, and the test does not change with any recent changes in diet, exercise, or medicines.

Two main functions of Glycosylated hemoglobin viz HbA1 or HbA1C are commonly used in diabetes monitoring. The normal range for HbA1C is 4 – 6 % and values for HbA1 is 2 % higher as HbA1C is a smaller part of HbA1 levels are below 7.5 % and moderately controlled if HbA1C levels vary between 7.5 % - 8.5 % and values from 8.6 % to 10 % indicate poor control of the disease and the values over 10% is considered alarmingly high.(3)

Glycosylation of serum proteins mainly albumin has also been used in diabetes monitoring. The half – life of albumin is 2 – 3 weeks and the degree of Glycosylated albumin provides an index of glycaemic over shorter period time than HbA1C. one of the most widely used measurements of Glycosylated serum proteins is fructose amine assay.(4)

**Diabetes and Magnesium**

Magnesium is an important element for health and disease. It is the second most abundant intracellular cation; has been identified as a cofactor in over 300 enzymatic reactions involving energy metabolism, protein and nucleic acid synthesis. Approximately 50% is in soft tissues, the other half in bone and less than 1 % is present in blood. The clinical laboratory evaluation of magnesium status is limited to the level of magnesium in serum, 24 hour urinary excretion and percentage retention following parenteral administration.(5)

It has been shown that an increase in glucose in blood leads to the release, displacement of magnesium from RBC’s thus in the body hyper glycemia, high blood glucose, will cause total body magnesium deficiency. Magnesium deficiency both extra and intracellular is a characteristic of chronic stable mild type II diabetes and may predispose to the excess cardiovascular morbidity of the diabetic state. Diabetic nephropathy and other complications are most likely to be worse as a result of concurrent Magnesium deficiency.(6)

It has been proved that serum Magnesium level is significantly lower in diabetic subjects.

**Calcium and Diabetes**

In prospective studies, low calcium intake is consistently found to be inversely associated with incident type 2DM or the metabolic syndrome. In the Nurses Health Study, total (food + supplements) calcium intake was inversely associated with incident type 2DM after complete multivariate adjustment, including vitamin D intake. A similar inverse association was seen in the Black Women’s Health Study, a prospective cohort of ~59,000 women aged 21 – 69 at baseline.(5)

**LITERATURE REVIEW**

Often referred to simply as **diabetes** is a [syndrome](http://en.wikipedia.org/wiki/Syndrome) characterized by disordered [metabolism](http://en.wikipedia.org/wiki/Metabolism) and abnormally high [blood sugar](http://en.wikipedia.org/wiki/Blood_sugar) ([hyperglycaemia](http://en.wikipedia.org/wiki/Hyperglycaemia)) resulting from insufficient levels of the [hormone](http://en.wikipedia.org/wiki/Hormone)[insulin](http://en.wikipedia.org/wiki/Insulin). The characteristic symptoms are excessive urine production ([polyuria](http://en.wikipedia.org/wiki/Polyuria)) due to high blood glucose levels, excessive thirst and increased fluid intake ([polydipsia](http://en.wikipedia.org/wiki/Polydipsia)) attempting to compensate for increased urination, blurred vision due to high blood glucose effects on the eye's optics, unexplained weight loss, and [lethargy](http://en.wikipedia.org/wiki/Lethargy). These symptoms are likely to be less apparent if the blood sugar is only mildly elevated. (5)

The [World Health Organization](http://en.wikipedia.org/wiki/World_Health_Organization)(10) recognizes three main forms of diabetes mellitus: type 1, type 2, and gestational diabetes (occurring during [pregnancy](http://en.wikipedia.org/wiki/Pregnancy)), which have different causes and population distributions. While, ultimately, all forms are due to the [beta cells](http://en.wikipedia.org/wiki/Beta_cell) of the [pancreas](http://en.wikipedia.org/wiki/Pancreas) being unable to produce sufficient insulin to prevent hyperglycemia, the causes are different. Type 1 diabetes is usually due to [autoimmune](http://en.wikipedia.org/wiki/Autoimmune_disease) destruction of the pancreatic beta cells. Type 2 diabetes is characterized by [insulin resistance](http://en.wikipedia.org/wiki/Insulin_resistance) in target tissues. This causes a need for abnormally high amounts of insulin and diabetes develops when the beta cells cannot meet this demand.

Prolonged high blood glucose causes glucose absorption, which leads to changes in the shape of the lenses of the eyes, resulting in vision changes. Blurred vision is a common complaint leading to a diabetes diagnosis; type 1 should always be suspected in cases of rapid vision change whereas type 2 is generally more gradual, but should still be suspected. (11)

The classical triad of diabetes symptoms is [polyuria](http://en.wikipedia.org/wiki/Polyuria), [polydipsia](http://en.wikipedia.org/wiki/Polydipsia) and [polyphagia](http://en.wikipedia.org/wiki/Polyphagia), which are, respectively, frequent urination; increased thirst and consequent increased fluid intake; and increased appetite. Symptoms may develop quite rapidly (weeks or months) in type 1 diabetes, particularly in children. However, in type 2 diabetes the symptoms develop much more slowly and may be subtle or completely absent. Type 1 diabetes may also cause a rapid yet significant weight loss (despite normal or even increased eating) and irreducible fatigue. All of these symptoms except weight loss can also manifest in type 2 diabetes in patients whose diabetes is poorly controlled. (12)

Diabetes can cause many complications. [Acute](http://en.wikipedia.org/wiki/Acute_%28medical%29) complications ([hypoglycemia](http://en.wikipedia.org/wiki/Hypoglycemia), [ketoacidosis](http://en.wikipedia.org/wiki/Ketoacidosis), or [nonketotic hyperosmolar coma](http://en.wikipedia.org/wiki/Nonketotic_hyperosmolar_coma)) may occur if the disease is not adequately controlled. Serious long-term complications include [cardiovascular disease](http://en.wikipedia.org/wiki/Cardiovascular_disease) (doubled risk), [chronic renal failure](http://en.wikipedia.org/wiki/Chronic_renal_failure), [retinal damage](http://en.wikipedia.org/wiki/Diabetic_retinopathy) (which can lead to [blindness](http://en.wikipedia.org/wiki/Blindness)), [nerve damage](http://en.wikipedia.org/wiki/Diabetic_neuropathy) (of several kinds), and microvascular damage, which may cause [impotence](http://en.wikipedia.org/wiki/Erectile_dysfunction) and poor healing. Poor healing of wounds, particularly of the feet, can lead to [gangrene](http://en.wikipedia.org/wiki/Gangrene), which may require [amputation](http://en.wikipedia.org/wiki/Amputation).

The diagnosis of type 1 diabetes, and many cases of type 2, is usually prompted by recent-onset symptoms of excessive urination (polyuria) and excessive thirst (polydipsia), often accompanied by weight loss. These symptoms typically worsen over days to weeks; about a quarter of people with new type 1 diabetes have developed some degree of diabetic ketoacidosis by the time the diabetes is recognized. The diagnosis of other types of diabetes is usually made in other ways. These include ordinary health screening; detection of hyperglycemia during other medical investigations; and secondary symptoms such as vision changes or unexplainable fatigue. Diabetes is often detected when a person suffers a problem that is frequently caused by diabetes, such as a [heart attack](http://en.wikipedia.org/wiki/Myocardial_infarction), [stroke](http://en.wikipedia.org/wiki/Stroke), [neuropathy](http://en.wikipedia.org/wiki/Neuropathy), poor wound healing or a foot ulcer, certain eye problems, certain [fungal infections](http://en.wikipedia.org/wiki/Fungal_infection), or delivering a baby with macrosomia or [hypoglycemia](http://en.wikipedia.org/wiki/Hypoglycemia). (13)

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following:

* fasting plasma glucose level at or above 126 mg/dL (7.0 mmol/l).
* plasma glucose at or above 200 mg/dL (11.1 mmol/l) two hours after a 75 g oral glucose load as in a [glucose tolerance test](http://en.wikipedia.org/wiki/Glucose_tolerance_test).
* random plasma glucose at or above 200 mg/dL (11.1 mmol/l).
* Most physicians prefer to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete. According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/l) is considered diagnostic for diabetes mellitus.(14)

An elevated level of glucose irreversibly bound to [hemoglobin](http://en.wikipedia.org/wiki/Hemoglobin) (termed [glycosylated hemoglobin](http://en.wikipedia.org/wiki/Glycosylated_hemoglobin) or *HbA1c*) of 6.0% or higher (the 2003 revised U.S. standard) is considered abnormal by most labs; HbA1c is primarily used as a treatment-tracking test reflecting average blood glucose levels over the preceding 90 days (approximately). However, some physicians may order this test at the time of diagnosis to track changes over time. The current recommended goal for HbA1c in patients with diabetes is <7.0%, which is considered good [glycemic control](http://en.wikipedia.org/wiki/Diabetes_management#Glycemic_control), although some guidelines are stricter (<6.5%). People with diabetes who have HbA1c levels within this range have a significantly lower incidence of complications from diabetes, including [retinopathy](http://en.wikipedia.org/wiki/Retinopathy) and [diabetic nephropathy](http://en.wikipedia.org/wiki/Diabetic_nephropathy).(15)

Many studies have shown that both mean plasma and intracellular free magnesium levels are lower in patients with diabetes than in the general population. This magnesium deficiency, which may take the form of a chronic latent magnesium deficit rather than clinical hypomagnesemia, may have clinical importance because the magnesium ion is a crucial cofactor for many enzymatic reactions involved in metabolic processes.(16)

Many studies show that mean plasma levels are lower in patients with both type 1 and type 2 diabetes compared with non-diabetic control subjects. The concentration of intracellular free magnesium in erythrocytes is a more sensitive marker in people with diabetes and insulin resistance than are plasma levels of magnesium. Decreased levels of free intracellular magnesium in erythrocytes have been reported in the majority of patients with type 2 diabetes.(17)

Resnick and associates suggest that extracellular and intracellular magnesium deficiency is typical in chronic, stable, mild type 2 diabetes and may be a strong predisposing factor for the development of the excess cardiovascular morbidity associated with diabetes. These investigators showed that the levels of serum ionized magnesium and erythrocyte intracellular free magnesium were significantly lower in 22 untreated patients with type 2 diabetes and mild hyperglycemia than they were in 30 healthy control subjects(18). Serum total magnesium was not reduced. Among its many actions, insulin stimulates the transport of magnesium from the extra-cellular to the intracellular compartment. Using atomic absorption spectrophotometry and the euglycemichyperinsulinemic glucose clamp technique, Paolisso and associates showed that plasma magnesium level declined and erythrocyte magnesium levels rose significantly (*P*<.05) in response to insulin in fasting healthy adults with no family history of diabetes.(19)

Reduced magnesium levels in diabetes are caused by several factors. The link between magnesium deficiency and the development of diabetes is strengthened by the observation that several treatments for type 2 diabetes appear to increase magnesium levels. Metformin, for example, raises magnesium levels in the liver. Pioglitazone, a thiazolidinedione antidiabetic agent that increases insulin sensitivity, increases free magnesium concentration in adipocytes.(20)

Magnesium deficiency is associated with insulin resistance and increased platelet reactivity, but studies of oral magnesium supplementation and changes in glycemic status or lipid levels in diabetes have not been conclusive. Oral magnesium to 20 patients with type 2 diabetes, intracellular free magnesium concentration in erythrocytes normalized and the increase in platelet reactivity in response to thromboxanes decreased significantly. The oral dosage was 400 mg / d of elemental magnesium.(21)

Epidemiologic data suggest that populations with low magnesium intake are at increased risk for hypertension, stroke, and other manifestations of atherosclerotic disease. In the Atherosclerosis Risk in Communities (ARIC) Study, for example, dietary magnesium intake was inversely correlated with ultrasonographically measured carotid artery wall thickness, which is a surrogate marker for atherosclerosis.(22)

There is evidence to suggest that altered vitamin D and calcium homeostasis may play a role in the development of type 2 diabetes. The role of vitamin D in type 2 diabetes is suggested by cross-sectional studies showing that low serum concentrations of 25-hydroxyvitamin D [25(OH)D] are associated with impaired glucose tolerance and diabetes (1-3). The role of calcium in the development of type 2 diabetes is suggested indirectly by cross-sectional studies in which high calcium intake has been found to be inversely associated with body weight and fatness (4-6). The results from small clinical trials have been inconsistent (23-28).

The purpose of the present study was to prospectively evaluate the association between vitamin D and calcium intake a Vitamin D and calcium insufficiency may negativelyinfluence glycemia, whereas combined supplementation with bothnutrients may be beneficial in optimizing glucose metabolismand the risk of type 2 diabetes in a large cohort of women followed for 20 years.

**MATERIALS AND METHODS**

The subjects selected did not belong to established Diabetes mellitus patients, but patients attending the hospital for routine master health check up (MHC) in which after our study we found patients were found to be diabetic based on the results obtained.

50 patients comprising of both male and female in the age group of 23 to 72 years who reported to the outpatient clinic for MHC. The subjects selected consisted of 26 males in the age group of 23 to 67 years and 24 females in the age group of 17 to 72 years,in order to cover our study for a wide range of age and sex related subjects.

**SAMPLE COLLECTION& PROCESSING**

Fasting blood sample collection was done between 7- 9am from all the patients. Exact sample collection procedures were followed, such as use of sterile and disposable needles and vaccutainer for collecting the samples.All the blood samples were allowed to clot at room temperature for 30 minutes, the tubes were gently tapped to displace clot adhering to the tube and then centrifuged with the cap on in each tube for 10 minutes at 2500 rpm. Serum from each tube was transferred to another set of appropriate labeled tubes using disposable plastic dropping pipettes. The samples were either analyzed immediately or preserved at 2 - 8˚C if there is a delay in analysis.

The department is equipped with 2 discrete selective fully automatic analyzers (Olympus AU640 and Beckmann AU480)for measuring all general chemistries and metals and therefore we employed those 2 instruments for analyzing Calcium, magnesium, HbA1c and plasma glucose.

**RESULTS**

**TABLE I**

**STATISTICAL PARAMERS**

**All Patients( n = 50)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.NO** | **PAIRS COMPARED** | **t** | **r** | **P** |
| 1 | FPGVs HBA1c | 15.21 | 0.91 | <0.0001 |
| 2 | FPGVsCa | 5.93 | 0.65 | <0.0001 |
| 3 | FPGVs Mg | 5.2 | -0.60 | <0.0001 |
| 4 | FPGVsCa/Mg | 8.64 | 0.78 | <0.0001 |
| 5 | HBA1c VsCa | 5.2 | 0.6 | <0.0001 |
| 6 | HBA1c Vs Mg | 5.62 | -0.63 | <0.0001 |
| 7 | HBA1c VsCa/Mg | 8.36 | 0.77 | <0.0001 |

**TABLE II MALES (n=26)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.NO** | **PAIRS COMPARED** | **t** | **r** | **P** |
| 1 | FPGVs HBA1c | 10.75 | 0.91 | <0.0001 |
| 2 | FPGVsCa | 4.54 | 0.68 | <0.0001 |
| 3 | FPGVs Mg | 4.19 | -0.65 | <0.0001 |
| 4 | FPGVsCa/Mg | 9.57 | 0.81 | <0.0001 |
| 5 | HBA1c VsCa | 3.31 | 0.56 | 0.0015 |
| 6 | HBA1c Vs Mg | 5.23 | -0.73 | <0.0001 |
| 7 | HBA1c VsCa/Mg | 9.57 | 0.81 | <0.0001 |

**TABLE III FEMALES (n=24)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.NO** | **PAIRS COMPARED** | **t** | **r** | **P** |
| 1 | FPGVs HBA1c | 10.29 | 0.91 | <0.0001 |
| 2 | FPGVsCa | 3.81 | 0.63 | <0.0001 |
| 3 | FPGVs Mg | 3.43 | -0.59 | <0.0001 |
| 4 | FPGVsCa/Mg | 8.36 | 0.77 | <0.0001 |
| 5 | HBA1c VsCa | 3.91 | 0.64 | <0.0001 |
| 6 | HBA1c Vs Mg | 3.71 | -0.62 | <0.0001 |
| 7 | HBA1c VsCa/Mg | 8.64 | 0.78 | <0.0001 |

**STATISTICAL ANALYSIS**

For the purpose of statistical calculations like r, t, and probability, we have downloaded a software from [www.easycalculations.com](http://www.easycalculations.com) and comparison were made between Fasting plasma glucose (FPG), HbA1C, Ca, Mg and Ca/Mg ratio.

Table I presents the statistical data obtained for all the 50 patients. Very good correlation (p<0.0001) is observed between all the parameters. Compared, the highest for FPG Vs HbA1C (r=0.91, t=15.21 & p=<0.001) indicating that fasting plasma glucose is indeed associated with HbA1c. Similarly both FPG and HbA1C shows good correlation to both calcium and magnesium individually as well as to their ratios the highest being with Ca/Mg ratio, suggesting that both calcium and magnesium are important for the control of glucose metabolism.

Table I presents data similar to Tables I&II, but for the 24 female patients. The correlation coefficients, t and p values are identical to all patients, but less than that observed for male patients. One more interesting observations in all the three tables is that ; both FPG and HbA1c shows negative correlation with magnesium. This may be due to the fact that increase in magnesium level may induce better glucose utilization.

It is there proved beyond doubt that both FPG and HbA1c are indeed associated with both calcium and magnesium. Measurements of both calcium and magnesium occasionally may help the clinicians to get better information about the overall control of both plasma glucose and HbA1c.

The results obtained did not indicate either hypo or hyper metals concentrations. As this study was solely to find out the association between the 4 analytes, we did not select hypo or hyper calcaemia or magnesemia patients

**Discussion**

Some studies have proved positive correlation between FPG and HbA1c and we have confirmed this in ours study. (29) In previous studies delay in estimating plasma glucose after more than 6 hours may have given low values due to deterioration.

Extensive studies carried out in this aspect during the last 10 years have proved Glycosylated hemoglobin as a better index than fructosamine. We replaced fructosamine with calcium and magnesium and our attempt to find a correlation between Glycosylated hemoglobin and metals proved positive. Hence, calcium and magnesium if done with Glycosylated hemoglobin will certainly help to diagnose type II diabetes mellitus. It has been suggested that magnesium is associated with diabetes mellitus and in our study too we have proved this point with a positive correlation of <0.0001, and this same p was obtained when calcium and magnesium are put together as a ratio.

Several studies have been carried out to find an association between zinc, chromium, and magnesium in type II diabetes mellitus. But we could not undertake to prove this point as facilities for measuring zinc and chromium were currently not available at the centre where we undertook this study. Good correlation was observed among all the parameters compared namely FPG, Glycosylated hemoglobin, calcium magnesium and their ratios(29).

It has been conclusively proved that positive predictive tests like glycosylated hemoglobin and plasma glucose are very useful to diagnose diabetes and our study has proved such an earlier finding,

The use of glycosylated hemoglobin has been proved as a better index of diabetes mellitus(30).

A study undertaken in UK showed fasting plasma glucose and glycosylated hemoglobin correlates well

In our study we have proved that elevated HbA1C correlates well with raised fasting plasma glucose which is in accordance with an earlier study.

In a study undertaken in China all three parameter viz fasting plasma glucose, glycol HbA1C and fructosamine were found to correlate well.

We have assayed magnesium instead of fructosamine and got good correlation and concluded that HbA1C, glucose and magnesium if done together will help to diagnose type II diabetes mellitus.

It has been suggested that low serum magnesium are related to diabetes mellitus and in our study too we have observed majority of values are around 2.0 mg/dl and a strong relationship exist between plasma glucose and magnesium.

Several studies have been carried out earlier to find an association between zinc, magnesium and chromium levels in Type II diabetes.

As there was no facility to measure zinc and chromium, we undertook to find out association between magnesium and diabetes and its link to plasma glucose and glycosylated hemoglobin. We found out strong correlation with P values of < 0.0001 for all the pairs of results compared, the best being magnesium and HbA1C. As the project was done in a very short period of 2 weeks we were unable to include control subjects and purely worked out the association among biochemical tests used to monitor diabetes with a link to magnesium

Our attempt to include magnesium along with the best available diabetes diagnostic biochemical monitors glucose and HbA1C was found to be successful and we strongly recommend that serum magnesium be included along with the other two diabetic monitors.

**conclusion**

* This study was undertaken primarily to find out the association between macro metals calcium and magnesium to the 2 important markers for diabetes mellitus namely fasting plasma glucose and Glycosylated haemoglobin.
* As this study involves only relationship between two macro metals and two diabetic markers we did not select established diabetic patients but using an randomly selected population attending MHC in the hospital.
* Very good correlation between FPG and HbA1c to both calcium and Magnesium separately as well as to the ration between them.
* The above observation clearly demonstrate that both calcium and magnesium indeed linked to glucose metabolism.
* The outcome of the study suggests that for every patient who is screened for diabetes mellitus it is important to measure both calcium and magnesium.
* Except HbA1c assay the other biochemical measurements uses inexpensive reagents there by making cost effective in doing all the four tests to arrive at a meaningful diagnosis to help clinicians for proper treatment and prognosis assessment.
* Further studies are required in this aspect using established diabetes mellitus patients to confirm our outcome by including trace metals as well as short term diabetic monitoring tests such as fructosamine.

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