"A prospective study on the association of HbA1c and serum magnesium in Type 2 Diabetes Mellitus"

Running title: An Institutional study

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Abstract

Introduction: Diabetes is a pandemic health disease which is associated with increased risk of morbidity and mortality. Cellular magnesium is an important trace element which acts as co factor of various enzymes. It also acts as a secondary messenger for insulin action and plays a key role in regulating insulin mediated glucose uptake. Low serum magnesium level is frequently associated with Type 2 diabetes mellitus.

Materials and Methods: A prospective study was conducted on total 120 who were divided into 3 groups consisting of 40 patients in each group. Group 1 consisted on normal volunteers without type 2 diabetes mellitus. Group 2 consisted of patients with controlled Type 2 diabetes mellitus

with HbA1c ≤7 and group 3 consisted of patients with uncontrolled Type 2 diabetes mellitus with HbA1c ≥ 7. Serum magnesium levels were assessed in all 120 patients and were compared with FBS, PPBS and HbA1c.

Results: It was noted in our study that the difference in FBS, PPBS and serum magnesium values in comparison to the Hb1Ac levels was statistically significant ($p<0.05$). The independent t test showed that the difference of serum magnesium levels between controlled and uncontrolled type 2 diabetes mellitus was not statistically significant ($p>0.05$). We compared FBS, PPBS and HbA1c levels with serum magnesium and found a strong correlation. ANOVA test revealed that serum magnesium level showed a linear relationship with and age and HbA1c levels and was statistically significant ($p<0.05$). However, serum magnesium level with serum HbA1c and gender did not show any correlation (($p\geq0.05$).

Conclusion: Diabetic patients showed reduced magnesium level associated with increased HbA1c in uncontrolled type 2 diabetes mellitus than in controlled type 2 diabetes mellitus.

Keyword: Serum magnesium, HbA1c, Type 2 Diabetes Mellitus

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease resulting either due to decreased insulin production or decreased insulin activity. There are 2 clinical presentations of DM; Type 1 DM and Type 2DM; Type 2 DM (T2DM) is seen more commonly and tends to occur in adults and treated with oral glycemic.[1,2] It is believed to occur due to complex inheritance-environmental interactions with other risk factors sedentary like life style and obesity. The prevalence of this disease among general population is increasing day by day and is expected to reach 366 million by 2030.[3] Diabetes is considered is a global epidemic and silent killer of the current generation. People suffering from T2DM in China and India contribute to the majority of cases occurring world- wide thus Asia is the current "hot-spot" for diabetes epidemic.

T2DM is associated with serious microvascular and macrovascular complications leading to retinopathy, neuropathy, nephropathy along with cardiovascular system, thus associated with a greater risk of morbidity and mortality.[4,5] HbA1c analysis provides average blood glucose levels over 2-3 months. Hence it is now recommended as Standard of Care (SOC) for testing and monitoring diabetes especially the T2DM.[6,7]

HbA1c analysis has aided in the successful management of diabetes which is accurate and comparable internationally. A well controlled level of HbA1c has shown positive evidence in preventing complications of diabetes. The chief advantage of HbA1c is that it remains unaffected by fluctuating glucose levels after meals and other short term changes from medical conditions. Magnesium is an essential mineral for the proper functioning of brain and body and is also known to regulate blood glucose. Deficiency of magnesium is thought to result in increased blood glucose levels. Hypomagnesaemia occurring due to depletion of serum magnesium can lead to poorly controlled T2DM over the duration of the disease. Although the assessment of serum magnesium level has been reported in many studies from various countries, the comparative studies within the diabetic population based on glycemic control are limited. Thus, our present study aims to explore the correlation of glycated HbA1c and levels of serum magnesium in T2DM.[8]

Materials and methods

The present study was a prospective study conducted in a private clinical setting for over a period of one year. Patients reporting to the OPD with known diagnosis of type 2 DM were selected for the study. After obtaining a written consent from selected patients, clinical sample of blood was collected, processed and assessed in biochemistry laboratory. In the present study the study population of 120 were selected and were divided into three groups as follows

Group1: 40 cases without diabetes mellitus

Group2: 40 cases with controlled Type 2 diabetes mellitus with HbA1c ≤ 7

Group 3: 40 cases with un-controlled Type 2 diabetes mellitus with HbA1c ≥ 7

Inclusion and exclusion criteria:

For group 1 subjects without diabetes mellitus and without any history of co morbidities were selected. Whereas for group 2 and 3 patients suffering from type 2 diabetes mellitus of both gender between the age of 30-70years; without any known history of co morbidities were selected. Patients with HbA1c ≤ 7 were included in group 2 whereas patients with HbA1c ≥ 7 were included in group 3. Patients suffering from or with a known history of renal and cardiac involvement; malabsorption and chronic diarrhea were excluded from the present study. Patients with history of alcohol or patients on diuretic therapy or magnesium supplements or antacids containing magnesium were excluded from the present study.

Sample collection and testing:

After obtaining written consent from the patients their personal and clinical details were recorded and confirmed that they were on overnight fast. 5ml of venous blood sample was collected from patients under strict aseptic conditions. Serum samples were used to assess levels of magnesium, EDTA sample was used to measure HbA1c and sodium fluoride serum samples were used to measure FBS and PPBS.

The blood samples were centrifuged at low speed and serum samples obtained was collected and stored at 28ºC and then used directly for assessment. The magnesium levels were measured using colorimetric assay (Cobas 6000; Roche Mannheim Germany) and FBS, PPBS and HbA1c was measured by ELSIA assay. The values of FBS, PPBS, HbA1c and magnesium were entered into excel sheets and stored. They were later submitted to statistician for statistical analysis. The univariate comparisons between the 3 study groups were compared by independent t test; whereas ANOVA test was used to compare serum magnesium with age, gender and HbA1c. A p value of <0.05 was considered to be statistically significant.

RESULTS

In group 1 we noted that the mean and standard deviation of FBS was 111.20 and 11.867; of PPBS was 147.08 and 16.092; of HbA1c was 5.2748 and 0.39332; of serum magnesium was 2.1958 and 0.15417 respectively. In group 2 we noted that the mean and standard deviation of FBS was 150.63 and 71.287; of PPBS was 167.00 and 50.928; of HbA1c was 5.6818 and 0.51362; serum magnesium was 1.7750 and 0.12030 respectively. In group 3 we noted that the

mean and standard deviation respectively of FBS was 252.43 and 83.603; PPBS was 306.90 and 68.224; HbA1c was 8.510 and 1.1160; serum magnesium was 1.7668 and 0.25796. We found that the difference in FBS, PPBS and serum magnesium values in comparison to the Hb1Ac levels was statistically significant (p>0.05). (Table 1)

FBS								
Category	**Mean**	**N**	**Std. Deviation**	**Min**	**Max**	**Median**	**F**	**Sig.**
Group 1	111.20	40	11.867	82	135	111.50	52.181	0.000
Group 2	150.63	40	71.287	86	391	119.00		
Group 3	252.43	40	83.603	117	393	238.00		
PPBS								
Group 1	147.08	40	16.092	112	180	149.00	121.254	0.000
Group 2	167.00	40	50.928	111	312	152.00		
Group 3	306.90	40	68.224	143	421	306.00		
Serum Mg								
Group 1	2.1958	40	0.15417	1.99	2.50	2.1550	68.929	0.000
Group 2	1.7750	40	0.12030	1.61	2.01	1.7400		
Group 3	1.7668	40	0.25796	1.45	2.50	1.6800		

Table 1: Comparison of mean values of FBS, PPBS and serum magnesium levels with levels of HbA1c

We found in the present study that the mean level of serum magnesium in group 2 was 1.7750 and standard deviation was 1.7668. In group 3 the mean level of serum magnesium was 1.7668 and standard deviation was 0.25796. (Table 2)

Serum Magnesium			
Category	N	Mean	Std. Dev
Group 2	40	1.7750	0.12030
Group 3	40	1.7668	0.25796
p value >0.05			

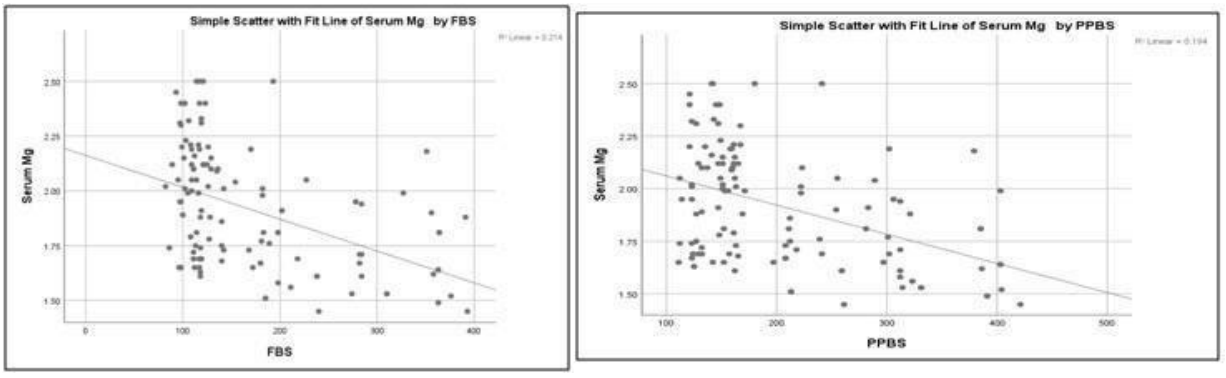
Table 2: Comparison of levels of serum magnesium in controlled and uncontrolled diabetics

The Independent t test showed that the difference of serum magnesium levels between controlled and uncontrolled type 2 diabetes mellitus was not statistically significant (p>0.05). (Table 3)

Independent Samples Test (Serum Magnesium)									
	F	Levene's Test for Equality of Variances		t-test for Equality of Means					
		Sig	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variance assumed	19.280	0.000	0.183	78	0.855	0.00825	0.04500	-0.08135	0.09785
Equal variance not assumed			0.183	55.197	0.855	0.00825	0.04500	-0.08193	0.09843
P value is 0.855 (not significant)									

Table 3 showing the Independent t test values of serum magnesium in controlled and uncontrolled type 2 diabetes mellitus.

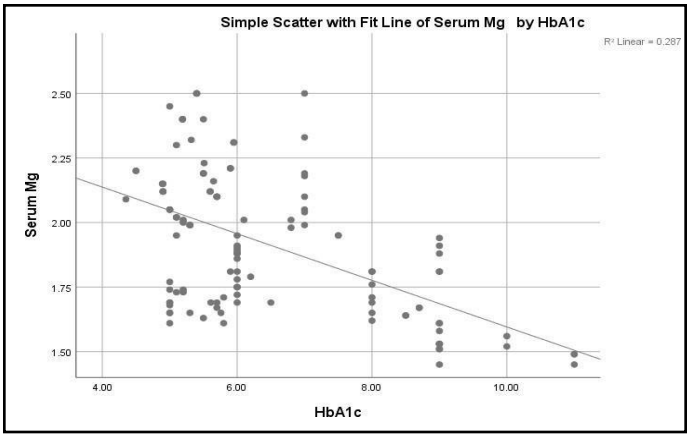
Simple scatter with fit line plot was used to show comparison of serum magnesium and FBS and found a strong correlation. Where x axis represents FBS/ PPBS and y axis represents serum magnesium. (Graph 1)



Graph 1: shows correlation between serum magnesium and fasting blood sugar level and post parandial blood sugar level

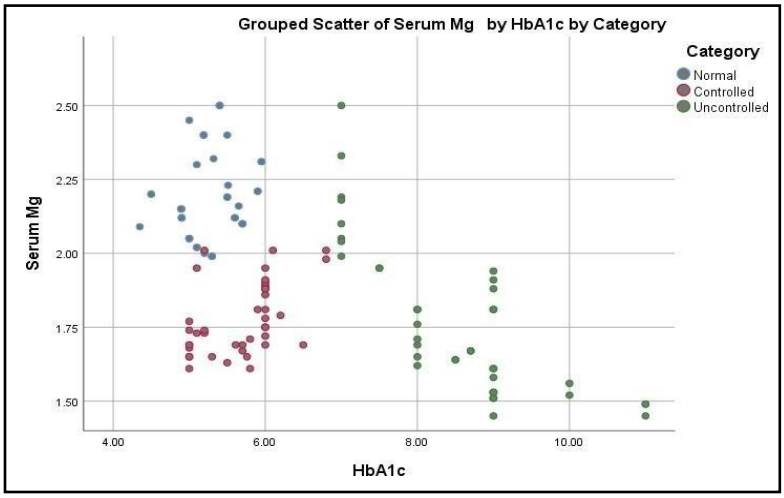
Simple scatter with fit line plot was used to show comparison of serum magnesium and HbA1c and there was a strong correlation. Where x axis represents HbA1c and y axis represents serum magnesium

.(Graph2)



Graph 2: shows correlation between serum magnesium and HbA1c

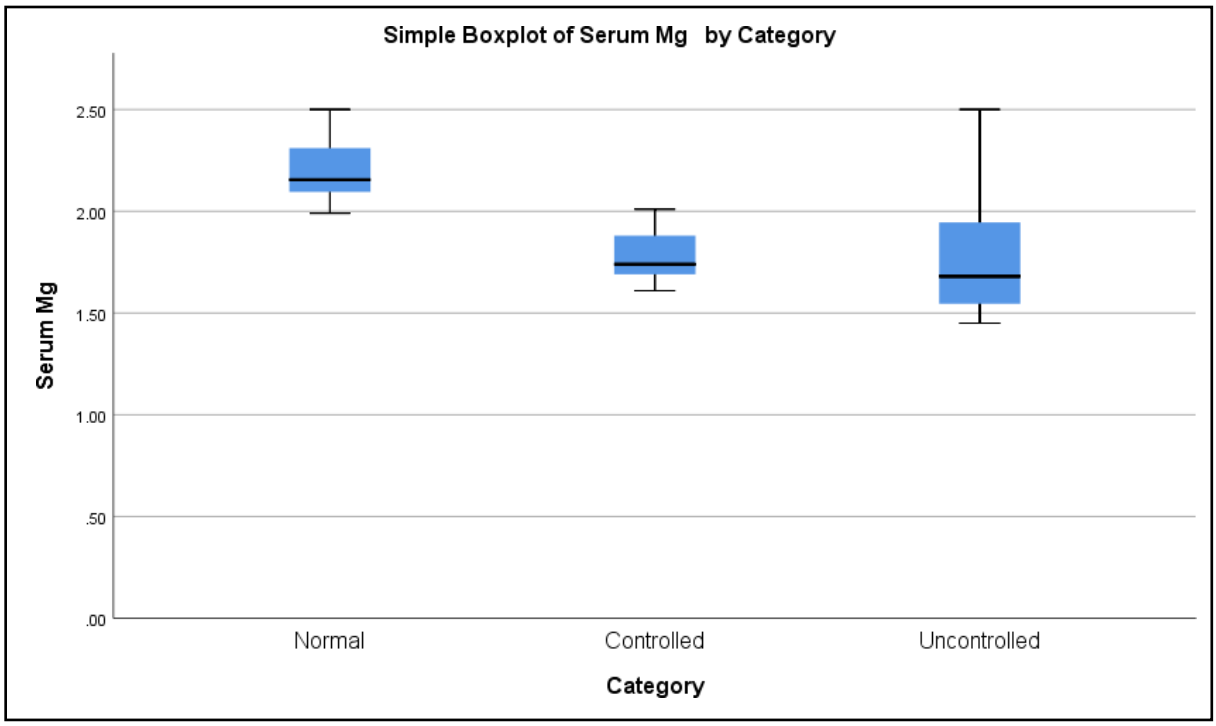
Group scatter plot to show the correlation of HbA1c and serum magnesium among group 1, group 2 and group 3 (where x axis represents HbA1c and y axis represent serum magnesium). We found a strong correlation between serum magnesium and HbA1c in group 2 and group 3. (Graph 3)



Graph 3 showing correlation of serum magnesium and HbA1c

In the present study we used simple box plot to compare the levels of serum magnesium with group 1, group 2 and group 3 (x axis represent 3 study groups; y axis represents serum

magnesium). We found that in group 1 (normal subjects) the simple box plot showed that the mean serum magnesium levels was 2.195 (minimum 1.99mg/dl and maximum 2.5mg/dl). In group 2 (controlled diabetics) the mean serum magnesium level was 1.775mg/dl (minimum 1.61mg/dl and maximum 2.01mg/dl). In group 3 (uncontrolled diabetics) the mean serum magnesium level was 1.766mg/dl (minimum 1.45mg/dl and maximum 2.5mg/dl). The box plot at different level suggests that there was an overall obvious difference in serum magnesium levels among the 3 study groups. It was also noted that box plot of uncontrolled type 2 diabetes mellitus was comparatively taller than others suggesting that variation of levels in serum magnesium is more. (Graph 4)



Graph 4 showing comparison of levels of serum magnesium with the 3 study groups

We found the correlation of dependent variable serum magnesium with predictor variables- age, gender and HbA1c through ANOVA test. We found that the levels of serum magnesium decreased as the levels of HbA1c increased thus displaying a linear relationship and was statistically significant ($p<0.05$).

We further found that with every 1 unit raise in HbA1c the serum magnesium level decreased by 0.073 units. Similar linear correlation was found between age and serum

magnesium levels; as age increased serum magnesium levels decreased. It was found to be statistically significant ($p<0.05$). However, gender did not contribute to the correlation of serum magnesium level with HbA1c with a p value of >0.05 and was not statistically significant. (Table 4)

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		
	B	Std. Error	Beta			Lower Bound	Upper Bound	p value
(Constant)	2.743	0.105		26.204	0.000	2.536	2.951	
Age[b]	-0.008	0.002	-0.310	-3.876	0.000	-0.012	-0.004	**<0.05**
Gender[b]	-0.033	0.042	-0.060	-0.780	0.437	-0.117	0.051	>0.05
HbA1c[b]	-0.073	0.013	-0.430	-5.392	0.000	-0.099	-0.046	**<0.05**
p≤0.05 (statistically significant)								
a. Dependent Variable: Serum Mg b. Constant (Predictors): Age, Gender, HbA1c								

Table 4 showing p value of constants and variables of ANOVA test

DISCUSSION

T2DM occurs due to resistance to insulin which is accompanied by increased dysfunction like excess visceral adiposity, dyslipidemia, hypertension, impaired fibrinolysis, increased platelet aggregation, vascular inflammation, endothelial dysfunction and premature atherosclerosis.[9] HbA1c is glycated hemoglobin formed by non-enzymatic reaction and is a normal physiologic function. HbA1c was 1st isolated by Huisman et al in 1958 and was characterized as a glycoprotein by Bookchin and Gallop in 1968. Koenig etal in 1976 1st proposed use of HbA1 as a biomarker for monitoring the levels of glucose among patients with diabetes. HbA1c provides a reliable measure of chronic glycemia and corelates well with the risk of long term diabetes complication and thus is the current choice in monitoring and management of type 1 and 2 diabetes mellitus.[6] HbA1c test is a non-enzymatic reaction between glucose and the N terminal end of the β chain which gives rise to HbA1c. Increase in plasma glucose results in increase of glycated hemoglobin and thereby increase of HbA1c levels in plasma. HbA1c is a reflection of glucose control over the period of red blood cell's life span (120 days). HbA1c can be assessed by separation method like chromatography, capillary electrophoresis or by chemical methods like immunoassays and enzymatic assays.[8]

Numerous trace elements play an important role in basic cellular reactions required to maintain energy production and life. It is noted that metabolism of several minerals is altered in diabetes mellitus which may play a specific role in pathogenesis and progression of diabetes.Magnesium (Mg) is the second most abundant intracellular cation and fourth most abundant cation in the human body that serves as a co-factor for all enzymatic reactions that require ATP. It is an essential enzyme activator for neuromuscular excitability and cell permeability, a regulator of ion channels and mitochondrial function, a critical element in cellular proliferation and apoptosis, and an important factor in both cellular and humoral immune reactions. Magnesium is involved in cardiac excitability, gating of calcium ion channels, transmembrane ion flux and neurotransmitter release.[10] Cellular Mg is a crucial cofactor for various enzymes involved in glucose transport, glucose oxidation, insulin release, and is a cofactor in the phosphorylation of glucose and helps in carbohydrate metabolism.[11,12]

Magnesium plays the role of a second messenger for insulin action; on the other hand, insulin itself is an important regulatory factor of intracellular magnesium accumulation.[13] Normal serum magnesium value is 1.8-2.3mg/dl and serum magnesium less than 1.7mg/dl is considered as deficiency or hypomagnesia. Intracellular Mg plays a key role in regulating insulin action, insulin-mediated-glucose-uptake and vascular tone. Reduced intracellular Mg concentrations result in a defective tyrosine-kinase activity, post receptorial impairment in insulin action and worsening of insulin resistance in diabetic patients. A low Mg intake and an increased Mg urinary loss appear the most important mechanisms that may favour Mg depletion in patients with type 2 diabetes.[14]

Thus in this present study we explored the correlation of glycated hemoglobin HbA1c and levels of serum magnesium in T2DM.

In the present study we compared serum magnesium levels in patients with controlled DM and uncontrolled DM and found that they were not statistically significant.

We found in this present study that the comparison of serum magnesium and FBS showed a negative correlation that is levels of serum magnesium was found to be decreased as FBS increased. This finding of ours is similar to the findings of Dipankur Kundu et al 2013 who additionally found low serum magnesium associated with increase in urine total proteins in patients with retinopathy.[15] Pratibha Misra 2019 and Geeta et al 2020 also found that serum

magnesium had significant correlation with FBS levels. However, our findings were in contrast to the findings of Kareem et al who found no correlation between serum magnesium and FBS.[9]

In the present study we found that comparison of serum magnesium and HbA1c in controlled and uncontrolled diabetics existed but was found to be not statistically significant. This finding of our study is in contrast to the studies of Jain et al who found that serum magnesium level in poorly controlled diabetic was lower than that of controlled diabetics.[12] Similarly we found that comparison of serum magnesium and HbA1c showed a strong correlation. We compared the mean values of FBS, PPBS and serum magnesium with the levels of HbA1c and found that they were statistically significant. The findings of our study correlate with the findings of Pujar S 2014, Labban 2019, John Thomas 2020.[16]

Further we found that the levels of serum magnesium expressed a linear relationship with HbA1c that is levels of serum magnesium decreased along with increase in levels of HbA1c. The finding of our study was similar to findings of previous studies by Schlienger et al, S.Ramadas et al, Senthil Manikandan TJ et al, Sharma A et al and de Lordes et al who found decline in serum magnesium levels with rise in HbA1c levels.[17] However our findings are in contrast with the findings of Al-Osali 2009; Pratyush Kumar 2019 who found that there was no significance in comparison of HbA1c and serum magnesium levels of T2DM. Hajar Saeed et al 2019, Masood et al and Walter et al found that serum magnesium level was normal in both T2DM and healthy controls.[18-20]

Weglicki WB 2012 and Saris NE et al 2000 showed a link between Mg deficiency and reduced insulin sensitivity in the presence of oxidative stress and increased free radicals in DM2.[21,22] Kumar et al 2019 found that diabetic retinopathy occurred significantly higher in patients with low serum magnesium than in patients with normal serum magnesium level.[23] Kumar et al in 2018 found that low serum magnesium levels was associated with diabetic nephropathy significantly higher in patients with hypomagnesemia as compared to those with normal magnesium levels.[24] Corsonello et al 2000, Corica et al 2006, Arpaci et al 2015 found that diabetic patients with low serum magnesium level showed significantly overt proteinuria.[25-27] Srinivasan et al 2012 and Labban et al 2019 showed that low serum magnesium level was linked with low levels of HDL.[28,29]

Considering the significant role of Mg in the maintenance of blood glucose level, it has been

used therapeutically as oral supplements. Rodríguez-Morán 2003 showed that oral magnesium supplementation improves both sensitivity and metabolic control in T2DM.[30] Ramadass 2015observed that when magnesium supplements were given there was a significant decrease in HbA1c values.[31] Djurhuus MS et al 2001 found that magnesium supplementation was associated with decrease in atherogenic lipid fractions and reduced insulin stimulated glucose uptake.[32] Lal J et al 2003 established that magnesium supplementation resulted in significant decrease in serum total cholesterol, LDL and triglycerides along with rise in HDL.[33]

Conclusion

Encouraged by these findings we stress on the importance of evaluation of serum magnesium as it reflects the glycemic status and HbA1c. We also recommend oral supplementation of magnesium in order to reduce the high glycemic index as well as delay the onset as well as progression of T2DM.

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