The diagnostic significance of calcium, phosphorus, magnesium and uric acid in type 2 diabetes mellitus and their association to HBA1C

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ABSTRACT

Divalent macrometals Calcium (Ca) and Magnesium (Mg) plays a significant role in the metabolism of carbohydrates, especially diverting assimilable glucose for utilization. Phosphate ions are essential during phorylation, a key enzyme reaction which diverts glucose to metabolic pathways. Urate, an end product of purine metabolism is altered in uncontrolled diabetes mellitus (DM) particularly in T2DM. As blood Glycosylated Hemoglobin (HbA1c) serves as an Index of long term Diabetic Control and is considered a gold standard, its level will indeed be associated with metals like Ca, Mg along with Phosphate and Urate, all of which are altered. Hence there must exist some association between HbA1c and Ca, Mg, P and Uric acid. Uric acid level is always low in DM and is associated with metabolic syndrome (metS) and serves as a potential biomarker for DM as its level is directly proportional to albuminuria. This study is an attempt to find out the association of HbA1c to Ca, Mg, P and Urate and to suggest that these 4 tests must be done for all T2DM patients.

Keywords: HbA1c, Ca, Mg, P, Urate, T2DM.

INTRODUCTION

Experimental diabetic rats were found to have low serum 1,25-dihydroxyvitamin D, deficiency in intestinal malabsorption of Ca, secondary hyperparathyroidism and bone loss. Absorption of Ca in controls was 60 ± 3% and in diabetics was 56 ± 3%. No derangement of calcium metabolism in adults with insulin-requiring juvenile- and adult-onset diabetes regardless of treatment status. The experimental diabetic rat model does not appear to be useful for determining the pathogenesis of adult human diabetic osteopenia [1]. Mean serum levels of some electrolytes were significantly lower in diabetics and HIV/AIDS patients than in controls, but were much lower in diabetics than in HIV/AIDS patients. The greater disturbances in serum electrolytes in diabetics improved with glycaemic control. In addition to restoring electrolyte status, highly active antiretroviral therapy (HAART) use in HIV/AIDS patients significantly improved serum total protein. Diabetic patients exhibit greater electrolyte disturbances than normal people suggesting an early detection and treatment of these abnormalities will enhance the quality of life of patients [2].

Increased urinary Ca excretion in 'functional hypoparathyroidism' is observed during poor blood glucose control, and may be one of the factors leading to reduced bone mass in DM [3] Accumulating data from animal models with DM reveals that intracellular Ca levels are increased in most tissues. The activities of the Adenosine Triphosphatase (ATPase) associated cation pumps, which determine intracellular Ca level (i.e., calcium-ATPase and [sodium + potassium]-ATPase) are also altered. Altered intracellular Ca metabolism may represent a common, underlying abnormality linking the metabolic, cardiovascular, ocular and neural manifestations of the diabetic disease process [4]. Cumulative evidence reveals that diabetes is a condition in which cell Ca**+** homeostasis is impaired. Defects in cell Ca**+** regulation were found in erythrocytes, cardiac muscle, platelets, skeletal muscle, kidney, aorta, adipocytes, liver, osteoblasts, arteries, lens, peripheral nerves, brain synaptosomes, retinal tissue and pancreatic beta cells confirming that this defect in cell Ca**+** metabolism is a basic pathology associated with the diabetic state. Better
understanding of the impairment in cell Ca\(^{2+}\) metabolism in diabetes may markedly enhance our understanding of this condition [5]. Serum calcium level was significantly lower in patients with T2DM compared to normal healthy controls. Hypocalcaemia was seen in 43% of patients with T2DM. Hypocalcaemia is significantly associated with uncontrolled hyperglycemia in patients with T2DM and should be looked for and corrected to have a better control of the blood sugar [6].

Level of potassium (K) in the biological samples of non-hypertensive diabetic patient was found to be higher, but it was not significant. The urinary level of K was found to be higher in both hypertensive diabetic and non-hypertensive diabetic patients than in the age-matched healthy controls. These results are consistent with those obtained in other studies, confirming that deficiency and efficiency of some essential trace metals may play a role in the development of DM [7]. A suboptimal vitamin D and Ca status has been associated with higher risk of T2DM in observational studies, but evidence from trials is lacking. In adults at risk of T2DM, short-term supplementation with cholecalciferol improved β-cell function and had a marginal effect on attenuating the rise in HbA1c [8].

Asymptomatic subjects with Extensive Coronary Artery Calcification (ECAC) are not firstly manifested as acute coronary events but presented a high level of Chronic Coronary Artery Disease (CCAD) related events during the 6.3 ±3.4 year follow-up. In contrast, first acute CAD-related events occurred mostly in subjects with mild and moderate Coronary artery calcification score [9]. The concentration of serum Alkaline Phosphatase (ALP) and Ca showed a significant increase in T2DM patients with periodontitis when compared to control, suggesting that increased serum ALP and Ca level could be often associated with the alveolar bone loss and tooth loss in T2DM with periodontitis among the south Indian population [10].

A paradoxical metabolic imbalance in phosphate (P) occurs from the early onset of diabetes and may lead to a reduction of high energy phosphates and tissue hypoxia. These changes take place in the cells and tissues in which the entry of glucose is not controlled by insulin and particularly in poorly regulated diabetes patients in whom long-term vascular complications are more likely to occur. Several therapeutic intervention trials have been carried out, including assessment of optimal glucose regulation, the effect of dietary inclusion of calcium diphosphate and pharmaceutical intake of etidronate disodium (EHDP), but none of these modalities wholly overcome the problem. The potential therapeutic application of fructose-1, 6-diphosphate, however, which also acts as human bioenergy holds a great deal of promise as an efficacious and well-tolerated therapeutic regimen [11].

A disturbance in phosphate handling occurs in the kidney tubules, where the excessive sodium-dependent glucose entry in diabetics depolarizes the electrochemical sodium gradient and consequently impairs P reabsorption. Similar changes may occur in other cells and tissues in which glucose entry is not controlled by insulin and particularly in poorly-regulated diabetic patients in whom long-term vascular complications are more likely [12]. In an Appropriate Blood Pressure Control in Diabetes cohort study, serum P, but not serum Ca or Ca*P product, was associated with cardiovascular mortality in time-dependent Cox regression models. Thus, serum P levels may be more reliable in predicting cardiovascular mortality in patients with T2DM [13].

This diminution in urinary P loss may have been due to diminished glycosuria but equally could have been influenced by a direct action of insulin on the renal tubule or suppression of glucagon and parathyroid hormone secretion. Under such conditions reduced urinary P may have been sufficient to cause a rise in serum P despite the known effects of insulin on the cellular influx of P [14]. A significant negative correlation between age and serum P and lower parathyroid activity in diabetic hemodialysis (HD) patients, which implies more prevalence of bone disease in elderly diabetic HD patients. Further study of bone disease in this group of patients is required to evaluate its effect on outcome and different therapeutic interventions [15].

Hyperglycemia causes excess urinary Ca and P excretion in patients with T2DM. In response to urinary Ca loss, parathyroid hormone (PTH) secretion is mildly stimulated. Bone formation seems to be suppressed in the hyperglycemic state in spite of increased PTH secretion [16]. The inhibition of the hypercalcemic response to phosphorus depletion (PD) in DM rats is mainly due to an inhibition of the resorptive response of bone to PD, and that insulin either directly or indirectly may play a permissive role in the development of the resorptive response of bone to PD [17]. Hyperphosphatemia is common in diabetic ketoacidosis (DKA) before therapy. The increase in serum P is likely to be due to a transcellular shift. Potential factors responsible for the shift are serum glucose, through its osmotic effect, and the organic anions [18].

The inorganic P is essential for the resynthesis of 2,3-diphosphoglycerate (DPG) and ATP, therefore phosphate depletion results in tissue hypoxia and decrease of energy rich P with disturbances of various organ systems. Regular controls of plasma P levels and a prophylactic substitution of P are recommended [19]. The level of P in the aqueous humor and serum of diabetics was significantly increased, especially in diabetics with proliferative diabetic
A large body of evidence demonstrates the prevalence and adverse clinical consequences of Mg deficiency in patients with DM. It would be prudent for physicians who treat these patients to consider Mg deficiency as a contributing factor in many diabetic complications and in exacerbation of the disease itself. Repletion of the deficiency or prophylactic supplementation with oral Mg may help avoid or ameliorate such complications as arrhythmias, hypertension, and sudden cardiac death and may even improve the course of the diabetic condition. No effect of sex, glycemic control or presence of microalbuminuria could be demonstrated on plasma concentration of trace elements in T1DM patients.[30]. Change in serum Mg level may have a bearing on the complication and morbidity in patients of DM.[31]. Untreated maternal DM reduces fetal Ca and Mg accretion by an effect on the expression of placental transport components involved in the maternofetal transfer of these cations.[32]. Zinc and Mg levels are not altered in DM, but the increased copper levels found in diabetes may merit further investigation of the relationship between copper and retinopathy. This result may be related to hydrophilic acrylic intraocular lens (IOL) opacification. Future studies regarding the pathogenic role of a high concentration of aqueous humor and serum phosphorus are required.[20].
T2DM [33]. Increased Mg intake was a significant protective factor for the incidence of T2DM in the general Japanese population, especially among subjects with insulin resistance, low-grade inflammation and a drinking habit [34].

The contents of Mg, K and Zinc in plasma did not correlate with the corresponding concentrations in skeletal muscle or circulating blood cells, as investigated in healthy controls, diabetics and in all subjects together, implying that the plasma concentrations are not useful in the assessment of electrolyte status. Hence, deficiency of electrolytes frequently occurs and should be looked for, in subjects with T2DM [35]. Decreased intracellular Mg can also result from a dietary deficiency of Mg or from an abnormal accumulation of saturated fatty acids in cell membranes, which inhibits the entrance of Mg into the cell; thus it is also the etiology not only of diabetes caused by Mg deficiency, but also of the "lipotoxic" T2DM. Although these pathologies cannot be corrected by the Mg-binding promoters, they can be corrected, respectively, by dietary Mg supplementation or by exercise plus dietary caloric and lipid restriction. Theoretically, the disease syndrome containing T2DM may involve approximately 30% of the population [36].

The serum Mg levels were significantly lower in the insulin treated group compared to the oral hypoglycemic (OHA) treated group [37]. Meta-analysis provides further evidence supporting that Mg intake is significantly inversely associated with risk of T2DM in a dose-response manner [38]. Hypomagnesemia is reported in T2DM; Mg deficiency may play a role in the development of endothelial dysfunction and altered insulin function [39]. It seems that in diabetic patients, kidney plays a key role in the regulation of serum Lipoprotein(a) levels and no significant correlation between serum Mg with serum HDL and Triglycerides were found, which needs further investigation [40]. Low serum Mg status is common in T2DM patients when compared to non-diabetic controls. It may be prudent in clinical practice to periodically monitor plasma Mg concentration in diabetic patients. If plasma Mg is low, an intervention to increase dietary intake of Mg may be beneficial to prevent the complications [41].

Uric Acid is independently associated with diabetes outcome, considerably more in women than in men [42]. Serum uric acid has been shown to be associated with cardiovascular disease, hypertension, and chronic kidney disease. However, few studies have examined the association between serum uric acid and DM and their findings are not consistent. Higher serum uric acid levels were inversely associated with DM in a representative sample of US adults [43].

Uric acid levels in patients with MetS1DM and T2DM and the mechanisms that influence uric acid levels in these patients and their potential links between uric acid and diabetic complications needs further studies. The effect on uric acid levels of drugs commonly prescribed for T2DM and the risk of uric acid nephrolithiasis in patients with MetS or DM needs more detailed study [44]. A positive relationship between glucose and uric acid concentrations up to about 8.0 mmol/L were observed and at higher levels of glucose, serum uric acid decreased. Uric acid levels were significantly reduced in T1DM and in those on oral hypoglycaemics and also in 'non-diabetics' with casual glucose levels greater than 10 mmol/L. Both uric acid and glucose concentrations were positively related to body mass index (BMI); only uric acid was positively related to alcohol intake. Men on antihypertensive treatment had raised levels of uric acid (significant) and glucose (non-significant). The positive relationship between serum uric acid and serum glucose could not be explained by the association with BMI, alcohol intake, age, social class, gout or treatment for hypertension. It probably reflects the biochemical interaction between serum glucose and purine metabolism, with increased excretion of uric acid during hyperglycaemia and glycosuria [45].

Higher serum uric acid concentrations were associated with a greater probability of albuminuria in patients with T2DM [46]. Plasma uric acid levels were elevated in men and women with impaired glucose tolerance in both ethnic groups. The lowest plasma uric acid levels were found in diabetic patients, especially in diabetic men. Even though obesity was positively associated with plasma uric acid, it did not explain the high plasma uric acid level in persons with impaired glucose tolerance. BMI had a significant and independent impact on plasma uric acid levels both in non-diabetic and diabetic men and women. Many predictive variables and their interactions were analysed along with the reasons for the high plasma uric acid levels in persons with impaired glucose tolerance and for the low plasma uric acid levels in diabetic patients [47].

Patients undergoing operation for primary hyperparathyroidism had a decreased relative survival during a 10-year follow-up compared to the general population. This decrease in relative survival is associated with DM and increased levels of uric acid pre-and postoperatively [48]. During normal pregnancy, plasma concentrations of creatinine and uric acid normally decrease as a consequence of their increased glomerular filtration. Hyperuricemia in pregnant women has been associated with several pregnancy complications. Patients with gestational diabetes had significantly higher levels of creatinine than normal pregnant women [49]. Serum uric acid values were in positive correlation with serum C-peptide values considering, among the diabetic subjects, only those with duration of
diabetes less than 5 years and without micro-macrovascular complications suggesting that these data lead to presume that diabetic patients with short duration of disease and without complications show a different serum uric acid pattern, strictly related to beta-cellular secretion [50].

Uric acid concentration is higher in subjects at high risk of DM with abnormal glucose tolerance and is independently determined by various components of the MetS [51]. Multiple regression analysis demonstrated that serum uric acid concentration, duration of diabetes, Glycosylated Hemoglobin (HbA1c), serum triglyceride concentration and systolic blood pressure were independent determinants of logarithm of urinary albumin excretion. Serum uric acid concentration is associated with microalbuminuria and subclinical atherosclerosis in men with T2DM [52]. Studies investigating the prognostic role of uric acid in patients with T2DM have given conflicting findings. Patients with T2DM and confirmed CAD, elevated levels of uric acid predict mortality independently of known cardiovascular risk factors [53].

The prevalence of hyperuricaemia was dependent on age and duration of the disease in T1DM patients, whereas in recently diagnosed T2DM patients, the prevalence of elevated uric acid levels was higher than in patients with long-standing T1DM, without any further increase with longer duration of the disease. An elevated uric acid level was also associated with body weight, hypertension and nephropathy in both types of diabetes and in both sexes. Elevated uric acid levels are correlated with the presence of coronary heart disease in female rather than in male diabetic patients, independently of hypertension and nephropathy [54]. Serum uric acid had positive association with cholesterol, triglyceride, non-HDL cholesterol and a negative association with Fasting Plasma Glucose (FPG), HbA1c and HDL cholesterol. Possible independent biochemical predictors of hyperuricemia were cholesterol, triglyceride, creatinine and FBG.

High serum uric acid levels are associated with the MetS, T2DM and cardiovascular disease. It is largely unknown whether there are gender-specific differences regarding the association between uric acid and pre-diabetic states. Serum uric acid concentrations were associated with different categories of impaired glucose regulation in individuals from the general population, particularly in women. Further studies investigating the role of uric acid in the development of derangements in glucose metabolism are needed [57]. Serum concentration of uric acid showed a positive relationship with the total phase of insulin secretion; even in states prior to hyperuricemia, uric acid can play an important role in the function of the beta cell in patients with T2DM [58]. The correlation between uric acid and other parameter was not very relevant. A study showed significant increase in serum uric acid with the increase in blood glucose value in T2DM. Elevated uric acid levels are associated with increased risk of cardiovascular mortality in T2DM [59].

Serum uric acid concentration raised with increasing FPG levels up to FPG of 7.0 mmol/L, but significantly decreased thereafter with further increase in FPG levels. Uric acid may serve as a potential biomarker of deterioration in glucose metabolism, but its clinical implication need to be further studied [60].

**EXPERIMENTAL SECTION**

The study population consisted of 100 non-hospitalised diabetic patients, both males and females in the age group of 50 to 70 years, who underwent routine Master Health Check Up.

**Inclusion Criteria**
Patients with HbA1c values more than 6.5% were included for this study.

**Exclusion criteria**
Results available from other clinics such as cardiac and endocrine were excluded for this study.

**Measurements of Analytes used in this study**
Fully Automatic DIRU CS 1300B analyser and DiaLab kits were used to measure Calcium, Phosphorus, Magnesium and Uric Acid.
The following were the principle of the kits used for measuring the analytes.

- Calcium: Arsenazo 111 dye binding
- Phosphorus: UV Molybdate
- Magnesium: Xylydyl Blue dye binding

HbA1c was measured using Bio-Rad D 10 analyser and kit supplied by them. This uses HPLC Methodology.

For all tests listed above, calibrators used were supplied by the kit manufacturers and the accuracy of the results obtained for all analytes were validated by using Bio-Rad accuracy controls at two different levels.

**Statistical Analysis**

For statistical analysis of data, a software downloaded from the website http://www.vassarstats.net was used to calculate correlation coefficient (r), student’s distribution (t) and probability (P) between HbA1c and Ca, P, UA and Mg.

**Table – I: Patients (HbA1c >6.5%) Vs Controls (HbA1c <6.5%)**

<table>
<thead>
<tr>
<th>Patient Groups</th>
<th>Statistical Parameters</th>
<th>HbA1c</th>
<th>Uric acid</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with HbA1c &gt;6.5% (n=50)</td>
<td>Mean</td>
<td>8.85</td>
<td>5.22</td>
<td>9.38</td>
<td>4.13</td>
<td>2.02</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.62</td>
<td>1.64</td>
<td>0.39</td>
<td>0.58</td>
<td>0.37</td>
</tr>
<tr>
<td>Patients with HbA1c &lt;6.5% (n=50)</td>
<td>Mean</td>
<td>5.63</td>
<td>5.03</td>
<td>9.47</td>
<td>4.10</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.42</td>
<td>1.26</td>
<td>0.33</td>
<td>0.68</td>
<td>0.36</td>
</tr>
</tbody>
</table>

**Table – II: Patients with HbA1c < 6.5%**

<table>
<thead>
<tr>
<th>Analytes Compared</th>
<th>R</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c Vs Ca</td>
<td>0.253308</td>
<td>1.814</td>
<td>0.03796</td>
</tr>
<tr>
<td>HbA1c Vs P</td>
<td>0.435362</td>
<td>3.35</td>
<td>0.000789</td>
</tr>
<tr>
<td>HbA1c Vs Mg</td>
<td>0.27225</td>
<td>1.96</td>
<td>0.027895</td>
</tr>
</tbody>
</table>

**Table – III Patients with HbA1c > 6.5%**

<table>
<thead>
<tr>
<th>Analytes Compared</th>
<th>Groups</th>
<th>r</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c Vs UA</td>
<td>All patients (n=50)</td>
<td>-0.2213</td>
<td>-1.572</td>
<td>0.06124</td>
</tr>
<tr>
<td></td>
<td>Female (n=25)</td>
<td>-0.334187</td>
<td>-2.52</td>
<td>0.00755</td>
</tr>
<tr>
<td>HbA1c Vs Mg</td>
<td>Males (n=25)</td>
<td>-0.29863</td>
<td>-2.168</td>
<td>0.0176</td>
</tr>
<tr>
<td></td>
<td>Females (n=25)</td>
<td>-0.38382</td>
<td>-2.88</td>
<td>0.002965</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

Table I presents the mean and Standard Deviation (SD) results for patients (HbA1c >6.5%) and controls (HbA1c<6.5%) for all the analytes studied. Except HbA1c, no changes in other analytes were observed between patients and controls.

However when statistical analysis were done (as shown in Tables II and III), we found out some associations between HbA1c and the analytes compared in this study as per ‘P’ values.

Table II presents the statistical parameters for the controls studied. HbA1c shows significant association to Ca, P and Mg, but no association was observed with Uric Acid, indicating that Ca, P and Mg analytes are mainly involved in regulating glucose metabolism.

The statistical parameters obtained for the patients, whose HbA1c values were> 6.5 % are presented in Table III.

While HbA1c shows moderate association with Uric Acid, for all patients, its association for female patients was found to be highly significant ( P= 0.00755). When HbA1c was correlated to Ca and Mg, it shows significant association to Mg in the case of males and to Ca for females.

Hence it is clear that HbA1c is certainly associated to Ca, P and Mg and to less extend to Uric Acid.

This research paper presents more detailed data for bringing out the available informations on the association between HbA1c and Ca, P, Mg and Uric acid, all of which are involved in regulating glucose metabolism. Since HbA1c is the Gold Standard to monitor diabetic control and also emerging as the first line of screening test for diagnosing DM, we undertook this experimental work as an extension of the literature survey that we did to find out if some useful associations exist between HbA1c and the analytes involved in DM.
The literature contains lot of data linking Ca with alterations in the metabolic pathways of DM patients [6,7,18] and hence it is possible that HbA1c must be linked to Ca. Similarly extensive research have been done linking Mg to uncontrolled DM and hence HbA1c is indeed linked to this metal also.[26,27,29] P is the main non-metal that works to initiate glucose metabolism and many works have been carried out in this field and hence HbA1c must be linked to P and our finding is in consistent with earlier observation .[13,14,18]. Uric Acid levels are altered in uncontrolled DM and its circulating level in plasma serves as an index of DM status and hence it must been linked to HbA1c as observed in this study for all patients and females, indicating that females are more prone to gout[43,45,47,48 50].

CONCLUSION

This research article has brought out extensive literature review on the role of Ca, P, Mg and Uric Acid in DM and brought out some research works linking HbA1c to the above analytes. The research work carried out in this topic predicts the usefulness of measuring Ca, P, Mg & Uric Acid in all T2DM patients as per the association found between HbA1c and the analytes measured. Our attempt to link HbA1c to all the four analytes has given some significant inverse association of HbA1c with Ca, Mg and Uric Acid for DM patients and to Ca, P and Mg in the case of controls. Further studies are needed to make solid conclusions and may be an extension of our research.

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