Study of the Serum Levels of Iron, Ferritin and Magnesium in Diabetic Complications

Renuka P*, M Vasantha

Department of Biochemistry, SRM Medical College Hospital and Research Centre, Kattankulathur, Kancheepuram District - 603203, Tamil Nadu.

ABSTRACT
Alteration in mineral status has been found to be associated with impaired insulin release, resistance and dysglycemia. To study the mineral status in diabetic complications - thirty patients each with diabetic neuropathy, nephropathy and retinopathy along with thirty uncomplicated diabetes mellitus patients and age matched thirty healthy controls were recruited for this study. Estimation of glycemic status, magnesium, iron and ferritin levels was done. Magnesium levels were found to be significantly decreased in the microvascular complications which correlated negatively with glycated hemoglobin. Iron and ferritin levels were found to be significantly increased in diabetic neuropathy, nephropathy and retinopathy. Hypomagnesemia, increased iron levels and hyperferritinemia seem to be associated with microvascular complication of diabetes mellitus as either causative factors or as a consequence of the disease. Screening of patients for these factors might help in the management of progression of the disease.

Key words:

INTRODUCTION
Long standing metabolic derangement in Diabetes mellitus is associated with permanent and irreversible functional and structural changes in the vascular system resulting in development of complications affecting the kidney, eye and nervous system. Impaired insulin release, insulin resistance and glucose intolerance have been found to be associated with alteration in mineral status in diabetic patients. Various mechanisms have been proposed for the onset of diabetes and the development and progression of diabetic complications one of which is alteration in the mineral status. Magnesium and iron are very important cations needed for the optimum cellular process. Magnesium is an abundant intracellular ion playing a vital role in insulin secretion and activity. Insulin is involved in the transport of magnesium through the cellular membrane and in the intracellular supply. Hypomagnesemia may enhance endothelial cell dysfunction and thrombogenesis by increased platelet aggregation and vascular calcification. Low magnesium levels also seem to result in proinflammatory and profibrinogenic response reducing the protective enzymes against oxidative stress. Iron is a transition metal that acts as an oxidant. Increased accumulation of iron affects insulin synthesis and secretion in the pancreas. Iron deposition in the liver may cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose production. Catalytic iron converts poorly reactive free radicals like H₂O₂ into highly reactive ones like hydroxyl radical and superoxide anion that can initiate and propagate the cascades leading to oxidative damage. Several studies have shown the role of oxidative stress in diabetic patients with iron overload. Ferritin is an index of body iron stores and is an inflammatory marker. Body iron stores are positively associated with the development of glucose intolerance, type 2 diabetes mellitus. Studies involving the levels of magnesium, iron and ferritin in microvascular complications of diabetes mellitus have yielded controversial results. In this study, we evaluated the magnesium, iron and ferritin status in patients with diabetic neuropathy, retinopathy and nephropathy.

MATERIAL AND METHODS
The study was conducted at SRM Medical College Hospital & Research Centre after obtaining the approval from the Institutional Ethical Committee. Thirty patients each diagnosed with diabetic retinopathy, neuropathy and nephropathy were recruited along with 30 diabetic patients without complications. Control group comprised of thirty healthy volunteers. All the subjects were in the age group of 35 - 70 years. Subjects with ESRD (end stage renal disease), anemia, those on mineral and iron supplements, drugs like aminoglycosides, amphotericin B, Cetuximab, cyclosporine, digoxin, diuretics were excluded from the study. After obtaining consent from the patient and eliciting the relevant history, fasting blood samples were collected for the estimation of glucose, HbA₁c, lipid profile, iron, ferritin and magnesium. Urine samples were collected for microalbumin analysis. Plasma glucose, lipid profile, iron, magnesium and urine microalbumin were estimated using standard kits in Beckman Coulter AU 400 chemistry

*Author for Correspondence
The development of microvascular complications of diabetes, including retinopathy, nephropathy, and neuropathy, is often attributed to the presence of hypomagnesemia. Reduced tubular reabsorption of magnesium due to diabetes mellitus can lead to significant hypomagnesemia in patients with diabetic retinopathy, nephropathy, and neuropathy. Patients with these complications had significant hypomagnesemia compared to healthy controls. Hypomagnesemia is considered as a possible risk factor for the development and progression of retinopathy, nephropathy, and neuropathy.

In our study, we observed that serum magnesium levels were significantly lower in diabetic nephropathy patients compared to diabetic patients without complications. The results also indicated that magnesium depletion might be linked to the pathogenesis of nephropathy. Our study demonstrated an increase in iron and ferritin levels in diabetic nephropathy subjects with improvement in nerve conduction following supplementation, suggesting that magnesium depletion might be linked to the pathogenesis of this complication.

RESULTS AND DISCUSSION

Our study shows that serum magnesium levels are decreased in micro vascular complications of Diabetes mellitus - Retinopathy, Nephropathy and Neuropathy. Similar results were observed in inverse correlation with glycemic control and a strong association with diabetic retinopathy. The various causes of low magnesium in diabetes include diets low in magnesium, osmotic diuresis causing high renal excretion of magnesium, insensitivity to insulin affecting the intracellular magnesium transport causing loss, rampant use of diuretics promoting magnesium wasting and reduced tubular reabsorption due to insulin resistance. There are reports of higher incidence of retinopathy in diabetics with hypomagnesemia. In our study, diabetic retinopathy patients had lower magnesium values compared to diabetic patients without any complications. Hypomagnesemia is considered as a possible risk factor for the development and progression of diabetic retinopathy. Diabetic nephropathy patients had significant hypomagnesemia in our study. Sakaguchi et al. observed that hypomagnesemia was significantly associated with progression of ESRD in patients with diabetic nephropathy but not in those with non-diabetic CKD. Serum magnesium levels were significantly lower in diabetic nephropathy patients compared to diabetic patients without complications. Low intracellular levels were reported in diabetic peripheral neuropathy subjects with improvement in nerve conduction following supplementation suggesting that magnesium depletion might be linked to the pathogenesis of this complication. A negative correlation was observed between serum magnesium levels and glycated hemoglobin in all the three studied microvascular complications emphasizing its role in the progression of the disease. Our study demonstrated an increase in iron and ferritin levels in diabetic micro vascular complications. Ferritin is the storage form of iron and it releases iron in a controlled fashion which plays a vital role in the maintenance of intracellular iron balance. Iron is a potent pro-oxidant and reactive oxygen species have been shown to interfere with insulin signaling at the cellular level. Shi et al. used PC-12 cells as an invitro model of peripheral neuropathy and demonstrated that iron overload aggravates oxidative stress injury in neural cells under high glucose concentration and that the Nrf2/ARE signaling pathway might play an important role in the pathogenesis of neuropathy. Extensive glycation of basement membrane/internal elastic lamina proteins generates adduct capable of binding adventitious iron.

### Table 1: Demographic and biochemical parameters of diabetic patients with and without complications and healthy controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n= 30)</th>
<th>Diabetes (n= 30)</th>
<th>Neuropathy (n= 30)</th>
<th>Nephropathy (n= 30)</th>
<th>Retinopathy (n= 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dL)</td>
<td>83.8 ± 8.2</td>
<td>123.1 ± 22.4 *</td>
<td>148.1 ± 19.9 *</td>
<td>158.7 ± 27.3 *</td>
<td>187.5 ± 93.8 *</td>
<td>&lt; 0.05 *</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>14.6 ± 1.5</td>
<td>14.8 ± 1.56</td>
<td>14.7 ± 1.49</td>
<td>14.1 ± 1.19</td>
<td>14.0 ± 1.06</td>
<td></td>
</tr>
<tr>
<td>T.Chol (mg/dL)</td>
<td>165.7 ± 10.37</td>
<td>242.3 ± 24.45 *</td>
<td>242.3 ± 28.46</td>
<td>241.9 ± 27.1</td>
<td>244.8 ± 46.2</td>
<td></td>
</tr>
<tr>
<td>TGL (mg/dL)</td>
<td>94.3 ± 16.7</td>
<td>164.3 ± 32.77 *</td>
<td>166.0 ± 20.47</td>
<td>182.1 ± 41.33 *</td>
<td>180.1 ± 38 *</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>41.0 ± 3.41</td>
<td>54.5 ± 5.52</td>
<td>60.8 ± 7.05</td>
<td>56.9 ± 11.41</td>
<td>57.0 ± 11.1</td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>99.7 ± 15.9</td>
<td>130.3 ± 22.3</td>
<td>145.2 ± 23.4</td>
<td>140.4 ± 40.85</td>
<td>140.7 ± 32.8</td>
<td></td>
</tr>
<tr>
<td>Microalbumin (mg/L)</td>
<td>9.2 ± 4.06</td>
<td>101.3 ± 77.01 *</td>
<td>116.3 ± 67.43</td>
<td>423.1 ± 63.49</td>
<td>129.9 ± 58.2</td>
<td></td>
</tr>
</tbody>
</table>

* * p < 0.05 is considered statistically significant

### Table 2: HbA1c, Iron, Ferritin, Magnesium levels in diabetic nephropathy in comparison with diabetic and control subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n= 30)</th>
<th>Diabetes (n= 30)</th>
<th>Diabetic nephropathy (n= 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>5.0 ± 0.45</td>
<td>6.7 ± 0.11</td>
<td>7.5 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>145.3 ± 21.25</td>
<td>200.9 ± 19.9</td>
<td>225.5 ± 34.2</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>192.2 ± 60.5</td>
<td>304.0 ± 41.22</td>
<td>351.9 ± 24.2</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Magnesium (mg/dL)</td>
<td>1.9 ± 0.11</td>
<td>1.5 ± 0.11</td>
<td>1.4 ± 0.12</td>
<td>&lt; 0.01 *</td>
</tr>
</tbody>
</table>

* p < 0.05 is considered statistically significant

### Table 3: HbA1c, Iron, Ferritin, Magnesium levels in diabetic nephropathy in comparison with diabetic and control subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n= 30)</th>
<th>Diabetes (n= 30)</th>
<th>Diabetic nephropathy (n= 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>5.0 ± 0.45</td>
<td>6.7 ± 0.11</td>
<td>7.4 ± 0.74</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>145.3 ± 21.25</td>
<td>200.9 ± 19.9</td>
<td>261.9 ± 27.5</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>192.2 ± 60.5</td>
<td>304.0 ± 41.22</td>
<td>357.5 ± 28.5</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Magnesium (mg/dL)</td>
<td>1.9 ± 0.11</td>
<td>1.5 ± 0.11</td>
<td>1.4 ± 0.1</td>
<td>&lt; 0.001 *</td>
</tr>
</tbody>
</table>

* p < 0.05 is considered statistically significant

subendothelial glycochelates catalytically destroy endothelium derived nitric oxide leading to imbalance of vasodilator vs vasoconstrictor factors resulting in a relative state of chronic vasoconstriction. The diminished blood flow to peripheral nerves leads to neuronal hypoxia and eventual nerve death. oxidative stress factors such as hyperglycemia, advanced glycation end products and hyperlipidemia contribute to the availability of intracellular iron that can generate and viciously worsen oxidative stress and renal damage. Iron content in the kidney has been shown to be increased in animal models of diabetes. There is considerable evidence that one renal insufficiency develops, regardless of etiology, it tends to progress over time. The pathogenic role of iron in progression of kidney disease is indicated by the observation that progression can be prevented either by an iron-deficient diet or chelators. Hsu et al found that hyperferritinemia may be an independent risk factor of nephropathy in patients with type 2 diabetes. In the diabetic eye, there is an impairment of iron homeostasis, leading to iron overload. The mechanisms involved in this include: (1) Destruction of heme molecules induced by hyperglycemia (2) intra retinal and vitreal hemorrhages (3) Overexpression of the renin-angiotensin system. The main consequences of iron overload are the following: (1) Retinal neurodegeneration due to the increase of oxidative stress (2) increase of AGE-RAGE binding (3) Defective phagocytosis of retinal pigment epithelium, which generates the accumulation of autoantigens and the synthesis of pro inflammatory cytokines. Iron catalyses the binding of the AGEs to the specific receptor, a crucial step in the pathogenesis of Diabetic retinopathy. The observational nature of our study design precludes proving a causative relationship between the altered mineral status and the diabetic microvascular complications. The cause of deficiency of magnesium is not established as quantification was not done for magnesium in dietary sources nor was urinary magnesium measured in this study. Serum albumin level was not measured to reflect accurate level for serum magnesium. Therefore, further studies are needed to address these issues. Hypomagnesemia, increased iron levels and hyperferritinemia seem to be associated with microvascular complications of Diabetes mellitus. Routine screening of these elements might help in mitigating the progression of diabetes mellitus towards complications by either magnesium supplementation or iron chelation therapy.

REFERENCES