INFLUENCE OF MODIFIED LEVELS OF PLASMA MAGNESIUM, Cu, Zn AND Iron LEVELS ON THIOLS AND PROTEIN STATUS IN DIABETES MELLITUS AND DIABETIC RETINOPATHY

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ABSTRACT: The clinical research suggests that the homeostasis of trace elements can be disrupted by diabetes mellitus. Conversely, research also suggests that early imbalances of specific elements may play an important role in upsetting normal glucose and insulin metabolism. This study evaluated the levels of Cu, Zn, Mg & Iron and its influence on protein oxidation which reflects the extent of oxidative stress and the thiols, which protects the proteins from oxidation in plasma of diabetic (n=60) and diabetic retinopathy (n=40) patients. 30 healthy non-diabetic subjects were studied for comparative analysis. There was a significant increase in the copper (p < 0.001) and AOPP (p < 0.01), & iron (p<0.05), and decrease in Zinc (p<0.001), Mg (p < 0.001) and total thiols (p<0.001) in diabetic patients without complications when compared with normal controls. Further, an increase in copper (p < 0.001), iron (p<0.001) and AOPP (p<= 0.005) and decrease in Mg (p< 0.001), Zinc (p< 0.001), total thiols (p< 0.001) in diabetic retinopathy patients when compared to the controls. A significant decrease in Total thiols, Zn and Mg and an increase in Iron levels was observed in DR patients when compared with DM.

Key words: Diabetes mellitus, Diabetic Retinopathy, Mg, Trace elements.

INTRODUCTION

Type-2 diabetes is a chronic disease characterized by a disorder of the glucose metabolism associated with a reduced ability of tissues to respond to insulin (insulin resistance). The resulting chronic hyperglycemia damages blood vessels and nerve cells throughout the body, producing micro-vascular diseases such as retinopathy, neuropathy, and nephropathy. Moreover, the risk for cardiovascular disease is considerably elevated in patients with type-2 diabetes compared to the general population. As a consequence, type-2 diabetes represents a major public health problem.

The clinical research suggests that the homeostasis of trace elements can be disrupted by diabetes mellitus [1]. Conversely, research also suggests that early imbalances of specific elements may play an important role in upsetting normal glucose and insulin metabolism. With regard to essential trace elements, the main clinical interest and the majority of publications focus on deficiencies of a single element or certain combinations of elements. Trace-element deficiencies are frequently associated to chronic diseases or to problems with its absorption. Chronic hyperglycemia may cause significant alterations in the status of some micronutrients, and on the other hand, some of these nutrients can directly modulate glucose homeostasis [2]. Deficiencies of certain minerals such as Mg, Zn and Cr have been shown to predispose a person to glucose intolerance and to promote the development of diabetic complications [3]. It was reported that Zn is involved in the synthesis, storage, secretion, and conformational integrity of insulin monomers and that Zn assembles it to a dimeric form for storage and secretion as crystalline insulin [4]. There are also reports of altered metabolisms of other micronutrients such as Cu, Fe, and Mg in diabetes.
Altered insulin metabolism, poor glycemic control and osmotic diuresis may be the contributory factors for the development of the associated complications of DM. Fung et al [5] found an inverse association between Mg intake and fasting insulin level. Several experimental studies suggest a protective role of Mg intake against diabetes using a rat model of spontaneous type-2 diabetes. Baton et al [6] demonstrated significant reduction in the incidence of diabetes after 7 weeks of feed with Mg rich diet. In humans, some [7] but not all [8] experimental studies have shown benefits of Mg supplementation on glucose metabolism or/and insulin sensitivity. Diabetes mellitus is associated with altered iron homeostasis in both human and animal models. Iron is capable of generating reactive oxygen species and contributes to diabetic nephropathy. Excess Fe has been implicated in the pathogenesis of diabetes and its complications [9]. In view of these facts, the work was carried out to study the correlation between Mg, Fe, Cu, Zn, AOPP and total thiol levels with respect to diabetes and diabetic retinopathy, so that any significant outcome of the work, can be applied to avert the complication of diabetes mellitus in clinical practice.

MATERIALS AND METHODS

The study group consisted of a total of 130 subjects which included 30 healthy controls, 60 diabetic patients without any complications and 40 diabetic retinopathy patients in the age group between 40 – 60 years of both sexes.

30 age and sex matched healthy individuals chosen as controls had no history of diabetes mellitus, hypertension, epilepsy, acute or chronic inflammatory conditions, psychiatric disorders, history of drug intake, smoking or alcohol consumption. Diabetic patients recruited for the study had no secondary complications of diabetes and they were all on oral hypoglycemic drugs. Diabetic retinopathy patients had no other secondary complications of diabetes. None of the subjects were on antioxidant supplements. History of all the subjects regarding the duration of diabetes, presence or absence of hypertension or dyslipidemia were taken into account. Type 2 diabetic patients were diagnosed based on the history, biochemical investigation and according to the biochemical criteria laid down by WHO. Cases of diabetic retinopathy were diagnosed by the ophthalmologist by ophthalmoscopy and included both proliferative and non-proliferative diabetic retinopathy. The study protocol was approved by the institutional ethical committee and informed consent was obtained from all subjects. 5 ml of venous blood was collected in heparinized vacutainers under aseptic precautions after taking informed consent. The blood samples were centrifuged at 3000 rpm for 10 min. Plasma Mg level was estimated by photometric method [10] using xylidyl blue, iron by dipyridil method of Ramsay [11], copper [12] and zinc [13] by spectrophotometric method. Total thiol level was estimated by the method of Ellman [14] and oxidative products of proteins by Witko’s method [15].

RESULTS

Table-1: Total thiols, Zn and Mg and an increase in Iron level were observed in DR patients

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 30)</th>
<th>DM (n = 60)</th>
<th>DR (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total thiols (mg/dl)</td>
<td>0.623±0.086</td>
<td>0.463±0.046</td>
<td>0.344±0.068</td>
</tr>
<tr>
<td>ACP (mmols/L)</td>
<td>0.017±0.00</td>
<td>0.275±0.07</td>
<td>0.352±0.06</td>
</tr>
<tr>
<td>Iron (µg/dl)</td>
<td>120.3±31.6</td>
<td>138.06±28.6</td>
<td>159.84±32.8</td>
</tr>
<tr>
<td>Copper(µg/dl)</td>
<td>51.22±19.38</td>
<td>76.40±18.09</td>
<td>79.15±17.84</td>
</tr>
<tr>
<td>Zinc(µg/dl)</td>
<td>106±12.75</td>
<td>70.5±20.8</td>
<td>59.23±12.6</td>
</tr>
<tr>
<td>Mg(mg/dl)</td>
<td>2.22±0.392</td>
<td>2.08±0.464</td>
<td>1.49±0.572</td>
</tr>
</tbody>
</table>

DM = Diabetes mellitus    DR = Diabetic retinopathy
a: p < 0.001, b: p<0.01, c: p<0.05    Control vs. DM,
d: p < 0.001, e: p<0.005,    Control vs. DR
‡: p < 0.001, #: p < 0.01   DM vs. DR
There was a significant increase in the copper (p < 0.001) and AOPP (p < 0.01), & iron (p<0.05), and decrease in Zinc (p<0.001), Mg (p < 0.001) and total thiols (p<0.001) in diabetic patients without complications when compared with normal controls. Further, an increase in copper (p < 0.001), iron (p<0.001) and AOPP (p<= 0.005) and decrease in Mg (p< 0.001), Zinc (p< 0.001), total thiols (p< 0.001) in diabetic retinopathy patients when compared to the controls. A significant decrease in Total thiols, Zn and Mg and an increase in Iron level were observed in DR patients when compared to DM.

DISCUSSION

Trace elements have a strong influence on metabolism in the body and alterations in the trace element levels have been found as either the cause for or the result of disorders like diabetes and altered insulin metabolism, poor glycemic control and osmotic diuresis may be the contributory factors [16]. Although many authors have suggested that diabetes per se may induce hypomagnesemia, others have reported that higher Mg intake may confer a lower risk for type 2 diabetes [17]. Not only has hypomagnesemia been associated with type 2 diabetes, but also numerous studies have reported an inverse relationship between glycemic control and serum Mg levels [18]. It is interesting that the induction of Mg deficiency has been shown to reduce insulin sensitivity in individuals without diabetes, whereas Mg supplementation during a 4-wk period has been shown to improve glucose handling in elderly individuals without diabetes. In patients with type 2 diabetes, oral Mg supplementation during a 16-wk period was suggested to improve insulin sensitivity and metabolic control [19]. The mechanisms whereby hypomagnesemia may induce or worsen existing diabetes are not well understood. Nonetheless, it has been suggested that hypomagnesemia may induce altered cellular glucose transport, reduced pancreatic insulin secretion, defective post receptor insulin signalling, and/or altered insulin–insulin receptor interactions. Not all studies, however, observed a correlation between glycemic control and serum Mg levels or improvement of diabetic control with Mg replacement [20]. The conflicting data may reflect different study designs and populations studied. In the present study, there was a significant decrease in the serum Mg level in patients with retinopathy when compared with normal as well as diabetics without retinopathy. Several studies reported that increased urinary Mg excretion in type 1 and 2 diabetes mellitus and impaired intestinal absorption [21] might contribute to the observed hypomagnesamia. Mg depletion has a negative effect on glucose homeostasis and insulin sensitivity in persons with diabetes and may be one of the reasons for evolution of diabetic retinopathy.

Diabetes Mellitus is an endocrinological disease having metabolic and oxidative stress in high quantity. In the presence of free radicals, glucose can undergo a process called auto-oxidation. Findings show that oxidative stress has the greatest role in developing complications. There are several potential sources of increased free radical production in diabetes including auto-oxidation of plasma glucose and increased transition metal bioavailability. The radical – scavenging antioxidant activity of the serum of people with DM is lower than that of age-matched controls. This may be attributed with the trace elements [22]. Iron promotes glycoxidation reaction in the presence of glucose and other sugars at alkaline pH forming enediols and dicarbonyls leading to the formation of advanced glycation end products. It has been reported that there is an increased accumulation of these glycation end products in long and short lived proteins in DM, thereby affecting their normal functioning.

The retina has high content of PUFA, highest uptake of oxygen and glucose oxidation relative to other tissues. This phenomenon renders retina more susceptible to oxidative stress. Biomarkers of protein oxidation are often applied when a battery of markers of oxidative stress status are being studied. Elevated biomarkers of protein oxidation have often been associated with diseases such as Alzheimer’s, diabetes mellitus and cancer. Chronic hyperglycemia favors glycation reactions and non-enzymatic glycation that leads to the alteration in function, activity and degradation of both intracellular and extra cellular proteins via chemical rearrangement and cross linking. Oxidized proteins functionally inactive and their unfolding are associated with enhanced susceptibility to proteinase. Thus, cells can generally remove oxidized proteins by proteolysis. However, poor handling of certain oxidized proteins by cells together with possible alterations in the rate of production of oxidized proteins increases their levels in blood. Attack by reactive oxygen species upon proteins can damage several amino acid residues. This type of alteration was characterized as metal catalyzed oxidation [23].
Excess iron has been implicated in the pathogenesis of diabetes and its complications. Free iron serves as a catalyst for lipid and protein oxidation and the formation of reactive oxygen species. In addition, iron indices are correlated with obesity and insulin sensitivity [24]. In the presence of hyperglycemia and inflammation, iron may contribute to the development and progression of oxidative injury. Iron may also negatively impact on glucose control [25]. What is new in the field is the recognition that iron plays an important role in the pathophysiology of disease in the absence of systemic iron overload [26]. The concept of iron contributing to diabetes is supported by a few important recent animal studies. Cooksey et al. [27] have demonstrated that, in obese mice with type 2 diabetes treated with an iron-restricted diet as well as an iron chelator, there were improvements in glucose metabolism without causing overt iron deficiency. Thus, even at normal levels, iron exerts a detrimental effect on b-cell function that may be reversible with removal of iron, either through phlebotomy or possibly iron chelation. This concept lends itself to exploring phlebotomy or iron chelation as potential treatments for diabetes. This may be an underlying link between the observed increase in serum AOPP and Fe in diabetic retinopathy. Serum Cu level used to determine oxidative stress status which is the cofactor of other enzymes in oxidative pathways [22]. Increase in the Cu ion levels in patients with DM might be due to hyperglycemia, that may stimulate glycation and release of copper ions and this accelerate the oxidative stress and can result in the formation of AGEs [28,29]. An increase in Cu concentration has been linked to disorders in the structure of the arterial walls, stress, infection and diabetes mellitus. The relationship between an increase in Cu concentration and the oxidation of low-density lipoproteins has been confirmed [30]. But a general consensus exists about the increased level of copper, the most important cofactor of oxidative and reductive reactions.

A large body of experimental and clinical evidence supports alteration of Zn homeostasis in diabetics. The potential antioxidant effects of Zn in diabetes [22] could be related to several mechanisms. Level of Zn, an activator of insulin, was investigated in diabetic patients and showed reduced insulin response to glucose challenge. It has been suggested that Zn metallothioneine complexes in the islet cells provide protection against immune-mediated free-radical attack [31] and at specific sites where it can compete for iron and copper. Zinc could also aid in protecting sulfydryl groups against oxidation and participate in the inhibition of the free radical in Haber-Weiss cycle by competing with transition metals. Regarding the serum zinc levels, there were significant difference between groups. There are some investigators who found decrease in serum Zn levels as well as the other investigators like D’Ocon found that serum Zn levels increased in diabetes mellitus (32). Concerning diabetes, Zn is considered important mainly because of its major role in the stabilization and the pancreatic storage of insulin [33] and acts as an efficient antioxidant [34], while oxidative stress is considered to be a main component in initiation and progression of insulin resistance and diabetes. Several modes of action have been described to explain the improved action of insulin by Zn. It appears that Zn can have direct insulin-like effects, which may be due to inhibition of the important glycogen-regulating enzyme GSK3, stimulation of the postreceptor proteins Akt and PI3-kinase, decrease in cytokines such as IL-1β as well as NFκB [31,35].

Animal studies showed that Zn has protective effects against the development of type 1 diabetes induced by alloxan or streptozotocin [31]. In a study on Zn restriction during pregnancy in rats, off-springs presented reduced body weight, increased fat mass and low lean mass and showed reduced insulin response to glucose challenge. Zn supplementation corrected the situation only in females [36]. In db/db mice Zn reduced hyperglycaemia and hyperinsulaemia. Administration of Zn for 3 months reduced proteinuria [37]. Zn restriction increased oxidative stress and the levels of isoprostanes [38], in line with antioxidant effects of Zn. Although some investigators suggest that decreased serum zinc levels can be prevented by oral zinc replacements, later search indicated that different representations of serum zinc level are independent from diet (39). The decrease in Zn may potentiate the toxicity of other metals such as iron and copper. Zinc deficiencies in diabetics are associated with excess free-radical activity and the increased oxidation of lipids, damaging the heart, arteries, and other integral parts of the vascular system [40].

It can be concluded that impaired trace-element metabolism may have a role in the pathogenesis and progression of type-2 diabetes mellitus. The increases of Fe and Cu, together with decreases of Zn, and Mg concentrations in blood of diabetic patients may be involved in disturbances of insulin secretion or its action. The high levels of Cu and Fe in diabetic patients disrupt the antioxidant functions and enhance the protein oxidation. These oxidative modification may be at least one cause of vascular complications of diabetes. The low level of zinc seen in the patients is possibly due to increased urinary excretion of this essential trace element. These studies indicate that type-2 diabetics are in need of Zn and Mg supplements as part of their maintenance.
REFERENCES


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