A study of trace elements in serum of diabetic patients

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I.   Introduction

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (Sarkar et al., 2010). The aetiological types of disorders of glycemia includes Type 1 diabetes (TID), TID indicates the process of ß-cell destruction that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of keto acidosis , coma, and death. Type 2 diabetes (T2D), T2D is the most common form of diabetes and individuals are characterized by disorders of insulin action and secretion , either of which may be the predominant feature. Gestational diabetes mellitus (GDM), Gestational diabetes is carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy (Zargar et al., 2002). Chronic hyperglycemia leads to microvascular complications such as nephropathy, retinopathy, and neuropathy (Bennett, 1994). The number of patients suffering from diabetes mellitus was reported to be over 200 million people worldwide , a big part of it being Non –insulin – dependent diabetes mellitus (NIDDM), patients (Olantunbosun, 2004).

Trace elements are required in small quantities for specific functions in the body. Clinical research seems to show a link between trace elements and glucose homeostasis. Studies suggested that imbalances of several trace elements may play important roles in glucose and insulin metabolism, and chronic hyperglycemia may cause significant alterations in the status of some trace elements (Friedrich et al., 2009, Viktorinova et al., 2009). In addition, disturbances in trace element status and increased oxidative stress in diabetes mellitus may contribute to the development of diabetic complications (Vincett et al., 2004). Copper (Cu) deficiency was an indicator of impaired glucose tolerance in an earlier study (Cohen & Miller, 1986)). Zinc (Zn) homeostasis affects the synthesis, storage, and secretion of insulin, and glycemic control as a result (Kelleher et al., 2011). High iron (Fe) levels were linked to insulin resistance and were reported to have contributed to an increase in T2DM incidence (Sheu et al., 2003). Hypomagnesemia has a negative impact on glucose homeostasis and insulin sensitivity in humans (McNair et al., 1978).

The aim of study was to determine the serum level of zinc, chromium, manganese , magnesium , copper and iron in patients of Diabetes mellitus of type I or II followed by comparison of results with control, duration and complications of diabetes mellitus.

II.   Materials & Methods

The study was conducted among the patients of either type I or II DM of age group more than 14 years residing in and around the city Bikaner, Rajasthan attending medical out patients/ in patient department of Sardar Patel Medical College & A.G.H Bikaner. Samples were taken at least 8 hrs overnight fast or RBS. A team comprising of 4 doctors, 1 technician and 3 volunteers was constituted and each member was assigned his/her task. Every technical detail was meticulously checked out. A total of 100 patients of either type I or II Diabetes mellitus (DM) and equal control were examined. Diabetes was diagnosed according to American Diabetes Association Criteria revised.

Samples of venous blood were drawn for blood glucose, CBC, ESR, Serum bilirubin total & conjugated, SGOT & pt, serum alkaline phosphatase, total protein and albumin, serum cholesterol, blood urea, serum creatinine.

Normoglycemia	IFG or IGT	DM
FPG<110 mg/dl.	FPG>110mg/dl. & <126mg/dl.(IFG)	FPG>126mg/dl
2hr PG<140mg/dl.	2hr PG>140 & < 200 mg/dl.(IGT)	2hr PG>200mg/dl, Symptoms of Diabetes

				and RPG≥ 200		mg/dl

FPG=Fasting plasma glucose, IFG= Impaired Fasting Glucose, IGT =Impaired glucose tolerance, 2 hr.PG= 2hr. Post Glucose load, RPG=Random Plasma Glucose

Samples of venous blood were drawn for blood glucose, CBC, ESR, Serum bilirubin total& conjugated, SGOT & PT, serum Alkaline Phosphatase, Total Protein & Albumin, Serum Cholesterol, Blood urea, Serum Creatinine. First morning sample of urine was collected in clean Vail for detail examination. The drawn samples were labeled carefully and taken to the central laboratory/ Biochemistry department, S.P. M.C. and A.G.H. Bikaner.

All biochemistry assay were carried out using a Atomic Absorption Spectrophotometer (AAS) available at the biochemistry department, S.P.M.C. Bikaner. First, equal amount of triacid was mixed and kept for 24hrs, and then digestion was done and run on AAS.

Plasma glucose (GOD-POD Method). Serum cholesterol (CHOD-PAP method) and serum triglycerides (GPO-PAP method) were measured. Cholesterol was estimated in serum by using Phosphotungstat method.

Patient Examination: Each selected patient was subjected to a detailed history and physical examination. History regarding age, sex, personal were also asked. Complaints with duration and findings were noted in standard Performa (Appendix).

Blood Pressure (B.P.) was recorded in sitting position at least three times at interval of 5 minutes. Compared in both arms and the mean were taken. Patients were categorized as hypertensive if they were on anti H.T. treatment of if they had systolic pressure> 140mm Hg and diastolic pressure was the appearance of Kortakoff's sound (Phase I) and disappearance of sound (Phase V) respectively. Coronary artery disease was diagnosed by history of angina, M.l. and documented by previous treatment records and by a standard 12 lead ECG. Interpretation of ECG was recorded as per Minnesota codes:-

1. Pathological q waves (major q wave abnormalities in an ECG recording. (Minnesota codes 1. 11-1.27)
2. ST Segment depression (codes 4.1-4.2)
3. T wave abnormalities (codes 5.1-5.4)

Malabsorption syndrome was ruled out by history and if necessary by stool examination and GI Biopsy. Sickle cell anemia was ruled out by history and P.B.F. and or bone marrow examination. Alcoholic cirrhosis was ruled out by history, liver function tests and USG abdomen. Patient were deemed unfit if they were taking oral contraceptive pills.

III. Observation

The present study first of its kind in Rajasthan was conducted on 100 patients of DM I or II at medicine Department in collaboration with Biochemistry lab at S.P.M.C. & A.G.H. Bikaner (Rajasthan). In this study 54 males and 46 females of either types I or II were investigated for Diabetes mellitus and Serum concentration of Zn, Cr, Mn, Mg, Fe, Cu were measured and reading were taken to compute statistics by students T test with Satterthwaite correction for assumption of homogeneity of variants. Same numbers of healthy control were also studied for comparison. All the detail descriptions of individual patients have been recorded in master chart appended in the end. The observation clinical results are as follows.

Table 1. Variation of trace elements in control and cases studied

	Zn	Cr	Mg	Mn	Fe	Cu
Mean of control in PPM	2.78	4.38	28.28	1.02	13.66	0.84
Mean of Cases in PPM	3.28	2.66	45.66	1.59	11.68	1.1

Mean Zn level in control was 2.78 PPM while in cases it was 3.28 PPM. This was significantly higher with P level 0.02 (that is P level<.05) that means serum Zn levels was higher in DM patients in comparison to control.

The P value for Cr was 0.002 that was significant to say that Cr level was less in DM patients. Mg with P value 3.04×10^{-14} was significantly higher in DM patients.

P value for Mn was 1.49×10^{-7} which indicates that serum level was higher in DM patients. The P value for Fe was 0.05 that means that level of serum Fe in cases and control was not significantly different. Cu was significantly higher in DM patients with P value 3.29×10^{-8}.

Table 2. Variation of trace elements with duration of Diabetes mellitus in patients

Duration	No. of patients	Complication of DM	Zn	Cr	Mg	Mn	Fe	Cu
<5yrs.	57	Gastropathy(4) P Neuropathy (3)	3.08	2.31	45.53	1.32	9.38	1.11
5to10yrs.	27	Retinopathy (7) P Neruopathy (5)	3.57	2.36	53.18	1.56	13.52	1.07
>10yers.	16	Ratinopathy (3) Gastropathty (3) P Neuropathy (1)	3.19	3.33	38.27	1.90	12.15	1.13

According to duration of DM serum Cr level was decreased as the duration of DM increased. While no statistically significant change was found in level of Zn, Mg, Mn, Fe, Cu.

IV. Discussion

Trace elements have important physiological effects when present at concentrations other than those associated with classical toxicity or with extreme deficiency (Agget, 1985). Many studies have reported significant though variable alteration in trace element concentration in type-1 and type-2 DM patients (Fujimoto, 1987, Hannan & Raines, 1991; Muradian and Moorley, 1987; Walter et. al., 1991).

In a study, Zargar et al., (2002) documented that plasma copper, zinc and magnesium levels were comparable between patients with fibrocalculous pancreatic diabetes and normal control subjects. (Zargar et al., 2000). Copper, an essential trace element, plays an important role in cytochrome oxidase function in the mitochondria. Copper deficiency results in swelling and subsequent disruption of the mitochondria of metabolically active tissues like hepatocytes and pancreatic acinar cells. In their study, copper levels were comparable between type-1, DM patients and non-diabetic controls. Glycemic control and presence of microalbuminuria did not affect the plasma copper levels. Earlier, Zargar et al. (1998) documented elevated plasma copper levels in type-2 DM patients. Conflicting results have been reported regarding the copper levels in type-1 DM, both elevated as well as decreased plasma copper concentrations had been reported (Isbir et. al., 1994; Brun et. al., 1992). Studies on zinc status in type-1 DM have shown contradictory results. Zinc is an essential trace element and it is important in glucose metabolism. Zinc is essential for many enzymes involved in the human metabolism and plays a role in the biosynthesis and storage of insulin in the β-cells. Significantly, low plasma zinc levels have been reported earlier (Isbir et. al. 1994). It is a component of many enzymes and it plays an important role in the maintenance of several tissue functions (Zargar et. al., 1998). The relationship between diabetes, insulin and zinc is complex, with no clear cause and effect relationships. Zinc plays a clear role in the synthesis, storage and secretion of insulin, as well as conformational integrity of insulin in the hexameric form. It has the ability to regulate insulin receptor intracellular events that determine glucose tolerance and the ability to support a normal pancreatic reaction to a glucose load (Andrews, 2005). It has a protective effect against β cell destruction and it has well known antiviral effects. The complications of diabetes may be mediated, at least in part, through oxidative stress, and zinc plays a key role in the cellular anti oxidative defence (Chausmer, 2005). Hence, it has been suggested that an abnormal zinc metabolism may play a role in the pathogenesis of diabetes and some of its complications (Rai et. al., 1997).

In fact, one study has suggested that low zinc consumption through drinking water is associated with later development of childhood onset DM. Surprisingly; we found significantly elevated levels of zinc in our type-1 DM patients compared to controls. Increased serum zinc levels have been documented in patients with type-1 DM previously treated with insulin (Hanna & Raines, 1991)

Magnesium is an essential ion which is involved in glucose homeostasis at multiple levels. A complex interplay exists between magnesium and glucose metabolisms. It plays an important role in the activities of various enzymes which are involved in glucose oxidation, and it may play a role in the release of insulin (Zargar et. al., 1998). It is mainly intracellular and its uptake is stimulated by insulin (Vikotorinova et. al., 2009). In our study we found significantly higher levels of magnesium than control in DM patients.

Diabetes mellitus as such has been reported to alter copper, zinc and magnesium status, although changes in trace elements occurring as a result of diabetes had not been confirmed (Walter et. al., 1991).

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