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Original Article

Treatment Regime and Trace Metals Homeostasis in Female Diabetic Patients

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ABSTRACT

Introduction: Trace elements are supposed to play a role in carbohydrate metabolism as reported by many researchers**.** A number of studies have reported that imbalance of certain trace metals cause diabetes and vice versa

Objective: The aim of the study was to compare serum level of Chromium (Cr) and Zinc (Zn) in young adult female type-1 and type-2 diabetic subjects treated with Combination insulin and Sitagliptin/Metformin combination therapy in Khyber Pakhtun Khwa Pakistan.

Methodology: The study population includes 124 type-1 and 137 type-2 female diabetic subjects aged 18-45 years (n=261) .Data from the study subjects regarding their age, weight, height, and medical history was collected on a well-designed proforma after their informed consent. Fresh samples of blood were taken from 261 female diabetic subjects in a Gel tube. HbA1c was determined on BIO-RAD VARIANT II TURBO using HbA1c Kit –2. Serum Zinc and Chromium were determined by atomic absorption spectrophotometer using acetylene flame (Model Perkin Elmer AAS 700) under standard conditions.

Results: The mean value of HbA1c of type-1 study population (10.62%) was higher as compared to the mean HbA1c of type-2 (8.32%). The mean level of serum Zn in type- I study population (0.04 mg/L) was higher than type-2 (0.03 mg/L) study population. The mean level of serum Cr in type-I study population (0.04 mg/L) was higher than type-2 study population (0.02 mg/L). Bivariate Pearson's Correlations analysis of serum Zn (mg/L) with HbA1C (%) shows a highly significant correlation (p=0.00).

Conclusion: The findings of this study suggest that treatment with insulin may be a better option in term of trace metal homeostasis in this selected group of patients**.**

Key words: Trace metals; Diabetes; Zinc; Homeostasis

Introduction

Pakistani population mostly lives in rural areas and is in a state of transition. Remarkable changes in economic and social sector as well as urbanization has brought many changes in lifestyle, dietary habits, lack of physical activities and stressful condition, all these have increased the risks of developing diabetes mellitus many fold. Moreover the polluted environment in urban areas of Pakistan has resulted in metabolic disease like diabetes mellitus [1].

The mean prevalence rate of T_2DM in Pakistan has been increased during the last 25 years.

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The current prevalence rate in various regions of Pakistan are 16.2% and 11.70 % in male and females in Sindh; 14% in males and 9.83% in female in Punjab; 13.3% among males and 8.9% in females in Baluchistan; while in Khyber Pakhtun Khwa (KPK) it is 9.2% in males and 11.60% in females respectively. The prevalence rate of type-2 diabetes mellitus is higher in urban areas (14.81%) than in rural areas (10.34%) of Pakistan [2].

Trace elements are supposed to play a role in carbohydrate metabolism as reported in many research studies[3].It is hypothesized that these elements potentiate the action of Insulin by activating insulin protein receptor sites, acting as cofactors or a part of enzyme systems, involved in carbohydrate metabolism, decrease insulin resistance and act as an antioxidants. Deficiencies of trace metals are mostly associated with chronic diseases. Chronic diabetes

may alter status of some micronutrients and alternatively some nutrients may directly disturb glucose homeostasis[4]. Deficiencies of some important minerals like Magnesium (Mg), Zinc (Zn), Chromium (Cr), Copper (Cu), Iron (Fe) may be a risk factor for the onset of diabetes and its associated complications. Zn is an important trace metal which plays a central role in insulin hemostasis. It is involved in its synthesis, storage, secretion, and maintaining its functional integrity as a dimer form for storage and secretion as crystalline insulin. In type-2 diabetes lower levels of Zn may affect the ability of pancreatic islet cells to produce and secrets insulin. Several population based studies have reported lower level of Zn concentrations in diabetic patients and increased urinary Zn excretion[5]. Lower dietary intake of Zn in subjects with type-2 DM increases the risk of coronary heart disease by a factor of two to four times and is a major cause of mortality among diabetic patients.

Role of Chromium as a blood-sugar modulator is well established[6]. Insufficient dietary Cr intake has also been implicated as a possible risk factor for the development of diabetes[7].

The present study was designed to evaluate the serum level of chromium and zinc in young female with type-1 and type-2 diabetes.

Materials and Methods

Purposive sampling method was used for the selection of study population size. The study population includes 124 type-1 and 137 type-2 female diabetic subjects aged 18-45 years (n=261). Type -1 study population was using combination insulin (Humulin70/30) 0.5-1.2 units/kg/day) while type -2 study population was using dipeptide peptidase-4 (DPP-4) inhibitors (Sitagliptin100- 200 mg; Metformin 1000- 4000 mg /day) for more than five years. The study was carried out from March 19, 2018 to April 5, 2018 in AIMS (Abaseen Institute of Medical Sciences), Phase 5 Hayatabad Peshawar, Pakistan, a tertiary care hospital and research Centre. The study was approved through letter no18/006. Data from the study subjects regarding their age, weight, height, and medical history was collected on a well-designed proforma after their informed consent.

Fresh samples of blood were taken from 261 female diabetic subjects in a Gel tube at the Abaseen Institute of Medical Sciences (AIMS), Diabetes Hospital and Research Center (DHRC) Hayatabad Peshawar. The blood samples were centrifuged for 10 minutes at room temperature. The resulting plasma samples were separated from red and white blood cells in efficient way. Platelets were also removed by centrifugation process. For this purpose the plasma samples were transferred into a second vial for another centrifugation for 15 minutes at room temperature. After centrifugation, the supernatant (blood serum) was transferred in serum cups with the help of dropper. After that the samples were kept in a freezer at low temperature within 30 minute in order to avoid the spoilage of trace elements present in the samples.

HbA1c was determined using principles of ion-exchange High-Performance Liquid Chromatography (HPLC).on BIO-RAD VARIANT II TURBO [8].

Serum Zinc (Normal Serum Levels of Zn**:** 0.5-1.2 mg/L) and Chromium (Normal serum level of Cr: 0.0008 - 0.0051 mg/L) were determined by atomic absorption spectrophotometer using acetylene flame (Model Perkin Elmer AAS 700) under standard conditions [9]. Chromium standards were prepared by diluting the stock standard solution to 2 ppm, 4 ppm and 6 ppm described in the "Standard Conditions" for Chromium, with 5% (v/v) glycerol.

Zinc standards were prepared by diluting the stock standard solution to 0.5 ppm, 1.0ppm and 1.5 ppm, described in the "Standard Conditions" for zinc, with 5% (v/v) glycerol. A 5% (v/v) glycerol solution was used as a blank.

Data was analyzed on SPSS for windows 21.0 software (SPSS Inc. Chicago, IL, USA) and Microsoft Excel. The obtained values were reported as Mean ± Standard Deviation (SD). Pearson's correlation analysis was carried to determine association between desired parameters. A two-tailed p value <0.05 was considered statistically significant.

Results

The information related to the patients and their family medical history is shown in Table 1. The percentage of type-I and type-II subjects were 39.34% (n=124) and 60.65% (n=137) respectively. The prevalence rate of, hypertension, renal disorders and liver disorders were 54.09%, 8.19% and 11.47% respectively in the study population.

The prevalence rate of diabetes, hypertension, renal, and liver disorders in the patient's family was 52.45%, 40.98%, 13.11% and 4.91% respectively.

Table I: Medical History of the Study Population

S. No	Parameter(disease)	%age of Patient(n)	%age of Family
01	Diabetic	100	52.45
02	Hypertensive	54.09	40.98
03	Non-hypertensive	42.62	54.09
04	Renal	8.19	13.11
05	Liver	11.47	4.91
06	Type-1(n=124)	39.34	-
07	Type-2(n=137)	60.65	-

The base line and biochemical parameters of the study population are shown in Table II.The mean age of type-1 study population (40.25years) was higher than the mean age of type-2 (36.94 years). The mean BMI of type-1 study population (24.66 Kg/m2) was almost equal to the mean BMI of type-2 (24.02Kg/ m2). The mean daily dosage of Combination insulin's for type-1 study population was 0.85 unit's /kg/day).The mean daily dose of Sitagliptin and Metformin for type-2 study population was 150 mg and 2500 mg/day respectively. The mean value of HbA1c of type-1 study population (10.62%) was higher than the mean HbA1c of type-2 (8.32%). The mean level of serum Zn in type- 1 study population (0.04mg/L) was higher than type2 study population (0.03mg/L). The mean level of serum Cr in type-1 study population (0.04mg/L) was higher than type-2 (0.02mg/L) study population.

Table 2: **Comparison of Base Line and Biochemical Characteristics of the Study Population**

Sr.#	Parameter	Type-1 (n=124)				Type-2 (n=137)			
		Min	Max	Mean	SD	Min	Max	Mean	SD
1	Age(years)	2.00	45.00	40.25	6.20	26.00	45.00	36.94	5.30
2	BMI(Kg/m^2)	0.24	32.03	24.66	2.86	17.92	35.63	24.02	2.82
3	Sitagliptin (mg) / day Metformin (mg) /day	- -	- -	- -	- -	100 1000	200 4000	150 2500	- -
	(Humulin70/30) (0.5-1.2 units/kg/day)	0.5	1.2	0.85	-	-	-	-	-
4	HbA1c (%)	6.80	13.80	10.62	2.43	5.00	11.30	8.32	1.40
5	Zn (mg/L)	0.02	0.08	0.04	0.01	0.02	0.06	0.03	0.00
6	Cr (mg/L)	0.00	0.11	0.04	0.04	0.00	0.11	0.02	0.03

SD= Std. Deviation, Max= Maximum, Min= Minimum

The Bivariate Pearson's correlation analysis of serum Zn and Cr with age, BMI, dosage (mg/day or units/day) and HbA1c in type-1 and type-2 diabetic population is shown in Table 3.

Serum Zn showed a highly significant correlation with dosage (units/kg/day) in type-1 study population (p=0.04) with age (p=0.01 and HbA1c (p=0.00) in type-2 study population. No significant correlation was found for serum Cr in both the study population with any parameter.

Table3: Bivariate Pearson's Correlations analysis of Serum Zn and Cr with age, BMI, dosage (mg/day or units/day) and HbA1c

Sr. #	Variable	Serum Zn(mg/L)				Serum Cr(mg/L)			
		Type-1 (n=124)		Type -2 (n=137)		Type1 (n=124)		Type -2 (n=137)	
		r	p	r	p	r	p	r	p
1	Age	0.36	0.07	0.41*	0.01	0.25	(0.22)	0.09	(0.56)
2	BMI	-0.16	(0.44)	0.09	0.58	0.38	(0.06)	0.03	0.82)
3	Dosage	0.41*	(0.04)	0.07	0.66	0.15	(0.46)	-0.21	(0.20)
4	HbA1c	0.15	(0.47)	0.54**	0.00	0.27	(0.19)	-0.04	(0.80)

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Discussion

Trace elements like Copper, Cobalt, Manganese, Zinc, Selenium, Iodine, Fluorine and Selenium are considered nutritionally significant. These are involved in various metabolic activities of the body including carbohydrate metabolism as reported by many studies[10].Trace elements along with vitamins are involved in hormone synthesis and enhance the metabolic processes in the body. The deficiencies of the elements are more frequently associated with a number of chronic diseases. Deficiencies of some minerals like Zn, and Cr are believed to cause diabetes and other associated complications as reported from many population based studies[11].In diabetic patients the serum level of trace metals is imbalanced as compared to healthy subjects as reported by a number of studies [12]. Thus it is assumed that disturbance in metabolism of certain micronutrient might have certain specific roles in the pathogenesis and progress of diabetes mellitus [13]. The exact mechanism how the trace metals increase insulin action is not known with certainty. It is hypothesized that these elements potentiate the action of Insulin by activating insulin protein receptor sites, acting as cofactors or a part of enzyme systems, involved in carbohydrate metabolism, decrease insulin resistance and act as antioxidants. Trace-element deficiencies are frequently associated with chronic diseases or to problems with its absorption. Chronic diabetes may alter status of some micronutrients and alternatively some nutrients may directly disturb glucose homeostasis[4].Trace metals also act as antioxidants and prevent tissues peroxidation[14].

In order to assess the level of two important trace elements, we conducted a cross sectional analysis of 124 type-1 and 137 type-2 female diabetic subjects to compare the serum Zn and Cr level and to look for a possible association of these trace metals and HbA1c in the study subjects.

We observed the mean level of serum Zn in type- 1 study population (0.04 mg/L) was higher as compared to the mean level of serum Zn in type-2(0.03 mg/L).

Islam etal conducted a study on 280 participants and found from blood sugar results that 51% were normal, 13% had prediabete and 36% had diabetes. They observed that the mean serum zinc level was higher in normal and diabetic subjects (65 ppb/L) than the pre-diabetes (33ppb/L. Similarly in multiple linear regressions, serum zinc level was found to be higher in normal than in pre-diabetes [15].

The effect of Zinc supplementation on glycemic control in some patients with type-2 diabetes mellitus is also reported by a number of studies [16-21].

The mean level of serum Cr in type-1 study population (0.04mg/L) was higher as compared to the mean level of serum Cr in type-2(0.02mg/L).

Jain et al. (2012) observed that Cr supplements have a beneficial effect as an adjunct therapy for patients suffering from type 2 diabetes [22]. Cephalic et al observed that positive effect of Cr supplementation in type 2 diabetic subjects may be related with their genetic phenotype [23].

Variable effects of chromium supplementation in normal and diabetic subjects have been reported from different studies on one or more components of the serum lipid profile [24-29].

HbA1c in type-I population was positively correlated with serum Zn, Cr in non-significant way. HbA1c in type-II population was positively correlated with serum Zn (p=0.00) in significant way and negatively correlated with Serum Cr in non-significant way. This correlation shows strong association of serum Zinc and status of diabetes in our study population.

According to our knowledge it is the first kind of study on the role of trace metals in diabetic subjects

from this part of Pakistan. The study has got some unavoidable limitations. The first and foremost is the small sample size including only young 261 female diabetic subjects visiting only one tertiary care health center in Peshawar city. As the population of this city is highly heterogeneous in nature, because of the influx of Afghan refugees and hence the outcome of this finding cannot be the true representative of the local inhabitants of KPK. Secondly the study period was also short due to lack of finances. Further studies are required involving lager populations for better results.

Conclusions

The findings of this study suggest that treatment with insulin may be a better option in term of trace metal homeostasis in this selected group of patients**.**

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C. Interpretation/ Analysis and Discussion