

*Full Length Research Paper*

# **A comparative study on the status of Zn and Cu in diabetic and non diabetic males in Punjab, Pakistan**

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Minerals play a considerable role in the prolongation of all biological and psychological processes in the body. The present study was aimed to determine and to compare the concentration of zinc and copper in the serum samples of diabetic and non diabetic males of different age groups, that is, between one to seventy five years. Fasting blood samples of diabetic males as well as of non diabetics of selected age groups were collected for the study. Blood serum was obtained after centrifugation of blood samples. Level of zinc and copper in blood serum samples were determined by using atomic absorption spectrophotometer. The data obtained was processed statistically by using student's t- test. A highly, significant ( $p < 0.01$ ) lower levels of zinc and highly, significant ( $p < 0.01$ ) higher levels of copper were found in blood serum of diabetic males when they were compared with non diabetic ones. The results reflect that the diabetic patients had a higher mean concentration of Zn (0.577 ppm), lower mean concentration of Zn (0.467 ppm), higher mean concentration of Cu (2.713 ppm) and lower mean concentration of Cu (2.207 ppm). It has also been represented by our results that the non diabetic males had higher mean concentration of Zn (1.94 ppm), lower mean concentration of Zn (0.937 ppm), higher mean concentration of Cu (1.31 ppm) and lower mean concentration of Cu (1.077 ppm). It has been concluded that the diabetic males of all age groups are deficient of zinc and they contain a higher level of copper in their blood serum.

**Key words:** Diabetic patients, minerals, blood serum, atomic absorption spectrophotometer.

## **INTRODUCTION**

Diabetes mellitus is characterized by higher glucose level, alterations in lipid and carbohydrate metabolism and many neurological and vascular complications. Diabetes mellitus is a heterogeneous group of disorders which results due to abnormal secretions of insulin (Mohini et al., 2012). The global estimate of people living with diabetes mellitus is 2.8% at 2000 and it is expected

to reach 4.4% by 2030 (Sarah et al., 2004). Minerals are very important naturally occurring and nutritionally valuable elements. Due to alteration in mineral levels in body, different diseases occur in body and diabetes is also one of them.

Zinc is required for the production of insulin and also for its storage. Structural integrity of insulin is also maintained by zinc (Chausmer, 1998). Copper acts as cofactor for various enzymes with antioxidant activity, which helps to protect the body from oxygen free radicals that are produced during oxidative stress. Copper

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maintains equilibrium between antioxidants and dangerous pro-oxidants (Rukgauer et al., 2001).

In the present study, one major objective was to determine the level of zinc and copper in the blood serum of diabetic males as well as in the blood serum of non diabetic males of five different age groups. Levels of aforementioned minerals in the blood serum of diabetic and non diabetic males were also compared to give awareness to the diabetic patients about the importance of alteration of mineral status in human body.

## **MATERIALS AND METHODS**

All the apparatus and glassware were washed with deionized water. Chemicals used were of analytical grade. Conc.  $\text{H}_2\text{SO}_4$ , conc.  $\text{HNO}_3$ , 63%  $\text{HClO}_4$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  were used during study.

### **Collection of blood samples**

Fasting blood samples (15 from each age group) of diabetic males was collected from Social Security Hospital Faisalabad, Pakistan. Five age groups, that is, from 1 to 15, 16 to 30, 31 to 45, 46 to 60 and 61 to 75 years were selected for blood sampling. Similarly fasting blood samples (15 from each group) of non diabetic males of respective age groups were also collected for comparison.

### **Preparation of blood samples**

Fasting blood samples (5 ml) was drawn from the study subjects for the evaluation of zinc and copper by disposable syringe. Blood samples were allowed to clot for about an hour and then centrifuged at 2500 RPM for 15 min. Serum was stored in contaminant free plastic tubes.

### **Wet digestion**

Serum samples were digested by using wet digestion method. A mixture of  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$  and 63%  $\text{HClO}_4$  in a ratio of 1:5:2 respectively; was prepared for digestion. Following is the method adopted for the digestion of each sample. 2.0 g of serum was taken in a conical flask and 20 ml of aforementioned mixture was added to it. On hot plate, first the solution was heated at low temperature and then at high temperature. The solution was heated until 2 to 3 ml of transparent solution was left behind. Then after cooling, deionized water was added to make 50 ml of solution. This solution was filtered through Whatmann filter paper to get transparent filtrate. The solution was stored in contaminant free bottles for elemental analysis.

### **Determination of minerals by using atomic absorption spectrophotometer**

Concentration of Zn and Cu in the serum samples were determined by using Atomic Absorption Spectrophotometer (Liang and Zhang, 2007; Xiao-Fang et al., 2006).

## **Preparation of standard solutions**

### **Preparation of standard solution of zinc**

Stock solution of zinc was prepared by weighing accurately 4.38 g of dried ( $110^\circ\text{C}$  for 5 h)  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ . The salt was dissolved in deionized water and the solution was transferred to 1 L flask and the volume was made up to the mark with deionized water. This solution contained 1000 ppm of Zn. Standard solutions containing 0, 0.5, 1.0, 1.5, 2.0, 2.5 ppm of Zn were prepared from stock solution, which were transferred to polybottles.

### **Preparation of standard solution of copper**

Stock solution of copper was prepared by weighing accurately 2.68 g of dried ( $110^\circ\text{C}$  for 5 h)  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ . The salt was dissolved in deionized water and the solution was transferred to 1 L flask and the volume was made up to the mark with deionized water. This solution contained 1000 ppm of Cu. Standard solutions containing 0, 2.0, 4.0, 6.0, 8.0 ppm of Cu were prepared from stock solution, which were transferred to poly bottles.

### **Calibration of instrument**

The instrument was calibrated and absorption, which is the characteristic of each element, was measured at their specific wave length (that is, for zinc 213.9 nm, for copper 324.8 nm).

### **Statistical analysis**

Statistical analysis was done by using Student's t-test for comparison between two groups and  $p < 0.05$  was considered to be statistically significant (Steel and Torrie, 1980).

## **RESULTS AND DISCUSSION**

Hyper glycaemia, which occurs due to disturbance in the metabolism of carbohydrates, has been associated with diabetes mellitus. Diabetic patients show abnormalities in the availability of insulin and insulin action in their body (Walter et al., 1991)

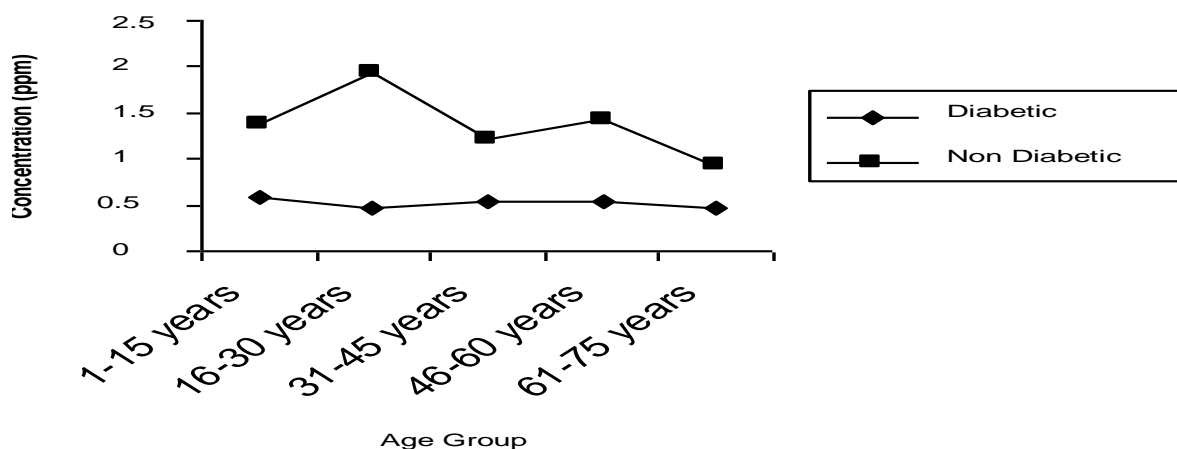
### **Concentration (ppm) of Zn**

Zinc is an essential micronutrient. Zinc deficiency has long been suggested to play a role in the development of diabetes in humans. The concentration of Zn in the blood serum of diabetic and non diabetic males is presented in Table 1 and Figure 1. Mean blood serum concentration (ppm) of Zn is 0.577, 0.467, 0.543, 0.547 and 0.477 in the diabetic males of age groups (years) 1 to 15, 16 to 30, 31 to 45, 46 to 60 and 61 to 75, respectively (Table 1 and Figure 1). Where as the level of Zn (ppm) in the blood serum of non diabetic males of corresponding age groups is 1.383, 1.940, 1.223, 1.433 and 0.937 respectively (Table 1 and Figure 1). The results of our study present that the blood serum concentration of Zn in diabetic males of all age groups are highly, significantly lower than the non diabetic ones. There are also several studies reporting Zn levels in diabetic patients as high

**Table 1.** Concentration (ppm) of Zn in diabetic and non diabetic males of different age groups.

S/N	Age group (years)	Diabetic				Non diabetic				T-Test	
		N	Mean	SD	SEM	N	Mean	SD	SEM	t-value	Remarks
1	1-15	15	0.577	0.21	0.05	15	1.383	0.36	0.09	7.49	HS
2	16-30	15	0.467	0.09	0.02	15	1.940	0.58	0.15	9.63	HS
3	31-45	15	0.543	0.10	0.03	15	1.223	0.23	0.06	10.48	HS
4	46-60	15	0.547	0.16	0.04	15	1.433	0.29	0.07	10.36	HS
5	61-75	15	0.477	0.17	0.04	15	0.937	0.11	0.03	8.89	HS

$p < 0.01$  (HS = highly significant).

**Figure 1.** Concentration (ppm) of Zn in diabetic and non diabetic males of different age groups.**Table 2.** Concentration (ppm) of Cu in diabetic and non diabetic males of different age groups.

S/N	Age groups (years)	Diabetic				Non diabetic				T-test	
		N	Mean	SD	SEM	N	Mean	SD	SEM	t-value	Remarks
1	1-15	15	2.207	0.37	0.10	15	1.107	0.20	0.05	10.13	HS
2	16-30	15	2.240	0.26	0.07	15	1.260	0.24	0.06	10.73	HS
3	31-45	15	2.247	0.31	0.08	15	1.077	0.14	0.04	13.32	HS
4	46-60	15	2.513	0.43	0.11	15	1.120	0.18	0.05	11.57	HS
5	61-75	15	2.713	0.35	0.09	15	1.310	0.29	0.07	11.95	HS

$p < 0.01$  (HS= highly significant).

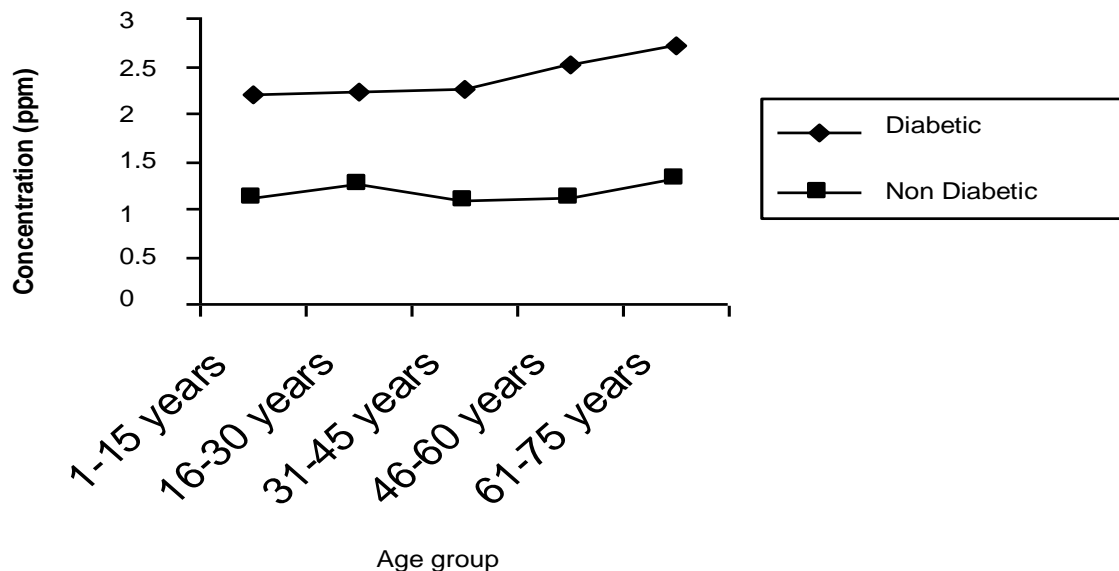
(Abdul et al., 2002) or low (Walter et al., 1991).

Significantly lower level of zinc in the serum of human subjects with diabetes mellitus than in control subjects was also reported in earlier studies (Pai and Prasad, 1988; Rai et al., 1997; Al-marouf and Al-Sharbatti 2006). Lower plasma and serum zinc levels in diabetics have also been reported by other studies (Anetor et al., 2002; Cunningham et al., 1994). In another study, a lower level of zinc was found in skeletal muscles of subjects with type II diabetes (Sjogren et al., 1988). Significantly lower levels of zinc in the serum of diabetics

than those of non diabetic ones and also higher levels of zinc in the urine samples of diabetics than non diabetics were found (Chausmer, 1998).

### Concentration (ppm) of Cu

Copper, an essential element plays an important role in the body. Copper has long been associated with disturbed carbohydrate metabolism (Frank et al., 2000). Level of Cu (ppm) in the serum samples of diabetic patients and non diabetic males is depicted in Table 2



**Figure 2.** Concentration (ppm) of Cu in diabetic and non diabetic males of different age groups.

and Figure 2. Mean blood serum level of Cu (ppm) in diabetic males of age groups, that is, 1 to 15, 16 to 30, 31 to 45, 46 to 60 and 61 to 75 years is 2.207, 2.240, 2.247, 2.513 and 2.713 respectively (Table 2 and Figure 2). While the blood serum level of Cu (ppm) in non diabetic males of respective age groups is found to be 1.107, 1.260, 1.077, 1.120 and 1.310 respectively (Table 2).

The findings of our study clearly demonstrate significantly higher level of Cu in the blood serum of diabetic patients than non diabetic ones. There are studies reported in serum and plasma copper levels as higher (Raz and Havivi, 1989), lower (Car et al., 1992) and normal (Mooradian and Morley, 1987). In literature, higher copper values were found in the sera of diabetic subjects in contrast to normal subjects (Rusu et al., 2005; Zargar et al., 1998; Isbir et al., 1994; Kruse and Rukgauer, 2000; Abou- Seif and Youssef, 2004). High mean values for Cu and Fe were detected in scalp hair and blood from diabetic patients when they were compared with non diabetic subjects however, the difference were not significant (Kazi et al., 2008). In another study, increased Cu levels were observed in diabetic patients (Ruiz et al., 1998).

## Conclusion

From present study, it is concluded that diabetic patients have significantly lower level of zinc in the blood serum as compared to normal subjects whereas diabetic patients of all age groups have significantly elevated level of copper in serum when compared with age matched

groups of non diabetic community. In conclusion, it is therefore suggested that supplements and diet containing higher levels of zinc and lower amount of copper should be given to the diabetic patients.

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