

*Full Length Research Paper*

# Comparative study on calcium, magnesium and cobalt in diabetic and non diabetic patients (males) in Punjab, Pakistan

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Minerals are very important because of their important role in the maintenance of human health. Our study was aimed at assessing and comparing the levels of Ca, Mg and Co in the blood samples of diabetic and non diabetic persons (males) of five age groups between one and 75 years. For this purpose, fasting blood samples of diabetic and non diabetic males of selected age groups were collected. The blood samples were centrifuged to get serum. The mineral elements in the serum were analyzed by using atomic absorption spectrophotometer. The results depicted that the diabetic patients had higher mean concentration of Ca (141.47 ppm), Mg (18 ppm) and Co (0.057 ppm) while non diabetic persons had higher mean concentration of Ca (201.33 ppm), Mg (36.15 ppm) and Co (0.047 ppm). Statistical analysis was done by applying Student's T-test. It can be concluded from the study that the diabetic patients of all five age groups are deficient of Ca, Mg, whereas the concentration of Co is higher in diabetic patients. In the further study physiological parameters along with other inorganic co-factors are being taken into consideration.

**Key words:** Diabetes mellitus, blood serum, Ca, Mg, Co.

## INTRODUCTION

Diabetes mellitus is characterized by high blood sugar level which explains numerous syndromes of abnormal carbohydrate metabolism. Absolute or comparative mutilation in the action of insulin and resistance in insulin secretions are associated with diabetes (Perez and Kohn, 1994). It is estimated that about 170 million people from

number is about 2% of the world population all over the world are badly affected by diabetes and this (Wokoma, 2002; Unwin et al., 2001). In the body, minerals play a considerable role in the prolongation of biological and psychological processes. Calcium is the mineral found in the body most abundantly. Better intake of calcium and vitamin D may protect against diabetes by improving the level of insulin (Stephan, 2009). Magnesium is mandatory for hundreds of biochemical reactions occurring in the body. It normalizes the blood

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pressure and sugar level of body (Wester, 1987; Saris et al., 2000). In insulin resistance, carbohydrate metabolism and in the complications of diabetes, magnesium deficiencies are of great concern (Resnick et al., 1991). Cobalt is essential in small amount to many living organisms, including humans. It is necessary for protein synthesis and for the metabolism of fats and carbohydrates.

According to a survey report, diabetes mellitus is increasing day by day in Punjab, Pakistan (Shera et al., 2010). Among the various factors, one is the inactiveness of the necessary enzymes due to alteration of inorganic co-factors.

In our study, one main objective was to investigate the level of calcium, magnesium and cobalt in the blood serum of diabetic persons and then to compare the level of the above mentioned elements in the blood serum of non diabetic persons. The study was conducted to give awareness to the diabetic patients that mineral imbalance in human beings may cause certain enzyme to show some abnormal activities which can result in diabetes.

## MATERIALS AND METHODS

During this study, all the apparatus and glassware were washed with deionized water. The chemicals used were concentrated  $H_2SO_4$  and  $HNO_3$ , 63%  $HClO_4$ ,  $CaCl_2 \cdot 2H_2O$ ,  $MgCl_2 \cdot 6H_2O$ ,  $CoCl_2 \cdot 6H_2O$ . All the chemicals were pure.

### Collection of blood samples

Fasting blood samples (15 from each age group) of diabetic males of five age groups (one to 15 years, 16 to 30 years, 31 to 45 years, 46 to 60 years and 61 to 75 years) were collected from DHQ Hospital Faisalabad, Pakistan. Similarly, fasting blood samples (15 from each age group) of non diabetic males of respective age groups were also collected for comparison.

### Preparation of blood samples

Fasting blood sample (5 ml) was drawn from the study subjects for the estimation of calcium, magnesium and cobalt with disposable syringe. For an hour, blood was allowed to clot and then centrifuged at 2500 rpm for 15 min. Serum thus obtained was stored in contaminant free tubes.

### Wet digestion

Serum samples were digested by using wet digestion method. A mixture of  $H_2SO_4$ ,  $HNO_3$  and 63%  $HClO_4$  in a ratio of 1:5:2, respectively, was prepared for digestion. The following method was adopted for the digestion of each sample. Two grams of serum was taken in a conical flask and 20 ml of the above mentioned 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 Whatman 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

Level of Ca, Mg and Co in the serum samples were determined by using atomic absorption spectrophotometer (Liang and Zhang, 2007; Shen et al., 2006).

### Preparation of standard solutions

Stock solutions of calcium, magnesium and cobalt were prepared by weighing accurately 3.67 g of dried ( $110^\circ C$  for 5 h)  $CaCl_2 \cdot 2H_2O$ , 8.47 g of dried ( $110^\circ C$  for 5 h)  $MgCl_2 \cdot 6H_2O$  and 4.04 g of dried ( $110^\circ C$  for 5 hours)  $CoCl_2 \cdot 6H_2O$ , respectively. The salts were dissolved in deionized water and the solutions were transferred to 1 L flask and the volume was made up to the mark with deionized water. These solutions contained 1000 ppm each of Ca, Mg and Co. Further standard solutions of Ca, Mg and Co were prepared by diluting the above solutions, which were then transferred to poly bottles.

The instrument was calibrated and absorption of different concentrations of metal standards was measured at specific wave length which is the characteristic of each element (calcium 422.7, magnesium 285.2 and cobalt 240.7).

### Statistical analysis

The data was analysed by using Student's t-test (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

Diabetes mellitus is a chronic metabolic disorder. There is accumulating evidence about the alteration in the metabolism of many elements in diabetes (Prasad, 1998).

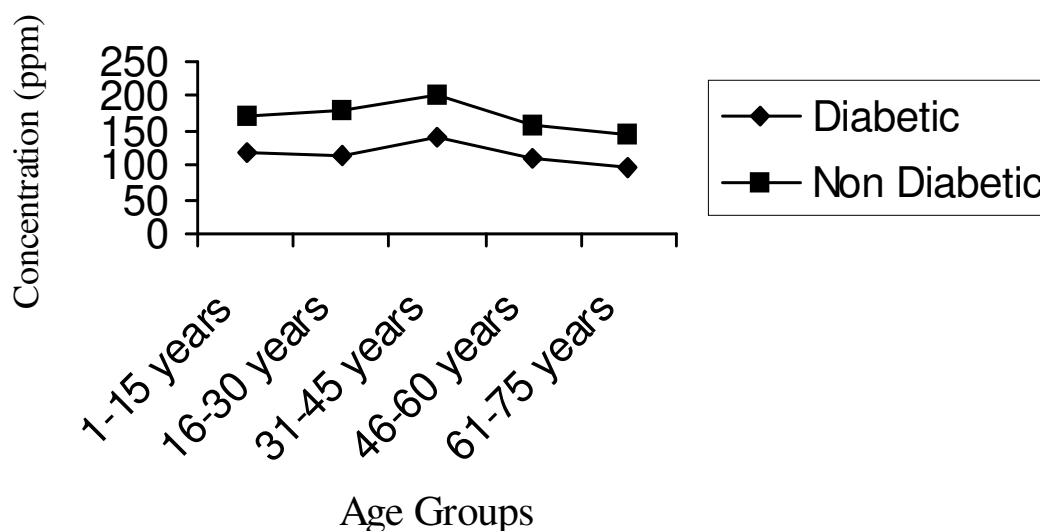
### Concentration (ppm) of Ca

Calcium is a versatile intercellular messenger that is used throughout the whole life of an organism to control diverse biological processes (Berridge et al., 2000). It has been suggested that diabetes and many other diseases like cardiovascular diseases are linked to the defects of calcium metabolism (Resnick, 1989). The concentration of Ca in the blood serum of diabetic and non diabetic persons (males) is presented in Table 1 and Figure 1. Mean blood serum level of Ca (ppm) was 119.93, 112.05, 141.47, 109.00 and 98.56 in diabetic males of age groups one to 15, 16 to 30, 31 to 45, 46 to 60 and 61 to 75 years, respectively (Table 1), while the level of Ca (ppm) in the blood serum of non diabetic males of age matched groups was 170.77, 181.90, 201.33, 156.17 and 143.33, respectively (Table 1). The results of the present study indicate that blood serum calcium of the diabetic patients

**Table 1.** Concentration (ppm) of Ca 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	Remark
1	1-15	15	119.93	13.39	3.46	15	170.77	8.21	2.12	12.54	HS
2	16-30	15	112.05	11.86	3.06	15	181.90	9.48	2.45	17.82	HS
3	31-45	15	141.47	11.31	2.92	15	201.33	9.25	2.39	15.87	HS
4	46-60	15	109.00	11.77	3.04	15	156.17	10.12	2.61	11.77	HS
5	61-75	15	98.56	6.41	1.65	15	143.33	11.77	3.04	12.94	HS

P<0.01; HS, highly significant.

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

(males) of all age groups is significantly lower than the non diabetic males of corresponding age groups. A significantly decreased level of calcium was reported in serum of diabetic patients (Skalnaya and Demidov, 2007). In another study, a significantly decrease in total ionized calcium was found in NIDD patients (Mohammad, 1991). Apparently, lower values of calcium was reported in type I diabetes (IDDM) (Fogh-Andersen et al., 1983).

### Concentration (ppm) of Mg

Magnesium is a crucial ion involved in insulin secretion, binding and activity. It is also an important cofactor for many enzymes involved in metabolism of carbohydrates. The concentration of Mg in diabetic and non diabetic males of different age groups is shown in Table 2 and Figure 2. The concentration (ppm) of Mg in the blood serum of diabetic males was 15.32, 14.80, 18.00, 13.24 and 14.36 in age groups one to 15, 16 to 30, 31 to 45, 46 to 60 and 61 to 75 years, respectively (Table 2), while its level in non diabetic males was 31.16, 30.60, 36.15, 28.43 and 29.49 ppm, respectively (Table 2). The results explain that concentration of magnesium in the blood

serum of diabetic males of all age groups are significantly lower than the values obtained for non diabetic males of respective age groups. Similar observations were observed in the past where significantly lower level of magnesium was found in serum of diabetic patients than non diabetics (Kisters et al., 2000; Sjogren et al., 1988; Diwan et al., 2006; Tripathy et al., 2004).

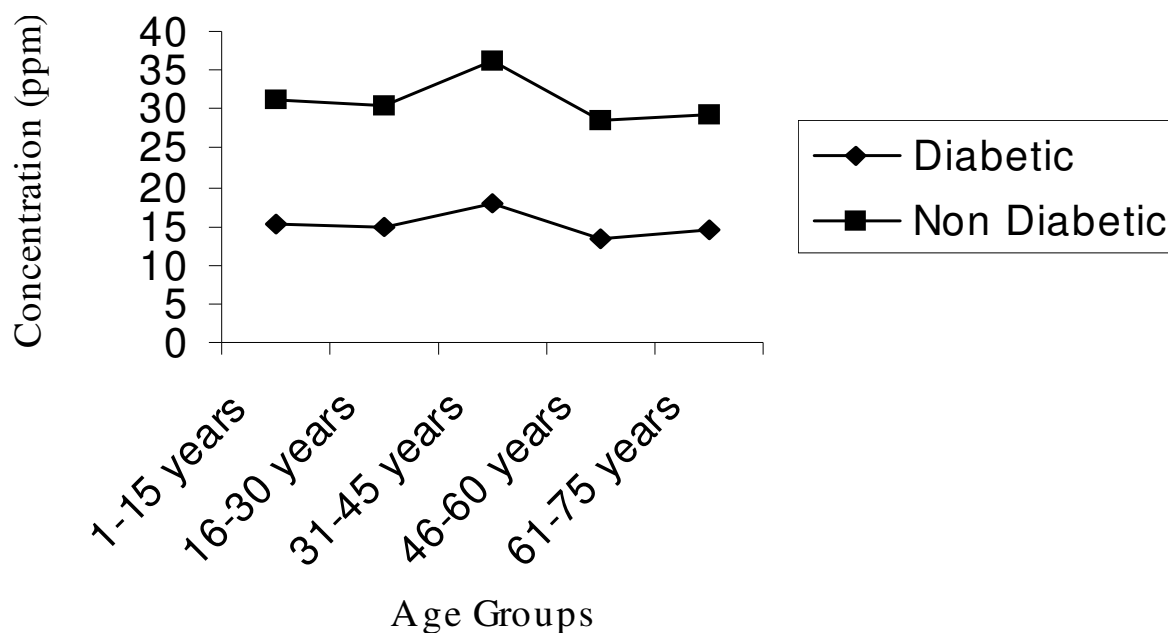
### Concentration (ppm) of Co

Cobalt is another essential mineral needed in diet in a very small amount. It helps to repair myelin sheath and thus increases transport of glucose from blood into body cells. The results given in Table 3 and Figure 3 reveal the concentration of Co in diabetic and non diabetic males of different age groups. The concentration of Co (ppm) in the blood serum of diabetic males of selected age groups was 0.040, 0.057, 0.047, 0.043 and 0.037, respectively (Table 3) and in the blood serum of non diabetic males of experimental age groups, it was 0.033, 0.047, 0.040, 0.033 and 0.026 ppm, respectively (Table 3). It is found that the blood serum of diabetic patients (males) of all

**Table 2.** Concentration (ppm) of Mg 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	Remark
1	1-15	15	15.32	3.52	0.91	15	31.16	5.62	1.45	9.25	HS
2	16-30	15	14.80	3.39	0.88	15	30.60	5.68	1.47	9.25	HS
3	31-45	15	18.00	1.52	0.39	15	36.15	4.00	1.03	16.43	HS
4	46-60	15	13.24	4.10	1.06	15	28.43	6.06	1.56	8.04	HS
5	61-75	15	14.36	3.59	0.93	15	29.49	3.59	0.93	11.54	HS

P<0.01; HS, highly significant.

**Figure 2.** Concentration (ppm) of Mg in diabetic and non diabetic males of different age groups.**Table 3.** Concentration (ppm) of Co 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	Remark
1	1-15	15	0.040	0.01	0.003	15	0.033	0.01	0.003	1.92	NS
2	16-30	15	0.057	0.04	0.010	15	0.047	0.03	0.008	0.78	NS
3	31-45	15	0.047	0.02	0.005	15	0.040	0.01	0.003	1.21	NS
4	46-60	15	0.043	0.01	0.003	15	0.033	0.02	0.005	1.73	NS
5	61-75	15	0.037	0.03	0.008	15	0.026	0.01	0.003	1.35	NS

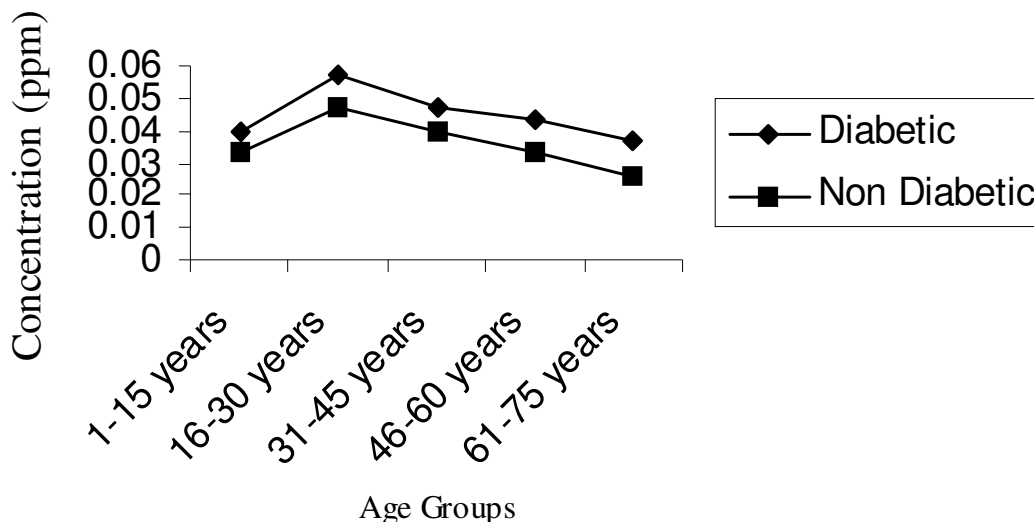
P>0.05; NS, non significant.

age groups contains a higher level of Co as compared to the healthy ones of identical age groups, but the difference is not statistically significant. Earlier, no significant difference in the contents of cobalt was reported in diabetic and non diabetic middle-aged and aged people with slight and severe diabetes (Liu et

al., 2000).

### Conclusion

Our study on the diabetic and non diabetic males of different age groups shows that the serum calcium and



**Figure 3.** Concentration (ppm) of Co in diabetic and non diabetic males of different age groups.

magnesium level are significantly lower in diabetic patients of all age groups as compared to non diabetic ones of respective age groups, whereas no significant difference is found in the serum cobalt level as compared to non diabetic of corresponding age groups. It is therefore recommended that foods and supplements rich in calcium and magnesium and have lower amount of cobalt should be given to the diabetic patients.

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