To Correlate The Serum Zinc And C-Peptide Levels In Patients With Type II Diabetes Mellitus With And Without Complications

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Abstract: Aims: Two prime leads that led to the present work are firstly, disturbances in Zinc metabolism are more pronounced in diabetics with complication and secondly, diabetic patient with increased C-peptide have an increased risk of coronary heart disease and peripheral vascular diseases. The aim of the study is to observe whether serum Zinc and /or C-peptide levels have any correlation in patients of type 2 diabetes mellitus (DM) with or without complications. Material and Methods: This prospective study included all newly diagnosed cases of type 2 DM with or without complications, of either sex between 30 to 80 yrs. Patients were clinically assessed and investigated, and their serum Zn and C-peptide levels were determined. Statistical analysis was carried out using unpaired students t-test. Results: During two year study period, 96 newly diagnosed consecutive type 2 DM cases were enrolled and they were divided into two groups. Group I included cases of DM without complications (n=54) and Group II cases of DM with complications (n=42). Neuropathy was present in 34 cases, nephropathy in 26 cases and retinopathy in 20 cases and all three complications were present in 10 cases. Mean serum Zn levels although slightly lower 70.96+1.54 µg/dl in group II cases compared to group I 72.28 +4.06 µg/dl was found statistically insignificant. Similarly mean serum C-peptide levels in group II (13.91+5.60 ng/ml) and group I (12.80+3.61 ng/ml) did not show a statistically significant alterations. Conclusion: It is concluded that serum Zn level and C-peptide levels bear no correlation and are of no predictive value in cases of type 2 diabetes mellitus with or without complications.[Agarwal S et al NJIRM 2013; 4(2) : 156-161]

Key Words: Diabetes mellitus, Serum Zn, C-peptide level.

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Introduction: Diabetes mellitus (DM) affects almost every system in the body. DM can cause both acute metabolic complications such as diabetic ketoacidosis and hyperosmolar non ketotic coma and late complications such as nephropathy, neuropathy, retinopathy, circulatory changes etc. These ultimately cause morbidity and premature mortality. Some patients never develop these problems while in others these begin early with the onset of disease. Disturbances in zinc metabolism are more pronounced in diabetic population with specific complications[1]. It is not clear whether difference in zinc status is a consequence of diabetes or whether altered zinc status contributes to expression of diabetes itself[2].

During conversion of human proinsulin to insulin, C-peptide are removed by proteolysis and equimolar amounts of insulin and C-peptide are secreted. In the past, C-peptide has been considered biologically inactive. However, recent studies have demonstrated that it is capable of eliciting molecular and physiological effects suggesting that C-peptide is infact a bioactive peptide[3]. Studies have investigated the relationship between hyperinsulinemia and diabetic complications, but the relationship between C-peptide and macrovascular and microvascular complications in type 2 diabetes millitus is still unclear and controversial[4,5]. Although diabetic patients with an elevated serum C-peptide level may have an increased risk of coronary heart disease and peripheral vascular diseases[5,6,7,8].

The present study has been undertaken to correlate the role of serum zinc and C-peptide levels in type 2 diabetes mellitus cases with or without complications.

Material and Methods: This prospective study of two year duration was carried out in the Departments of Medicine and Pharmacology S.N. Medical College, Agra and Rohilkhand Medical College & Hospital, Bareilly. Ninety six freshly diagnosed consecutive cases of type 2 diabetes mellitus who attended medical OPD, diabetic clinic or admitted to medical wards, who fit in the inclusion criteria, were included in the present study.

The inclusion criteria were age group more than 30 years; presence of clinical signs/symptoms of diabetes mellitus like polyurea, polyphagia and polydypsia and laboratory findings suggestive of type 2 diabetes mellitus or type 2 diabetes mellitus with complications. Exclusion criteria were severely malnourished cases, presence of proven hepatic or cardiac disease or chronic renal failure. Besides, pregnant and lactating mothers were also excluded.

Each case was evaluated with detailed clinical history pertaining to age at onset of diabetes, duration and clinical presentations, drug treatment and family history of diabetes. A particular stress was given to elicit the clinical signs/symptoms of neuropathy, nephropathy and retinopathy. In females a detailed obstetrical history regarding still birth and large babies (macrosomia) and shoulder dystocia (difficult delivery) was taken.

Each case was subjected to thorough physical examination and detailed systemic assessment in respect to neurologic deficit, renal and retinopathic changes and cardiovascular status of the patient was also noted. The patients were grouped into DM without complications (n=54) and DM with complications (n=42).

The routine investigations carried out in each patient included complete hemogram such as general blood picture, hemoglobin, total and differential leucocyte counts (TLC & DLC), erythrocyte sedimentation rate (ESR), complete urine analysis (including albuminuria, glycosuria, ketonuria and microscopic sediments), blood urea, serum creatinine, serum bilirubin, SGPT, SGOT, fasting and post prandial blood sugar, serum triglyceride and serum cholestrol.

Serum zinc was estimated by atomic absorption spectrophotometer (ECIL AAS 4139). Serum C-peptide estimation was done by electrochemiluminescence immunoassay (ECLIA) by using Roche Elecsys 2010 immunoassay analyzer.

Differences among groups in mean values were assessed by student's unpaired t-test. P value of <0.05 was considered statistically significant. Spearman's correlation was obtained between serum zinc and C-peptide levels.

Observations &Results: Of 96 type 2 diabetes mellitus cases, 54 (56.25%) cases did not have any complications (Group I) and 42 (43.75%) were suffering from complication of DM (Group II).

Age wise breakup of total 96 DM cases both in group I (DM without complications) and in group II (DM with complications) was shown in table-1. The average age of patients of group I was 48.15±4.90 yr. and of group II was 52.48± 4.53 yrs. In group I maximum numbers of

patients (n=32) were in the age group 40-50 yrs and in group II maximum number of cases (n=28) were of 50-60 yrs.

There was a preponderance of involvement of male patients in both groups. M:F ratio 1.29:1. In group I there were 30 male and 24 female patients (M:F ratio 1.25:1) while in group II there were 24 male and 18 female patients (M:F ratio 1.33:1). The average age of males was 47.93±4.75 years and of females was 48.93±5.07 years in group I and in group II the average age of males was 52.92±4.40 and of females was 51.89±4.63 years.

Table-1: Distribution of cases according to age

Age (yrs)	Group-I no. (%)	Group-II no. (%)
30 - 40	4 (4.17)	0
40 - 50	32 (33.33)	10 (10.42)
50 - 60	18 (18.75)	28 (29.17)
> 60	0	4 (4.17)
Total 96 (100%)	54 (56.25)	42 (43.75)

In group II, only neuropathy was observed in 8(19.05%) cases, nephropathy in 4(9.52%) and

retinopathy in 2(4.76%). 6 patients (14.29%) had both neuropathy and retinopathy, while neuropathy and nephropathy both were present in 10(23.81%) cases. Nephropathy and retinopathy both were present only in 2 cases (4.76%). 10(23.81%) cases had all the three complication neuropathy, retinopathy and nephropathy.

Total 34 (35.42%) cases had neuropathy. Mean serum zinc level in cases with neuropathy was 71.18±1.60 µg/dl and in cases without neuropathy was 71.98±3.87 µg/dl. The difference in mean serum zinc levels was statistically insignificant (P>.05). Table – 2

A total of 20 (20.83%) cases had retinopathy. Mean serum zinc level in cases with retinopathy was 71.06±1.69 µg/dl and in case without retinopathy was 71.87±3.56 µg/dl. The difference in mean serum zinc levels was not statistically significant. Table – 2

Table - 2: Comparisons of Serum Zinc levels (in µg/dl) in cases with & without Complications

Age Group (years)	Mean Serum Zinc levels (Mean± SD)							
	Without neuropathy	With neuropathy	Without retinopathy	With retinopathy	Without nephropathy	With nephropathy	Without any Complication	With any Complication
30 - 40	83.97±0.10 (n=4)	0	83.97±0.10 (n=4)	0	83.97±0.10 (n=4)	0	83.97±0.10 (n=4)	0
40 - 50	71.19 ±2.50 (n=34)	71.82±0.85 (n=8)	71.34±2.35 (n=34)	70.81±0.00 (n=2)	71.31±2.30 (n=42)	0	71.22±2.58 (n=32)	71.62±0.86 (n=10)
50 - 60	71.10±2.06 (n=24)	70.98±1.85 (n=22)	71.02±1.95 (n=32)	71.09±1.99 (n=14)	71.72±2.18 (n=24)	70.30±1.36 (n=22)	71.56±2.18 (n=18)	70.71±1.74 (n=28)
> 60	0	71.06±0.54 (n=4)	0	71.06±0.54 (n=4)	0	71.06±0.54 (n=4)	0	71.06±0.54 (n=4)
Total	71.98±3.87 (n=62)	71.18±1.60 (n=34)	71.87±3.56 (n=76)	71.06±1.69 (n=20)	72.17±3.64 (n=70)	70.42±1.30 (n=26)	72.28±4.06 (n=54)	70.96±1.54 (n=42)
	p>0.05 (NS)		p>0.05 (NS)		p>0.05 (NS)		p>0.05 (NS)	

Table - 3: Comparisons of Serum C- peptide levels (in ng/ml) in cases with & without Complications

Age Group (years)	Mean Serum C-peptide levels (Mean± SD)							
	Without neuropathy	With neuropathy	Without retinopathy	With retinopathy	Without nephropathy	With nephropathy	Without any Complication	With any Complication
30 - 40	12.09±2.64 (n=4)	0	12.09±2.64 (n=4)	0	12.09±2.64 (n=4)	0	12.09±2.64 (n=4)	0
40 - 50	13.17 ±3.77 (n=34)	10.94±4.91 (n=8)	13.08±3.91 (n=34)	5.96±0.00 (n=2)	12.75±4.11 (n=42)	0	13.62±3.41 (n=32)	9.94±4.82 (n=10)
50 - 60	11.76±4.08 (n=24)	17.19±3.71 (n=22)	14.31±4.73 (n=32)	14.46±4.83 (n=14)	13.15±4.71 (n=24)	15.68±4.45 (n=22)	11.50±3.72 (n=18)	16.19±4.44 (n=28)
> 60	0	7.88±5.72 (n=4)	0	7.88±5.72 (n=4)	0	7.88±5.72 (n=4)	0	7.88±5.72 (n=4)
Total	12.55±3.89 (n=62)	14.62±5.59 (n=34)	13.55±4.28 (n=76)	12.29±5.84 (n=20)	12.84±4.27 (n=70)	14.48±5.45 (n=26)	12.80±3.61 (n=54)	13.91±5.60 (n=42)
Signi.	p>0.05 (NS)		p>0.05 (NS)		p>0.05 (NS)		p>0.05 (NS)	

The mean serum zinc level in cases with nephropathy (n=26) 70.42±1.30 µg/dl and without nephropathy (n=70) was72.17±3.64 µg/dl in cases and this difference in serum zinc level was not statistically significant.

Mean serum zinc level in cases with complications (group II) was 70.96±1.54 µg/dl as compared to 72.28±4.06 µg/dl in cases without any complication (group I) and that the lower values in cases of type 2 diabetes with complications were found statistically insignificant. In the present study mean serum C-peptide in cases with neuropathy (n=34) was 14.62±5.59 ng/ml as compared to 12.55±3.89 ng/ml in cases without neuropathy (n=62) and difference in C-peptide levels in both groups was statistically insignificant. Similarly, a statistically insignificantly lower values of serum C-peptide was observed in cases of retinopathy (n=20) 12.29±5.84 ng/ml as compared to 13.55±4.28 ng/ml in cases without retinopathy. The mean serum C-peptide level in cases of nephropathy was 14.48±5.45 ng/ml whereas in cases without nephropathy was 12.84±4.27 ng/ml and that these values were statistically insignificant. Table – 3

Overall the mean serum C-peptide level in cases with complications (group II) was 13.91±5.60 ng/ml as compared to 12.80±3.61 ng/ml in cases without complication (group I) and difference in mean serum C-peptide levels in both groups was statistically insignificant (p>.05). Table – 3

In our study no significant correlation between mean serum zinc levels and mean serum C-peptide levels was found in both groups of DM. Table - 4

Table - 4: Correlation between Serum Zinc and Serum C- peptide levels in both groups

Group	r	t
I (n=54)	-0.115	0.577
II (n=42)	0.110	0.481
Total	-0.058	0.393

r = Spearman's Correlation

Discussion: Of various chronic complications observed in type 2 diabetes only microvascular complications namely neuropathy, nephropathy and retinopathy were studied in the present study. DM cases were subdivided into two groups. Group I consisted of type 2 diabetes mellitus cases without complication (n=54) and group II consisted cases of type 2 diabetes with complications (n=42). The average age of onset of type 2 diabetes mellitus in group I was found to be[8] lower (male 47.93±4.75 & females 48.72±5.07) as compared to group II cases (male 52.92±4.40& female 51.89±4.62). Inukai et al[5] observed mean age 53.4±14.6 years in their study in cases of Type II diabetes with complications. Sari et al[6] observed average age 55.5±8.4 years in their study groups with duration of diabetes 1 -27 years. Our observations thus are corresponding to the findings by other workers in the field.

The study observed predominance of male involvement with M:F ratio of 1.29:1 and that in both subgroups I & II there was greater involvement of males in DM. In contrast a female preponderance was noted by other workers in the field[5,6]. A strong relationship between the level of glycemic control and the diabetic complications especially microvascular have been observed[9].

The development and progression of neuropathy, nephropathy and retinopathy are directly related to the extent of glycemic control (measured as blood glucose levels and/or hemoglobinA1c). However, workers in the field have implicated the role of trace elements including zn2.In the present study mean serum zinc levels in cases with complication (group II) was 70.96+1.54 µg/dl as compared to 72.28+4.06 µg/dl[9] in cases without any complications (group I) and that the lower values in the cases of type 2 diabetes mellitus with complications were found statistically insignificant. This suggested no correlation between serum zinc levels between the two groups and in addition serum zinc levels were of no predictive values in respect to three complications studied. Our findings were in conformity with those of Walter et al[10] who estimated zinc levels in diabetic patients with and without complications. The authors concluded that there was no consistent association between plasma zinc and complications of diabetes. Further, Car et al[11] estimated serum Zn, Cu and Zn/Cu ratio in healthy volunteers and in type 1 and type 2 diabetic patients. The authors

noted that changes in Zn and Cu level as well as Zn/Cu ratio were not related to chronic diabetic complications. These observations were comparable to those of the present study. It may be mentioned that zinc is required for insulin synthesis and storage, and insulin is secreted as zinc crystals, it maintains the structural integrity of insulin[12] moreover, D'Ocon et al[13] observed that in diabetic group serum Zn levels were not correlated with insulin but were correlated with serum glucose levels.

Type 2 patients secrete high levels of proinsulin and they may have a lower levels of active hormone than radio immunoassay indicates, because the assay does not distinguish between proinsulin and insulin. Therefore, measurement of C-peptide provides a better index of insulin levels in type 2 diabetes mellitus.

Overall, the mean serum C-peptide level in cases with complications (Group II) was 13.91±5.60 ng/ml as compared to 12.80±3.61 ng/ml in cases without complications (Group I). Thus, it was observed that mean serum C-peptide level were insignificantly altered in the two groups of DM and that it did not exhibit any statistically significant correlation between group I and group II DM cases. It is therefore concluded that C-peptide values can not serve as a predictive parameter in respect to complications occurring in DM cases.

Our findings were well corroborated by Sari et al[6] who observed statistically insignificant difference in plasma C-peptide levels in cases with and without neuropathy, nephropathy and retinopathy. The authors further observed that serum C-peptide level was significantly associated with the presence of coronary artery disease (p=0.001), peripheral vascular disease (p=0.001) and autonomic neuropathy (p=0.001) indicating a relationship between C-peptide and macrovascular but not microvascular complications in patients with type 2 DM. Further there was no relationship between C-peptide level and duration of diabetes. However, contrasting views were expressed by Toyry et al [14] and Gottsater et al[15] who observed an association between parasympathetic neuropathy and elevated fasting C-peptide level. Regarding nephropathy, Shin et al[16] reported that C-peptide values in microalbuminuric type 2 DM patients were not different from those in normoalbuminuric patients. Klein et al[4] found no relationship between higher levels of C-peptide at baseline and lower 6 years incidence or progression of retinopathy. They suggested that glycemic control and not C-peptide was related to the incidence and progression of diabetic retinopathy. It is also observed that serum Zn and C-peptide levels were not significantly correlated with each other in cases of type 2 diabetes with and without complications. Table - 4.

Conclusion: it may be noted that though serum Zn levels were lower in group II compared to group I in type 2 diabetes mellitus cases but these differences in serum zinc levels were statistically insignificant[12].

Similarly serum C-peptide levels were insignificantly altered in the two groups of DM cases. It is therefore concluded that both serum Zn level and C- peptide levels can not be used as predictive factor for chronic complications of type 2 DM cases. However, a major limitations in our study was relatively small sample size with complications. Hence, larger randomized studies involving large number of DM cases are required to fully establish implication or predictive values of serum Zn and C-peptide levels in type 2 diabetes mellitus.

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