Quantification and Comparison of Some Heavy Metals in Scalp hair, Finger Nails and Plasma of Diabetic Patients of Sargodha Zone (Pakistan)

[1]IFTIKHAR HUSSAIN BUKHARI*, [2]MUHAMMAD SHOAIB, [2]MUHAMMAD JAWAD SARWAR, [3]SAMIA HAFEEZ, [1]TANVEER HUSSAIN BOKHARI AND [1]SYED ALI RAZA NAQVI

[1]*Department of Chemistry*, *Government College University*, *Allama Iqbal Road*, *Faisalabad-38000*, *Pakistan.*

[2]*Department of Chemistry*, *University of Sargodha*, *Sargodha-40100*, *Pakistan.*

[3]*Department of Chemistry*, *Bahauddin Zakariya University*, *Multan*, *Pakistan.*

ifti_61@hotmail.com*

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Summary: There is an accumulating evidence that metabolism of several trace elements is altered in diabetic patients and these micro nutrients have specific role in pathogenesis and progress of Diabetes Mellitus (DM). Seven heavy metals i.e. Co, Cd, Cr, Ni, Mn, Cu and Zn in nails, scalp hair and plasma of DM patients of Sargodha zone were quantified by Flame Atomic Absorption Spectrophotometer (AAS). Results were with the same age group healthy Non-diabetic mellitus (NDM) volunteers. In the *nails* of males Cr and Zn level higher but Cu and Ni concentration was lower than healthy controls significantly. In females Cu and Ni showed same pattern but Zn in order of vice versa. In *Scalp hair*, Ni, Cu, Zn and Mn level in male DM was reduced but Cr, Cd, and Co showed elevated concentration than healthy controls. In females DM again Ni, Cu, and Zn level was lower and vice versa for Cr, Cd, Co and Mn. In *Plasma* of male DM patients Cr and Ni showed lower level while Cd, Co, Cu, Zn and Mn were in higher concentration than healthy controls and same results were seen for females. Our findings are in good agreement with the previous literature data, yet extensive effort is still needed in order to avoid the prevalence of diabetes mellitus in Pakistan. It is concluded from the present study that maintenance of specific heavy metal profile, that is necessary for in healthy persons, can be reduced the risk of DM and it can prove to reduce the rate of morbidities by DM as well.

Keywords: Heavy metals, AAS, Diabetes, Scalp hair, Plasma sample

Introduction

Diabetes is a disorder in which a person has higher level of blood sugar. This may be either because the body does not produce enough insulin or because body cells do not responds to insulin which is produced [1]. There are many types of diabetes, the most common of which are Type 1 diabetes and Type 2 diabetes [2], Gestational diabetes [3], Pre-diabetes, Congenital diabetes, Diabetes Insipidus and Diabetes Mellitus [4]. In all these abnormalities beta cells have key role. Beta cells function is cracked by free radicals [5]. Copper metal exerts antioxidant effect on beta cells leading to their protection from destruction [6]. Chromium is the essential micronutrient which is present in several biological systems. It helps in the formation of Glucose Tolerance Factor (GTF) which acts as cofactor of insulin in all insulin dependent systems [7]. Cadmium exacerbated diabetes complications probably via Cd^{2+} antagonistic and chelating activity at nerve terminals [8]. Cadmium potentiates nephropathy and also it reduces insulin level and has direct cystotosic effect on pancreas. Zinc metal plays a key role in action as well as synthesis of insulin in the physiological and pathological state of diabetes [9]. Glucose intolerance will occur in case of the deficiency of manganese which can only be reversed by Mn supplementations [10]. A manganese deficiency produces defective cells in the pancreas and small number of pancreas islet cells which contain fewer beta cells that manufacture insulin [11]. Salt of cobalt ($CoCl_2$) is revealed to lessen serum glucose level considerably [12].

Abnormality in required concentration of trace elements and minerals in human body inevitably increases the risk of various diabetic complications [13]. The concentration effect of trace elements in diabetic patients has already been studied [14-17]. When one considers the associated risk factors present in Pakistani society, it looks no surprise for diabetic patients [18].

Literature revealed that there is a direct relation between heavy metals accumulation and diabetes. Therefore the main objective of the present study was to investigate and quantify some heavy metals profile in scalp hair, nails and plasma of diabetic patients of Sargodha Zone (Pakistan).

Results and Discussion

In the diabetic population 57.14% were male while 42.85% were female. Same proportion was for non-diabetic males and females. Diabetic male 40.48% belonged to younger age group (30-44years) while 59.52 % diabetics and non-diabetic females were belonging to elder age group (45-60 years) (Table-1). The numbers of male subjects who have been suffering from diabetes from less than 4 years, between 5-9 years, 10-14 years and above 15 years

were 61.90%, 16.66%, 11.90% and 9.52% respectively. Both diabetic smoker and non smoker were 50%, while no diabetic smoker and non smoker were 30.95% and 69.05% respectively.

Comparing the level of heavy metals in hair, only zinc manifested a small variation while other metal's level in diabetic is similar to no diabetic volunteers. It is also obvious from table that Zn level in nails of male is greater than female. In nails as well as plasma there is no considerable variation of cadmium in both sexes and same for Co, Cu, and Mn in case of scalp hair and finger nails. Zinc level in female diabetic is lower than male diabetic subjects which is contrary to the findings of Nsonwu *et al*. (2006) [19].

For Cr and Zn difference is significant as compared with controls but insignificant for Mn, Co, Ni, Cu and Cd metal in nails of diabetic male samples (Table-2). Chromium and zinc level is slightly higher in scalp hair in diabetic than non-diabetic volunteers. Copper and zinc were lower in hairs of male's diabetic subjects while Cd and Co showed insignificant difference than control. Although Cu and Zn concentration in plasma of diabetic are slightly higher than controls.

Only noteworthy variation for Cu, Ni, Cr and Zn is observed in nails of diabetic female's samples while Mn, Co and Cd showed no valuable difference compared with healthy volunteers (Table-3). Chromium level in hairs of women diabetic was elevated compared with non-diabetic healthy women. Here level of Zn in scalp hair is low in patients than healthy subjects. This fact supports Margarita *et al.* (2007) studies [20]. Obviously Co and Mn are showing very slight variation in plasma compared with controls but remaining metals are displaying significant difference**.**

Only level of Zn in diabetic mellitus smoker subjects is low compared with nonsmoker in nail and hair but remaining metals i.e. Cr, Cd, Co, Cu, Mn and Ni showed higher level in smokers, as also reported by Afridi *et al.*(2008) [21]. Cd in diabetic smoker has been found similar to nonsmokers but on the other hand Cr, Co, Cu, Ni, Zn and Mn concentration is found higher in smokers than nonsmokers. In plasma of diabetic smoker's subjects elevated Cr, Cd, Co, Zn and Mn level and reduced Ni level is observed when comparing with their corresponding nonsmoker diabetic volunteers (Table-4).

Table-5 shows that as diabetic subjects gets aged level of metals reduces. Note that Zn level is too much reduced in hair due to hair loss with age, and also in nails. It may be due to frequent urination.

Experimental

Selection of Subjects

Subjects from six tehsils of Sargodha district (Bhalwal, Kotmomin, Sahiwal, Silanwali, Shahpur and Sargodha) were selected. Among 42 diabetic patients 24 were males and 18 were females in age range of 30-60 years. Necessary data on occupational, physical activity, life style pattern as smoking or non smoking habit, past and present illness and medication was recorded. All the chemicals and reagents were procured from E. Merck, Germany.

Washing of Samples

Samples (nail and hair) were washed three times with acetone followed by water and again with acetone as recommended by IAEA Advisory Group, for the removal of gel, grease, dust and polish, followed by drying in electric oven at 60^{o}C [22].

Scalp Hair, *Nails and Blood Samples Collection*

More than 1g hair of diabetic patients and controls subjects were cut at the root of occipital area and stored in polyethylene bags; nails (more than 1g) were cut and stored in labeled polyethylene bags, Blood sample (5 mL) were collected from subjects aseptically via for determination of heavy metals with sterilized syringe, Blood samples (5 mL) were collected from subjects aseptically via vein-puncture for the determination of heavy metals with sterilized syringe and Na_2EDTA powder was used as anti-coagulation agent.

Scalp Hair, *Nail and Blood Samples Preparation for Analysis*

One gram of hair was cut into small pieces and each was soaked in ultrapure nitric acid (5 mL, 60%) overnight. Then one milliliter H_2O_2 was added and kept on hot plate at 180 ^{o}C for 2-3 minutes until white fumes started to evolve [23]. One gram of each nail sample was cut into small pieces and soaked in ultrapure nitric acid (5 mL, 60%) in digestion flask (100 mL) overnight followed by the addition of 1 mL H_2O_2 and digested on hot plate at 180 ^{o}C until white fumes started evolving. Volume of all samples were made upto 20 mL by double distilled de-ionized water and stored in samples vials for further analysis. Plasma was isolated from blood by Centrifugation at 3000 rpm. Plasma was then collected from each

centrifuged blood sample vial by micropipette and transferred to digestion flask. In each flask 5 mL of 60% HNO_3 was added and kept overnight. One milliliter of H_2O_2 was added into each flask and kept on hot plate at 180 ^oC for 2-3 minutes until white fumes started evolving [24-26]. Final volume of plasma samples were made 15 mL by adding double distilled water in each sample vial.

Table-1: Mean ± S.D. concentration (µg/g for Hair & Nail, mg/L for Plasma Samples) of Diabetic Subjects (Men vs. Women).

	Samples	Cd	Cr	Co	Cu	Mn	Ni	Zn
Diabetic Patients Men (N=24)	**Hair**	**0.52±0.01**	**2.68±0.53**	**6.74±1.80**	**18.73±3.01**	**8.62±2.31**	**2.61±0.07**	**35.42±5.64**
	Nail	**0.74±0.05**	**21.43±5.37**	**6.81±2.32**	**15.58±6.79**	**4.38±1.02**	**2.18±0.03**	**394.96±57.12**
	Plasma	**0.556±0.01**	**0.599±0.03**	**3.65±1.31**	**11.61±2.41**	**2.43±1.02**	**1.30±0.16**	**15.75±3.12**
Diabetic Patients Women (N=18)	**Hair**	**0.46±0.05**	**3.99±1.40`**	**6.59±1.03**	**19.18±1.71**	**9.13±2.10**	**2.52±0.19**	**38.90±4.21**
	Nail	**0.48±0.015**	**1.95±0.13**	**6.44±1.86**	**16.77±2.01**	**4.13±1.12**	**2.28±0.06**	**22.374±4.61**
	Plasma	**0.576±0.04**	**0.59±0.02**	**3.82±1.01**	**11.76±2.23**	**1.97±0.04**	**1.372±0.08**	**16.26±3.25**
S/NS	**Hair**	***NS***	***NS***	***NS***	***NS***	***NS***	***NS***	***S***
	Nail	***NS***	***S***	***NS***	***NS***	***NS***	***S***	**S**
	Plasma	***NS***	**S**	***S***	***S***	***S***	**S**	***S***

t-Test Analysis (*p<0.05=Significant Difference (S), p>0.05=Non-Significant Difference (NS)*

Table-2: Mean ± S.D. concentration (µg/g for Hair & Nail, mg/L for Plasma Samples) of Diabetic Subjects (Men) Vs Control (Men).

	Samples	Cd	Cr	Co	Cu	Mn	Ni	Zn
Diabetic Patients Men (N=24)	**Hair**	**0.52±0.01**	**2.68±0.53**	**6.74±1.80**	**18.73±3.01**	**8.62±2.31**	**2.61±0.07**	**35.42±5.62**
	Nail	**0.74±0.05**	**21.43±5.3**	**6.81±2.32**	**15.58±6.79**	**4.38±1.02**	**2.18±0.03**	**394.96±57.12**
	Plasma	**0.556±0.01**	**0.59±0.03**	**3.65±1.31**	**11.61±2.41**	**2.43±1.02**	**1.30±0.16**	**15.75±3.12**
Control Subjects (N=24)	**Hair**	**0.11±0.02**	**0.9±0.1**	**5.32±2.00**	**35.5±6.2**	**10.5±3.43**	**6.2±2.3**	**220±24**
	Nail	**0.9±0.03**	**0.5±0.021**	**5.31±1.21**	**37.5±3.21**	**5.60±2.04**	**6.1±1.54**	**99.5±9.87**
	Plasma	**0.09±0.05**	**2.65±1.00**	**2.2±0.1**	**0.96±0.10**	**1.06±0.34**	**2.5±0.2**	**1.55±0.055**
S/NS	**Hair**	**NS**	**S**	***NS***	***S***	***S***	***S***	***S***
	Nail	***NS***	***S***	**NS**	***S***	***NS***	***S***	***S***
	Plasma	***S***	***S***	***NS***	***S***	***NS***	***NS***	***S***

t-Test Analysis (*p<0.05=Significant Difference (S), p>0.05=Non-Significant Difference (NS)*

Table-3: Mean ± S.D. concentration (µg/g for Hair & Nail, mg/L for Plasma Samples) of Diabetic Subjects (Women) Vs Control (Women).

	Samples	Cd	Cr	Co	Cu	Mn	Ni	Zn
Diabetic Patients Women (N=18)	**Hair**	**0.46±0.05**	**3.99±1.40**	**6.59±1.03**	**19.18±1.71**	**9.13±2.10**	**2.52±0.19**	**38.90±4.21**
	Nail	**0.48±0.015**	**1.95±0.13**	**6.44±1.86**	**16.77±2.01**	**4.13±1.12**	**2.28±0.06**	**22.37±4.61**
	Plasma	**0.576±0.04**	**0.593±0.02**	**3.82±1.01**	**11.76±2.23**	**1.97±0.04**	**1.37±0.08**	**16.26±3.25**
Control Subjects Women	**Hair**	**0.10±0.001**	**1.21±0.03**	**4.32±1.31**	**31.7±4.1**	**6.52±1.89**	**6.6±1.02**	**198.21**
	Nail	**0.87±0.005**	**0.48±0.07**	**5.34±1.25**	**28.1±5.32**	**4.54±0.63**	**7.2±1.6**	**93.7±7.12**
	Plasma	**0.09±0.055**	**2.65±1.0**	**2.2±0.1**	**0.96±0.10**	**1.06±0.34**	**2.5±0.2**	**1.55±0.055**
S/NS	**Hair**	**NS**	**S**	***S***	***S***	***S***	***S***	***S***
	Nail	***NS***	***NS***	***NS***	***S***	***S***	***S***	***S***
	Plasma	***S***	***S***	***NS***	***S***	***NS***	***S***	***S***

t-Test Analysis (*p<0.05=Significant Difference (S), p>0.05=Non-Significant Difference (NS))*

Table-4: Mean ±S.D concentration (µg/g for Hair & Nail, mg/L for Plasma Samples) of male Diabetic smokers and nonsmoker subjects.

	Samples	Cd	Cr	Co	Cu	Mn	Ni	Zn
Diabetic Patients Smoker (N=21)	**Hair**	**0.72±0.09**	**3.37±1.21**	**7.37±1.24**	**21.21±3.02**	**7.29±1.69**	**3.34±1.29**	**39.83±5.61**
	Nail	**0.87±0.01**	**19.35±2.17**	**7.32±1.08**	**13.91±2.73**	**5.61±2.01**	**3.20±0.07**	**388.54±43.21**
	Plasma	**0.72±0.01**	**0.67±0.09**	**3.65±1.31**	**12.01±2.91**	**1.39±0.07**	**1.45±0.13**	**18.79±3.62**
Diabetic Patients Non-Smoker (N=21)	**Hair**	**0.61±0.80**	**2.78±0.06**	**5.32±0.27**	**17.58±2.15**	**6.35±1.96**	**3.30±1.27**	**42.50±4.22**
	Nail	**0.67±0.03**	**16.54±3.12**	**5.67±1.36**	**9.53±1.84**	**4.132±1.78**	**2.08±0.04**	**412.35±52.11**
	Plasma	**0.53±0.05**	**0.47±0.08**	**2.42±0.91**	**8.37±2.44**	**0.89±0.72**	**2.58±0.86**	**11.74±2.61**
S/NS	**Hair**	***NS***	***S***	***S***	***S***	***S***	***NS***	***S***
	Nail	***S***	***S***	***S***	***S***	***NS***	***NS***	***S***
	Plasma	***NS***	***NS***	***S***	***S***	***NS***	***S***	***S***

t-Test Analysis (*p<0.05=Significant Difference (S), p>0.05=Non-Significant Difference (NS)*

Table-5: Mean ± S.D. concentration (µg/g for Hair & Nail, mg/L for Plasma Samples) of diabetic subjects (Men< 45) Vs (Men > 45).

	Samples	Cd	Cr	Co	Cu	Mn	Ni	Zn
Diabetic Patients Men<45 (N=10)	**Hair**	**0.78±0.21**	**2.36±1.66**	**5.44±1.57**	**17.20±2.66**	**8.23±1.18**	**1.95±0.41**	**101.45±12.4**
	Nail	**0.73±0.11**	**2.67±1.28**	**8.65±2.06**	**22.36±2.84**	**7.35±0.34**	**3.01±0.61**	**74.51±7.86**
	Plasma	**0.37±0.01**	**0.65±0.03**	**2.06±0.33**	**6.34±1.05**	**0.78±0.01**	**0.91±0.05**	**57.62±3.98**
Diabetic Patients Men>45 (N=14)	**Hair**	**0.89±0.09**	**1.75±0.91**	**6.21±1.88**	**17.54±2.28**	**5.98±0.73**	**0.97±0.44**	**95.12±9.72**
	Nail	**0.47±0.17**	**1.78±1.68**	**7.32±2.34**	**17.32±3.76**	**7.32±0.61**	**2.68±0.76**	**57.45±4.29**
	Plasma	**0.34±0.03**	**1.87±0.02**	**2.34±0.31**	**5.14±0.91**	**0.89±0.01**	**1.35±0.07**	**56.69±3.36**
S/NS	**Hair**	***NS***	***S***	***NS***	***NS***	***S***	***S***	***S***
	Nail	***NS***	***S***	***S***	***S***	***NS***	***NS***	***S***
	Plasma	***NS***	***S***	***NS***	***S***	***NS***	***NS***	***S***

t-Test Analysis (*p<0.05=Significant Difference (S), p>0.05=Non-Significant Difference (NS)*

Analysis

Flame Atomic Absorption Spectrophotometer (FAAS) was applied for analysis which is considered as one of the most effective, modern and completely economical instrument. Significance of difference between controls and subjects was assessed using student's t test analysis. Due to sensitivity of instrument 5% error was encountered i.e. α=5%=0.05. Calculated t value was matched with tabulated t-values at 0.025 due to biasing of data. Difference was considered significant for $p<0.05$ and insignificant for $p>0.05$.

Conclusion

This study primarily demonstrated that trace elements levels in plasma, scalp hair and finger nails could significantly deviate from control ranges. Therefore it seems reasonable to analyze plasma, scalp hair and finger nails in clinically routine practices to monitor trace element status in patients with DM and other related disorders. These results obtained are consistent with those obtained in other studies confirming that metals may play a significant role in development of diabetes and progression of its metabolic complications. Nevertheless comprehensive studies covering large population are needed to elucidate a clear relationship between glucose metabolism disorders and plasma, hair and nails levels of trace elements.

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