Chromium, Manganese and Zinc Levels in the Biological Samples of Type 1 Diabetic Mellitus Children, Reside in Different Areas of Sindh, Pakistan

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Summary: T1DM is the most acquainted childhood immune-caused onset and may lead to early mortalities and morbidities. It can emanate in any age group but is highest reported in lesser than 18 years of age. In Pakistan only, T1DM institutes ~ 2% of the all diabetic population. This work was formulated to assess the concentration of manganese (Mn), zinc (Zn) and chromium (Cr) in blood, scalp hair and serum samples of diabetic mellitus type 1 children age ranged (1–5), (6-10) and (11-14) years of both genders (male and female), compared with similar sex and age referent subjects. For sample pretreatment, microwave-aided acid digestion procedure (MAD) was employed for elemental analysis in the biological samples of T1DM children. The employed method was validated by using certified reference samples of blood, scalp hair, and serum. Digests biological specimen were analyzed by AAS. The Zn was analyzed by FAAS, while the assessment of Mn and Cr were measured by ETAAS. The Mn, Zn and Cr, presents significantly low levels in the samples of scalp hair, blood and serum of female and male of age group of (1-5) (6-10) and (11-14) of diabetic mellitus type 1 children, compared with referent subjects. These data present an important hypothesis to doctors and other clinical experts to investigate insufficiency of these essential micronutrients (Cr, Mn, & Zn) in biological specimen of blood, scalp hairs and serum of T1DM children.

Keywords Zinc, Manganese, Chromium, Biological samples, Type 1 Diabetic Mellitus, Children.

Introduction

Diabetes mellitus (DM) results as an improper body functioning and failure of regulation mechanism. Insulin producing β cells damage due to autoimmune processes that may cause the trait of T1DM, Beta cell devastation consequence in high blood glucose, which requires exogenic insulin for endurance. Long term standing complications, because of Hyperglycemia, including neuropathy, retinopathy, heart disorder and renal failure, which may cause considerable incompetency and abbreviate life expectancy [1].

The DM is mainly divided into two types of diabetes, T1DM (Type 1 DM) and T2DM (Type 2 DM). T1DM occurs in childhood while type 2 diabetes occurs in adulthood, are caused by insulin resistance or insulin shortage. The T1DM results as a partial or complete impairment of the insulin fabricating beta cells. Diabetic nephropathy is the major imperative reason of death in T1DM patients, compared to T2DM. Positive family history, age and fatness especially central obesity are most important well-known threat factors [2].

T1DM is characterized by autoimmune disease, correlated to elevated glucose levels in blood. This hyperglycemia coerces to a variety of vascular snags like, retinopathy, neuropathy, and coronary artery disease. Insulin is one of very essential hormones that plays crucial role in the directive glucose utilization by the cells [3]. T1DM occurrence is more than individuals 20cases/y/one million individuals [4]. Caucasian population as compared populations of Asia is very high 0.4± 1.1 cases/y /one million individuals may be appropriate to diverse incidence distribution of HLA alleles in every population [5]. Rate of T1DM appears to be comparable in both genders male and female [6]. Even though numerous autoimmune diseases affect primarily females, with the cooperated effort of WHO-DAMOND, registered rate of T1DM in childhood under the 15 years age group has been diverse in different parts of the world [1].

The occurrence of T1DM has been increasing worldwide throughout the previous ten years, and if that continues tendency, rate of new cases of T1DM doubling-up in children younger than 5 years in Europe is predict among 2005 and 2020 [7]. Pakistan ranks 6th at present rate with the highest burden of diabetes mellitus among the countries. In our country, Pakistan more than 10% of its adult population from 185 million populations has diabetics and an equivalent quantity of inhabitants is suffering from improper glucose tolerance (IGT) [8]. The occurrence of DM and the prognostication of its

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populace with DM in Pakistan, in the years of 1995 and 2000 had been predicted to be 6.7% and 7.1% while for 2025, it has been predictable to be 8.7% correspondingly more than the age group of 20 years [9]. Total figure has been stunning too, mutually in rustic along with metropolitan areas. 2.16M, 2.45M and 4.23M will be diabetic in the rustic areas, while 2.18, 2.84 and 10.23 million are the expected numbers of diabetes cases in the metropolitan areas: During the 3 points in time indicated, 1.7, 2.1 and 5.8 in millions the respective figure for females, the whole numeral of males is probable to be: 2.63, 3.21 and 8.67 in millions. 4.34, 5.3 and 14.5 million will be the total expected figure of diabetics. From 1995 to 2025, Pakistan will have raise from 4.3 million in to 14.5 million [10].

The reported frequency of T1DM is very low in and institutes < 2% of the whole diabetic population [11]. In Pakistan, among children aged up to 16 years the occurrences of diabetes is found to be merely 1.02 out of 0.1M a year [12]. A newly published research by DIAMOND Project Group confirms a low frequency rate in Pakistan. Data available on T1DM in Pakistan are barely accessible particularly concerning medical presentation counting Diabetic ketoacidosis (DKA) and unrelieved complications , primarily because of Pakistan's deficient health care system [12].

The part of trace element and micronutrients is optimal for metabolic function in human beings supply a diversity of functions including regulatory, catalytic and structural actions where, they act together with enzymes, presecretory, granules, biological membranes and prohormones [13]. There is mark in T1DM that numerous trace metals alter the metabolism and these nutrients holds key role in pathogenesis and progression of this syndrome [13]. Trace elements which take part on cause of T1DM are zinc (Zn), manganese (Mn), and chromium (Cr). The Zn is pivotal for functions of growth and development, nervous system, hormones activity, immune systems, wound healing and taste and smell functions. It is also essential for nucleic acids and proteins synthesis, unbridling of vitamin A from cell wall structure and the liver and sexual maturation [14]. It also has a chief role in production, exudation and storing of insulin, bestowing it to be one of the most essential micronutrient, insulin functions and metabolism in diabetes [15]. The Zn deficiency and insulin resistance are interconnected [16]. The lipid and protein structures are changed, in diabetic patients, due to glycosylation induced chronic hyperglycemia and their per-oxidation. Lipid per-oxidation initiates the production of toxic malondialdehydes and aldehydes [17]. The Zn, an anti-oxidant element [18], may decrease lipid per oxidation status in T1DM patients [19].

Chromium (Cr) is most requirements for action and potential of insulin receptors. Activation of insulin receptors may be improved due to phosphorylation caused by Cr, which results in improved insulin sensitivity [20]. Mertz & Cornatzer, 2009 demonstrated that abnormal intake of Cr leads to diabetes and glucose intolerance. Subjects exposed to insulin tend to excrete higher amounts of chromium than those of controlled subjects, whereas not much of noteworthy difference in chromium excretion from urinary track of diabetic and controlled subjects. Lesser Cr concentration in humans are also known for the production of vascular complications with DM [21]. Manganese (Mn) is a cofactor for a numerous enzymatic system. It was recommended that Mn is essential for regular insulin secretion and synthesis [22].

Atomic absorption spectrometry (AAS), and inductively coupled with plasma mass spectrometry (ICPMS), extremely responsive spectroscopic techniques, were used to compute and access trace elements in environmental and biological samples. In the light of above mentioned facts, it is necessary to assess elemental concentrations of essential , trace elements in biological specimen of body fluids, urine, blood ,various biopsy, scalp hair and serum of human subjects, having physiological disorders, ,might be used as bio indicators [23]. Assessments of human hair samples for determination of exposed doses of trace elements have been extensively used evaluation of numerous types of environmental contaminants. Information provides by blood elemental analysis that which elements are absorbed by body in recent few hours, days or in the weeks in some cases [24].

Flame atomic absorption spectrometry (FAAS), a sensitive quantification technique, required for the assessment of biological samples for trace quantities of metals [25]. This method has a required convective system, microwave ovens, for whole or fractional decomposition of the matrix using ash drying for solubilization of the analyte [26]. To avoid the production of mineral acids in large amount and to control the nitrous vapors escaping into environment, microwave mediated digestion method is recommended [27, 28].

The objective of current research was to estimate the deviation in the concentrations of Cr, Mn and Zn in the biological specimens (scalp hair, blood and serum) of children suffering from T1DM and healthy children of either genders within the age

group of (1-14 years) of Pakistani children correlated to age and sex factors. Microwave-assisted acid digestion technique is used to prepare biological samples and validity of the analytical procedure were checked through the use of corresponding certified reference materials of blood ,scalp hair and serum [26].

Experimental

Materials and Methods

Instrumentation

The digestion of biological samples was mediated with Peel microwave oven (Osaka, Japan) (power upto 900 W), whereas, Analyst 700, AAS (Perkin Elmer) – (Norwalk, CT. USA) having background correction of Deuterium, HGA-400 graphite furnace (Perkin Elmer), a pyrocoated graphite laminar flame burner tube Perkin Elmer's autosampler (AS-800) were employed for elemental analysis of the sample. Table-1 elaborates the operating conditions of the instrument. The Cr and Mn were analyzed by using ETAAS while FAAS equipped with air–acetylene flame, under optimized operating conditions was used to determine the Zn. In flame absorption mode, absorbance peaks were measured as signals, whereas, in graphite furnace (peak area), computation of combined absorbance values was completed. Entire glasswares were acid washed with PTFE, while instrument conditions were set as recommended by the manufacturer.

Reagents and Standard Solutions

Ultrapure water was obtained with the help of ELGA (Bucks – UK). (65%) HNO_3 and (30%) H_2O_2 of Merck (Darmstadt, Germany) were used. Standard solutions of trace metals (Cr, Mn and Zn) were purchased from Fluka Kalmia (Buchs, Switzerland). These solutions were diluted to obtain a final solution of thousand ppm with 0.2M HNO_3. The solutions were stored in PTFE bottles at 4°C. All Certified reference materials were purchased from BREC (Brussels, Belgium) for methodology accuracy. These references include control lyophilized® human blood Recipe (Clincheck - Munich, Germany) control lyophilized® human serum Recipe (Clincheck - Munich, Germany) and certified human hair (BCR 397). All glass and plastic apparatus and materials were kept in 0.2 M HNO_3 for a day and washed with Milli-Q water followed by drying and stored in Laminar flow hood to avoid any contamination.

Diabetic mellitus Type 1 children and medical treatment Methodology

Approval for initiation of this work was granted from ERC of the University of Sindh, Jamshoro – Pakistan.

Study population

The study conscripted patients from different hospitals of Hyderabad and Jamshoro districts from January 2016 to June 2016. The blood, scalp hair and serum sample of 194 control subjects and 84 DM1 patients of both genders predominantly collected (Table-2). For all the controls and T1DM children, anthropometric parameter comprising height, waist, and weight circumstances were computed following standard protocol. Blood pressure, WBC's, RBC's Glucose, HbA1C, Platelets, Platelet distribution width, mean platelet volume, Weight, Height, and BMI were measured using standard protocol (Table-3). In order to collect particulars regarding health, duration of diabetes, ethnic origin, dietary habits, physical data and their consent, a questionnaire was provided to each person. Written consent form was furnished from each children's parents or guardians at the time of commencement of study. All the subjects and their parents or guardians were given an oral session about the purpose of this study. The reason for oral session was that most of the subjects belong to rural areas with very poor academic and literal background. Questionnaires, provided to the subject's parents, were to collect socio-economic conditions of their surroundings, their dietary habits and to obtain other information as shown in Table- 4.

Table-1: Measurement conditions for electrothermal atomization AAS 700.

Parameters	Chromium	Manganese	Zinc	
Lamp current (mA)	7.5	7.5	7.5	
Wave length (nm)	357.9	279.5	214.0	
Slit-width (nm)	0.7	0.2	0.7	
Drying temp (°C/ramp/hold(s)	140/15/5	140/15/15	Burner height (mm)	7.5
Ashing temp (°C/ramp/hold(s)	1,400/10/20	1,400/10/20	Oxidant (Air) L/min	17
Atomization temp (°C)/ramp/hold(s)	2,500/0/5.0	2,200/0/5.0	Fuel (Acetylene) L/min	2
Cleaning temp (°C)/ramp/hold(s)	2,600/1/3	2,600/1/3		
Chemical modifier	$Mg(NO_3)_2$	$Mg(NO_3)_2$		

Table-2: The number of subjects as control and diabetic mellitus type 1 children.

Age groups	Male		Female	
	Controls	DM I	Controls	DM I
1- 5	25	14	22	12
6- 10	31	16	26	14
11- 14	51	15	39	13
Total	107	45	87	39

Table-3: Biochemical parameters in referents and Diabetic mellitus type 1 children of both genders.

Parameters	1- 5 years		6-10 years		11-14 years		Normal range
	Referents	Diabetic mellitus type 1	Referents	Diabetic mellitus type 1	Referents	Diabetic mellitus type 1	
Male							
Weight (kg)	17.9±1.38	14.3±1.08	27.9±1.45	23.5±1.32	40.9±1.36	33.6±0.95	---------
Height (cm)	94.9±3.17	77.2±1.20	125.3±2.7	105± 2.08	151.7±1.95	138.2±1.35	---------
BMI(kg/m2)	19.7±0.85	24.0±1.15	17.7±1.24	21.3±0.83	17.8± 1.60	17.6± 0.99	---------
Hb (g/dL)	11.9±0.42	6.29±0.51	12.6±0.95	6.29±0.51	12.3 ± 0.50	5.62 ±0.38	11.5-14.8
Hct (%)	35.6±1.35	48.3±3.28	37.5±1.02	46.3±1.72	36.9 ±1.41	50.4 ±1.19	35- 55
Glucose (mmol/L)	4.39±0.40	6.50±0.37	4.68±0.62	7.43±0.52	4.85± 0.42	8.16± 0.50	3.4- 5.4
% HbA1C	4.37±0.50	8.62±0.61	4.82±0.48	9.15±0.60	4.65± 0.35	9.42± 0.70	> 5.6
RBC (mm3)	4.60±0.51	3.05±0.19	4.23±0.59	2.95±0.48	4.76±0.42	2.64±0.35	3.5- 5.5
WBC (mm3)	6.42±0.62	6.35±0.37	7.05±0.43	6.05±0.20	7.38±0.62	6.53± 0.45	3.5- 10
Platelets (mm3)	210± 10.6	235± 9.52	252± 25.9	298± 19.5	278± 30.9	316± 20.6	100-400
mean platelet volume (fL)	10.0±0.24	10.7±1.03	10.2±0.40	11.0±0.39	10.5± 0.28	11.8± 0.57	---------
Platelet distribution width [%]	11.5±0.96	12.4±1.63	11.9±0.52	12.7±1.30	11.7± 0.30	12.5± 0.65	---------
Female							
Weight (kg)	14.5±1.15	12.6±0.98	25.5±1.38	21.9±1.06	42.5±2.39	35.9± 1.24	---------
Height (cm)	93.0±4.02	84.9±1.30	127.8±2.08	112.6±1.82	151.7±1.44	144.9±1.07	---------
BMI(kg/m2)	16.8±1.21	17.8±1.04	15.6± 1.05	17.3± 1.60	18.5± 1.35	17.1± 0.68	---------
Hb (g/dL)	11.7 ±0.31	6.35±0.42	12.5 ± 1.05	6.18 ±0.36	11.9 ± 0.48	5.39 ±0.62	11.5-14.8
Hct (%)	36.4 ±0.89	49.2±2.71	36.9 ±0.87	50.7 ±1.35	38.2 ±1.58	51.9 ±1.35	35- 55
Glucose (mmol/L)	4.52± 0.69	6.79±0.52	4.75± 0.40	7.62± 0.70	4.60± 0.58	7.95± 0.72	3.4- 5.4
% HbA1C	4.24± 0.37	8.75±0.92	4.59± 0.35	9.07± 0.52	4.48± 0.47	9.29± 0.62	> 5.6
RBC (mm3)	4.72±0.39	3.18±0.36	4.39±0.45	3.06±0.28	4.59±0.39	2.76±0.40	3.5- 5.5
WBC (mm3)	6.65±0.55	6.47±0.72	7.18±0.60	6.29± 0.49	7.42±0.55	6.72± 0.67	3.5- 10
Platelets (mm3)	218± 20.5	249± 14.8	259± 30.8	298± 19.5	282± 26.4	328± 20.5	100-400
mean platelet volume (fL)	10.3± 0.18	10.9±0.62	10.5± 0.31	11.2± 0.35	10.7± 0.20	11.5± 0.40	---------
Platelet distribution width [%]	11.3± 0.72	12.3±0.90	11.6± 0.39	12.5± 0.89	11.9± 0.51	12.7± 0.48	---------

Biological samples

Heparinized lithium Vacutainer® (HLV®) tubes (Becton Dickinson) (7 mm) were used for the collection of venous blood samples (5 mL). For elemental analysis, intravenous blood specimens (~ 2mL) were in refrigerator with static temperature of −20 °C. For further scrutiny, collection of scalp hair samples at ~ 5 cm above hair root was taken from the nape of neck. Each participant's hair specimen was sealed in airtight plastic bags with a unique identification number and was attached to questionnaire of the respondent. At the time of sample pretreatment, specimens of hair were cut into ~ 0.3 cm. Later, these hair specimens were washed with Triton X-100 (1:200 v/v dilution) 4 times, followed by rinsing thrice with ultra-pure water and twice with analytical grade acetone. Then at 80–85 °C, the specimen was dried in an oven.

Microwave assisted acid digestion

3 replicates of each CRMs including BCR 397 human hair (0.2 g), Clincheck® control-lyophilized human whole blood (0.5 mL) and duplicate serum (0.5 mL) scalp hair (0.2g) and blood (0.5mL) samples were individually taken in PTFE flasks of 25mL. 3mL addition of HNO_3 - H_2O_2 (2:1, v/v), were added into each flasks. These flasks, with each sample and digestion mixtures, were then kept at room temperature in flow hood for 10 minutes. Later, these PTFE containers holding samples were given at 4 minutes at microwave oven at 900W, to digest the samples. Dilution of digested sample material was completed with addition of HNO_3 (0.1 mol /L) to reach 10 mL mark. Without sample (blank) extraction was used throughout the whole analysis.

Table-4: Questionnaire employed in sampling of Diabetic mellitus type 1 children.

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Serial No. _____ Dated:_____

Personal Information: Contact Nos:_____

Full Name _____ Caste _____

Blood Group: _____

Place of Birth _____ Age_____

Weight (Kg) _____ or (in Pounds) _____ Height _____ (Feet) _____ (Inches)

Ethnic origin (check **only one**):

Sindhi Punjabi Pathan Balochi

Sex **(Male)** ☐ **(Female)** ☐ (Tick Mark any one)

Please circle the **highest** year of school completed:

1 2 3 4 5 67 8 9 10 11 12

(Primary) (Elementary) (Secondary and higher Secondary)

Present Address _____

Parents /Guardian information

Occupation: ____ Socioeconomic Status: **Poor** ☐ **Mid. Class** ☐ **Rich** ☐

Disease and Treatment Information:

Has any family member suffered by any physiological disorder? (Yes/No) _____

Type of Diabetes (T2DM/Gestational Diabetes) _____

Health conditions (brief description by the doctor):

_____

_____

(3) Food habits and Life Style of parents (Brief description): ***(Fill through a to d)***

(a) Regularly (2-3 times/week) **(b) moderately** (1 time/week) **(c) Rarely** (1-2 times/month) **(d) Never**

1. Meat _____ 2**.** Fish _____ **3.** Chicken _____

4. Vegetables _____ **5.** Fruit intake _____ (Frequently used fruit(s) _____)

Table-5: Determination of Cr, Mn and Zn in certified samples by microwave digestion method (N=6).

Elements	Certified values	MWD Mean ± SD	(%) Recovery	Paired t-testat Experimental
Certified sample of serum (µg/l)				
Cr	**5.9±1.5**	**5.84±0.25 (4.28)b**	**98.9**	**0.875**
Mn	**2.8±0.7**	**2.75±0.16 (5.81)**	**98.2**	**0.0668**
Zn	**2.225±0.334**	**2.75±0.16 (5.81)**	**99.6**	**0.670**
Certified sample of whole blood (µg /l)				
Cr	**2.0 ± 0.5**	**1.94 ± 0.09 (4.64)**	**97.0**	**0.0042**
Mn	**25.0 ± 5.0**	**24.8±0.21 (8.47)**	**99.2**	**0.0302**
Zn	**2.27 ± 0.06**	**2.19±0.15 (6.61)**	**96.5**	**0.260**
Certified sample of human hair (µg g-1)				
Cr	**91.0 ± 10c**	**90.94 ± 5.99 (6.59)**	**99.9**	**0.0028**
Mn	**11.2 ± 0.3**	**11.0±0.92 (8.36)**	**98.2**	**0.458**
Zn	**199 ± 5.0**	**197.8±7.29 (3.68)**	**99.4**	**0.678**

[a] Paired t-test between certified values vs. found values, degree of freedom (n−1)= 5. $t_{Critical}$ at 95% confidence limit= 2.57.

[b] Values in parenthesis RSD.

[c] informative value

Table-6: Elemental concentration in biological samples of referents and Diabetic mellitus type 1 (DM1) children of both genders.

Biological samples	Age groups	Control	DM1	Control	DM1
		Male		Female	
		Chromium			
Scalp hair (µg/g)	1-5 yrs	3.17±0.54	0.63±0.10	3.25±0.35	0.67±0.12
	6-10 yrs	3.64±0.31	0.55±0.12	3.57±0.21	0.48±0.07
	11-14 yrs	3.40±0.25	0.40±0.08	3.52±0.14	0.45±0.05
Serum (µg/l)	1-5 yrs	1.9±0.12	0.82±0.09	2.0±0.09	0.85±0.05
	6-10 yrs	2.2±0.25	0.65±0.11	2.4±0.20	0.69±0.07
	11-14 yrs	2.6±0.20	0.42±0.07	2.7±0.26	0.45±0.16
Blood (µg/l)	1-5 yrs	68.7±6.31	24.9±5.59	63.5±8.27	21.5±4.14
	6-10 yrs	82.3±6.03	28.3±7.80	79.4±5.09	25.7±2.23
	11-14 yrs	95.5±7.44	31.8±8.30	89.8±9.02	28.4±4.62
Manganese					
Scalp hair (µg/g)	1-5 yrs	3.54±0.12	0.59±0.10	3.42±0.20	0.53±0.07
	6-10 yrs	3.80±0.15	0.52±0.07	3.73±0.27	0.47±0.06
	11-14 yrs	3.95±0.24	0.45±0.06	3.89±0.30	0.42±0.04
Serum (µg/l)	1-5 yrs	1.42±0.03	0.75±0.11	1.49±0.08	0.78±0.09
	6-10 yrs	1.65±0.13	0.54±0.10	1.72±0.06	0.59±0.11
	11-14 yrs	1.74±0.09	0.38±0.05	1.80±0.05	0.41±0.09
Blood (µg/l)	1-5 yrs	24.7±3.46	8.82±1.10	26.5±3.4	9.62±2.5
	6-10 yrs	29.3±1.31	11.25±1.58	32.9±2.71	12.8±1.35
	11-14 yrs	35.6±1.48	16.9±2.08	37.8±2.6	15.3±0.86
Zinc					
Scalp hair (µg/g)	1-5 yrs	165±8.26	53.8±5.15	152±7.65	49.5±3.57
	6-10 yrs	198±6.19	42.8±3.22	185±9.37	40.3±5.27
	11-14 yrs	239±7.05	35.8±4.28	219±9.15	33.5±5.02
Serum (mg/l)	1-5 yrs	1.3±0.04	0.59±0.12	1.35±0.05	0.62±0.09
	6-10 yrs	1.5±0.07	0.42±0.10	1.48±0.08	0.46±0.07
	11-14 yrs	1.6±0.10	0.35±0.10	1.52±0.12	0.38±0.05
Blood (mg/l)	1-5 yrs	11.5±2.32	3.45±0.55	13.2±1.83	3.75±0.32
	6-10 yrs	9.35±0.48	2.09±0.39	8.92±0.74	1.96±0.28
	11-14 yrs	7.85±1.15	1.65±0.28	7.58±0.95	1.48±0.22

Statistical analysis

Processing of data and statistical analysis of the obtained data were carried out using Minitab v.13.2 (Minitab Inc., PA), XLState (Addinsoft, USA) and Excel - 2003 (Microsoft Office ®). The analysis of variance was used to evaluate the consequence of alterations among the concentrations of Cr, Mn and Zn in the biological specimen of T1DM children and control subjects, calculated by the unpaired two-sample t-test. A $p<0.05$ was measured substantial alteration. For the evaluation of the substantial alteration of understudy elements in experimental and certified reference values, Student's t-test was used.

Analytical figures of merit

Calibration curve reached from the detection limit up to 10 µg /mL for the concentration range of Cr, Mn and Zn. The limit of quantification (LOQ) and detection (LOD) were found as $LOQ = \frac{10\sigma}{m}$ and $LOD = \frac{3\sigma}{m}$ respectively, where σ is the standard deviation of 10 readings of blank (n = 10) and m is the slope of the linear section of the calibration graphs. The LOQ and LOD obtained for Cr were 30.0 and 92.0 µg /L respectively. The MAD requires a very short time of 2-3 minutes to digest the samples. Accuracy and efficiency of the method was checked through certified samples of blood, serum and scalp hair (Table-5). From the certified values, the difference for the mean values of Cr, Mn and Zn was observed less than 1–2%. <2% of the coefficient of variation was observed and by comparing both procedures, non-significant differences ($p>0.05$) was perceived.

Results and Discussion

The nutritional elements needed for the normal physiological development and growth of the organs are called as trace elements. Deviation in the trace metal concentration from normal range can trigger by the chronic and uncontrolled hyperglycemia. Present hospital-based research was performed to find out the different Zn, Cr and Mn conc. in all scalp hair, blood, and serum samples of T1DM children. Categories of all analyzed biological samples were based on T1DM, gender, and controls.

In scalp-hair samples of T1DM children - male of 3 age groups (1-5), (6-10) and (11- 14), concentrations of Cr were found to be at 95 % confidence intervals (CI: 0.54-0.70 µg /g, 0.48-0.68 µg/g and 0.35- 0.46 µg /g) respectively. The Cr values were considerably lower than referent subjects of the same age group (1-5), (6-10) and (11- 14) (95 % CI 2.90-3.92 µg/g) with p value < 0.001. The

similar tendency was similar in females. The concentration of Cr in serum samples of male and female control subjects, age ranged (1- 14) years was found to be higher (CI: 1.78-2.79 µg /L) than, male (0.38- 0.89 µg / L) and female (0.37- 0.89 µg / L) T1DM children. The Cr concentrations in blood specimen of control children of both genders (CI 58.9- 99.3 µg /L) was significantly higher as compared to the Cr concentrations observed in blood samples of male and female T1DM children (CI 19.4- 36.0 µg / L) ($p < 0.01$) (Table-6).

In T1DM children of both male and female genders, the lower levels of Mn were found in scalp hair of three age groups (1-5), (6-10) and (11- 14) at (95 % CI: 0.39- 65 µg/g) versus controls (95 % CI: 3.33- 4.09 µg /g). The level of Mn is significantly lower in serum samples of male (CI: 0.35- 0.82 µg / l) and female (CI: 0.36- 0.85 µg / l) T1DM patients ($p < 0.001$) compared to control children (CI: 1.40- 1.82 µg / l). In the scalp hair samples of both genders suffering from T1DM of three age groups (1-5), (6-10) and (11- 14), the concentration of Mn were found to be lower (CI: 30.3- 56.0 µg/g), respectively as compared to control children (148- 243 µg / g), ($p < 0.001$) (Table-6).

The concentration of Zn in sera samples of male and female control subjects, age ranged (1-14), was found to be higher (CI: 1.28- 1.66 mg/L) than those obtained in sera samples of T1DM children (CI 0.29-0.67 mg/L). The similar pattern was observed in females. In controls of both genders, the concentrations of Zn in blood samples of control children of three age groups was found to be higher (CI 7.09- 14.2 mg/L) as compared to the Zn concentrations, observed in blood specimen of T1DM children (CI 1.36- 3.93 mg /L) of both genders ($p < 0.01$) (Table-6).

Student t test (unpaired) was performed between all studied groups was calculated at different probabilities. At 95% confidence intervals our calculated t value exceeds that of t critical value, which indicated that the difference among means values of these trace metals (Cr, Mn & Zn) in control and T1DM children of both genders exhibited significant differences ($p< 0.001$).

Diabetic mellitus occurs due to hypertrophy, LV dysfunction and coronary artery syndrome left ventricular (LV), but mechanisms still need the revelation [29]. Electronegativity of Cr, Zn and Mn currently work as flawless participants for the organ-toxicity. Trace elements homeostasis is tightly coordinated system due to different proteins involved in their absorption, consumption and elimination. Uptake of different Proteins is tightly coordinated involved in the regulation of different system of the body. Distorted levels of Cr, Mn and Zn, and are concerned in the progression of a number of disorders including diabetes mellitus [30].

Complications such as Parkinson's and Alzheimer's disease may be accountable for the due to development of diabetes cause by oxidative stress, Other complications which occur in diabetes may also be related to cell death, necrosis and oxidative stress is responsible for the formation of reactive oxygen species(ROS), lipid bio membranes resulting from ROS attack by lipid per oxidation [30]. The Zn play important role in physiological action of insulin, its contribution in the glucose metabolism [31]. Contrary levels of Zn were found to be fifty percent lower in DM (407µg/L) as compared to referent subjects (750µg/L) in current research. Pakistani subjects were shows similar results [32]. Saudi Arabia study also shows deficient concentration of Zn in DM [33]. Another Turkish study shows, 18.99 µmol/L level of Zn for T2DM (124 µg/L), related to 14.44 µmol/ L (94.4 µmol/L) for controls [34]. Insulin is used for the crystallization of the hormone, which is binding to Zn. Two Zn ions with hexametric units are sitting at the center hence, after a meal, it is believed that pancreatic β-cells release enough amount of insulin, which is stored in to permit adequate release [35]. With consistent of animal studies, where supplements of zinc was completed through Metallothionein (MT). In progression and development of DM 8 (hZnT-8), the Zn transporter concerned potentially due to its location in insulin emission of pancreatic vesicles [36]. The Zn transporter is the islet- restricted as an applicant of the manager of insulin secretion and storage, eventually leading to DM [33].

Threat of developing DM is increasing 53%, which is linked with the deficiency of Zn. The Zn is essential for many enzymes involved in the biosynthesis and storage of insulin in the ß-cells and plays important role in the metabolism considerably, little plasma zinc levels have been reported earlier [33].

Diabetes (hyperglycemia) associated with blood glucose fluctuations can be improve by Cr level in individuals with a trend towards improve glucose level [35]. Action of insulin is increased, and it is a cofactor in the insulin action blood glucose of control serum Cr levels. In the current study, Cr levels in sera samples were found to be lower in the comparison of control subjects [37]. The Cr excretion

is increased by high levels of insulin and hyperglycemia, it has been seen that the lower level of Cr in the sera of diabetics patients were attributed to insulin resistance [38].Glycosuria resulting from hyperglycemia and osmotic diuresis, which enhance excretion of Cr through urine. Insulin resistance and hyper insulinemia might be interrelated with diminution in insulin-receptors, or post-insulin-receptor signaling defect, reduced insulin binding [39]. Initial cause of T1DM and T2DM thought to be insulin resistance and diminish the action of insulin and sensitivity of target tissue (skeletal muscle and predominantly the liver) to the relative deficiency of endogenous insulin secretion [39]. Elevated blood glucose is also main cause of unnecessary glucagon or irregular and too much hepatic glucose production in some patients. Elevated fasting glucose levels are the output resulting in impaired insulin secretion and increased glucagon's. Regulation blood glucose also affected by cortical epinephrine, or another hormone. Somatostatin, an excess of growth hormone, may have a defect by Cushing's syndrome, pancreatitis, pheochromocytoma, and aldosterone's, pregnancy hyperthyroidism, cirrhosis, myocardial infarctions and are other factors, including emotional stress, that might be due to elevated blood glucose. It's probably multifactorial etiology. It also shows hormone secretion and its function may regulate by trace elements. A compound called 'GTF' (glucose tolerance factor) is part of Cr required for proper usage of glucose, insulin receptor sensitivity and lipid digestion [39]. It was found in an study that 500 mg Cr for 2 months twice a day results as a considerable perfection of glycosylated hemoglobin (HbAlc) standards, and indicates the rate of glucose digestion [39]. Here in this study it is shown that the probable associations among blood and serum Cr concentration sugar (glucose and HbA1c) in T1DM, it has been shown by several studies that there is a negative relationship between serum Cr concentration and serum glucose levels [39].

The Cr insufficiency upset carbohydrates metabolism, as, it is considered as a main cofactor in different enzymes, that regulate glucose metabolism. Insufficiency of Cr can make difficult transportation of glucose into the cells, by upsetting membrane elasticity and lipid metabolism [40]. The Cr shows key role in the synthesis of insulin and its function. Insulin connection shows glucose entrance to the cells. Hyperglycemia increases the oxidative stress in diabetic patients due to insufficiency of Cr. A number of studies have also verified that oxidative stress and lipid per oxidation is reduce though Zn supplement in diabetic patients, even though it does not extensively change the level of glucose. The Cr play promising role in improvement of DM patients through reducing oxidative stress and glucose levels [41-43]

The Mn plays a vital function in a numeral of physiological processes, as constituent in some enzyme activators and as a part of a few enzymes. The Mn is stimulated enzymes, which play an important role in uptake of carbohydrates, amino acids and cholesterol. During the synthesis of ATP's, one of reactive oxygen species is the super oxide radical, which is produced in mitochondria [44, 45]. Prime antioxidant enzyme of mitochondria is manganese superoxide dismutase, catalyzes the exchange of superoxide radical to hydrogen peroxide, and water can be reduced by further antioxidant enzymes [43]. The symptoms of Mn insufficiency include improper growth, defective impaired glucose tolerance, reproductive function, skeletal abnormities and variable lipid and carbohydrates metabolism. In human, it has been less clear neither lower dietary Mn nor low level of Mn in blood or tissues has been associates with numerous chronic disorders like epilepsy, diabetes mellitus and osteoporosis. An oxidative stress is a reason for development of diabetic complication [46].

The Mn is an essential trace mineral for human health. It works with the enzymes, which act as a catalyst, that promote the rate of biochemical reaction in cells [47]. Many enzymatic functions are assisted by the Mn including (i) manufacturing and powering an antioxidant enzyme – superoxide dismutase, which helps protecting cell membranes and reduces the degradation and disruption of tissues ;(ii) help the body in metabolizing fats, carbohydrates and proteins ; and (iii) assists in production of blood sugar and energy [46]. Micro albuminuria with vascular complication had observed in most of the patients. The T1DM occurrence of micro albuminuria in patients is a responsive future forecaster of development of diabetic nephropathy [48]. In T1DM, micro albuminuria is a known danger aspect for renal failure increase mortality and in risk factor for nephropathy includes poor glycemic control, hypertension, male sex, smoking positive family history and presence of coronary disease and retinopathy [49]. The Mn helps the body to metabolize the glucose level [50]. It is reported that T1DM people may often have a serious Mn deficiency [51]. Even though consequences have been contradictory [52].

Some researcher suggests that inhabitants with diabetes have considerably lower Mn concentrations than non-diabetic people. In other words, researcher are

yet to verify whether diabetes cause contribute to the development of the metabolic disorder actually [49]. Intra-arterial plaque development can lead to stroke and heart attack due to the contribution of LDL oxidation. Little evidence supported that the diabetic or non-diabetic individual's, Mn supplementation improve glucose tolerance [53]. Even though, Mn appear to a perform the key role in metabolism of glucose. For the normal insulin synthesis and secretion, an appropriate Mn level is required for insulin-resistant diabetic patients, Mn showed good response to oral doses [54].

Conclusion

We concluded that the understudied trace elements (Zn, Mn and Cr) were found in lesser concentration in the biological specimen (scalp hair, blood and serum) of type 1 diabetic mellitus children of both genders, age ranged 1- 14 years. The reason is the contrary long-term outcomes in the children at pregnancy time and during its early lifetime (0- 185 days) might be related with insufficiency of these trace elements. It was also noted that at the age of early 30s Pakistani girls/ female suffering from DM. The resulted data indicated that the lesser concentrations of essential elements in biological samples of T1DM children of all three age group than referent subjects because of insulin deficiency and glucose tolerance, dietary and environmental factor, family history. While enhanced the excretion of essential trace elements via urinary system due to poor absorption, disturbances/ lack of insulin secretion or its action. More studies are needed to find out if the supplementation of essential elements in T1DM may help to control diabetes and prevent oxidative injuries leading to diabetic complications.

To improve the outcomes, it was obligatory to monitor the insufficiency of these trace metals in the diabetic pregnant women, both for the newborns and their mothers, and also at the early time of children. Upcoming research should examine these patterns in relation to the medical progress of individual pregnancies in diabetic female, considering nutritional supplements by means of vital micronutrients and medical treatments received during the pregnancy. The better consideration desires to be given for satisfactory source of these trace elements in the food of childbearing females, toward the possible ill impacts for the neonates. Most of the comprehensive research, however, is necessary to classify the possible adverse effects of insufficiency of these trace metals in the childbearing females, in regular and sickness stages. Advance research in this direction is in process.

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Abbreviations

T1DM	Diabetes mellitus type - 1
T2DM	Diabetes mellitus type - 2
Cr	Chromium
Mn	Manganese
Zn	Zinc
IGT	Improper glucose tolerance
DKA	Diabetic ketoacidosis
FAAS	Flame atomic absorption spectrometry
PTFE	Polytetrafluoroethylene
HNO_3	Nitric acid
WBC	White blood cell
RBC	Red blood cell
LOQ-LOD	limit of quantification and detection
BMI	Body mass index
FAAS	Flame atomic absorption spectrometry
ETAAS	Electro thermal atomic absorption spectrometry
MS	Mass Spectrometry
PTFE	Polytetrafluoroethylene
BREC	Bureau of References of European Communities
ERC	Ethical Review Committee

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