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Interaction between Cadmium and Zinc Levels in the Biological Samples of Type 1 Diabetic Mellitus Children, Reside in Different Areas of Sindh, Pakistan

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Abstract

Type 1 Diabetes mellitus (T1DM) is one of the familiar childhood immune-mediated onsets and can lead to early mortalities and morbidities. It can arise at any stage, but the peak of occurrence is reported less than 18 years of age. T1DM cases in Pakistan were less than 2% of the total diabetic population. The current work designed to assess the concentration of cadmium (Cd) and zinc (Zn) in blood, scalp hair and serum samples of T1DM children, age ranged 1 - 14 years of both genders. For comparison purpose, the age-matched referent subjects of both genders were tested. The microwave-assisted acid digestion procedure was used to determine the elemental analysis in the biological samples of T1DM children and referent subjects. The resulted data of certified reference material of blood, scalp hair, and serum validated the certainty of the designed method. The analysis of Zn was performed by flame atomic absorption spectrometry, while the Cd contents were determined by electrothermal atomic absorption spectrometry. T1DM affected children of both genders have lower Zn level in the blood, scalp hair, and serum samples. Whereas, the levels of Cd were found to be higher in the biological samples of T1DM affected children as compared to referent subjects. The finding of the current study is a significant hypothesis for medical experts, to diagnose the deficiency of essential (Zn) and toxicity of heavy/toxic element (Cd) in the biological specimen of T1DM affected children.

Keywords

Zinc, Cadmium, Biological Samples, Type 1 Diabetic Mellitus, Children

1. Introduction

Diabetes Mellitus (DM) is a global issue. It is the most important cause of morbidity and mortality all over the world. Diabetes is related to high rates of blindness, hospitalization, renal failure, and non-traumatic amputation [1]. The elevated incidences of DM may cause by unhealthy lifestyles, maternal or fetal malnutrition, and genetic factors. Limited physical activity is a major risk for the prevalence of DM. The other risk factors of DM may include obesity and high caloric intake [2] [3]. The DM is mainly divided into two types of diabetes. Type 1 Diabetes Mellitus (T1DM) is insulin-dependent, occurs in childhood, results in partial or complete damage of the insulin-producing beta cells (β-cells) and Type 2 DM (non-insulin dependent) happens in adulthood, caused by insulin resistance or insulin shortage [1]. T1DM is one of the frequent immune-mediated childhood diseases and can cause early mortalities/morbidities [4]. T1DM can happen at any age, although the peak occurrence is observed under 18 years of age [5] [6]. Genetic factor and vulnerability of certain viruses may contribute to T1DM. The major risk factors for DM are identified as the positive family history, age, and fatness especially central obesity [7] [8]. The clinical presentations of T1DM in children and adolescence have merged increasingly over recent years. Conventionally, T1DM is described as an uncommon disorder of autoimmune origin, presenting acutely with ketosis, thirst, and polyuria and weight loss in a child [9]. Diabetic nephropathy is the major imperative reason for death in T1-DM patients, compared to T2DM. The occurrence of T1-DM is more than the individual's 20 cases/y/one million individuals [10].

Trace metals in T1DM patients are evidence to alter human metabolism. Moreover, these trace metals may have a key role in the progression and pathogenesis of such syndrome [10]. The role of trace element and micronutrients is optimal for metabolic function in human beings and supplies a diversity of functions including regulatory, catalytical and structural mechanisms in which, they act together with macromolecules such as enzymes, presecretory, prohormones, granules, and biological membranes [10].

Trace elements have a principal role in the prevalence of T1DM. Zn is an important micronutrient with the major role in the synthesis, storage, secretion, and function of insulin. Its metabolic function is altered in diabetes [11]. Zn deficiency is correlated with insulin resistance [12]. In diabetic patients, chronic hyperglycemia is due to glycosylation and peroxidation leads to increased oxidative stress and thereby proteins and lipids structure are changed [13].

The intake of toxic elements may promote DM, hypertensive and atherosclerosis disorders by increasing oxidative stress due to the deficiency of Zn [14]. The deficiency of essential nutrients, lack of homeostatic control or an excess intake of some toxic elements causes chronic physiological disorders [15]. The toxic metals can interfere with the normal functioning of essential trace elements in the enzymatic systems. The toxic metals can be replacing the nutritional minerals in the enzymes and inactive to them. Whereas, a higher dietary level of essential minerals and vitamins helps to prevent toxic metal toxicity, as well as

helps to eliminate them from the body [15]. Metal ions bonded to melanosomes according to atomic weight and volume. Heavy metals can effectively compete to same binding sites as foreign ions to replace previously bound metals [15]. However, these bonded heavy metals cannot easily manageable to displacement [16]. The Cd is an inhibitor of the enzymes with sulfhydryl groups and disrupts the pathways of the oxidative metabolism [17]. These facts were also confirmed by in vitro studies, reported elsewhere [18].

To keep in view of these facts, it is necessary to analyze the essential trace and toxic elemental concentrations in the biological samples of T1DM children, and to monitor their impact on the human metabolism. In many cases, whole blood, serum, plasma, and urine were analyzed [19] [20] [21]. The atomic absorption spectrometry (AAS) is mostly used for the clinical assay due to their specificity, sensitivity, precision, simplicity, and relatively low cost per analysis [22]. Hair analysis has many advantages as compared to serum, blood or other body fluids such as it is easily collected, stable on storage and has a higher concentration of elements, but many individuals and external factors can influence element levels [23] [24].

The rate of T1DM has been increasing worldwide over the last ten years [25]. Pakistan numbered 6th among the list of top ten countries having highest burden of DM. More than 10% of the adult population of Pakistan from 185 million populations is the witness of diabetes. Similarly, an equivalent quantity of inhabitants is suffering from improper glucose tolerance (IGT) [26].

Among children aged up to 16 years, the occurrences of diabetes are found to be merely 1.02/100,000 per year in Pakistan [27]. A published research study by the DIAMOND Project Group confirms that the low-frequency rate of T1DM in Pakistan are barely accessible particularly concerning medical presentation counting Diabetic ketoacidosis (DKA) and unrelieved complications. It is primarily because of deficient in appropriate health care system in Pakistan [27].

The main objective of this study was to evaluate zinc and cadmium concentrations in the biological samples (scalp hair, blood, and serum) of T1DM and referent children of both genders, ages ranged between (1 - 14) years.

2. Materials and Methods

2.1. Instrumentation

Biological samples were digested through a domestic microwave oven, PEL (Osaka, Japan) (maximum heating power of 900 W). The elemental analysis was carried out by means of A. Analyst 700, Perkin Elmer (Norwalk, CT, USA) atomic absorption spectrometer, equipped with deuterium background correction, and graphite furnace HGA-400 (Perkin Elmer), laminar flame burner a pyrocoated graphite tube with an integrated platform and an autosampler AS-800 (Perkin Elmer). **Table 1** shows the operating condition of the atomic absorption spectrometer. The Cd was measured by electrothermal atomic absorption spectrometry (ETAAS), whilst flame atomic absorption spectrometry

Table 1. Measurement conditions for electrothermal atomization AAS 700.

Parameters	Cadmium	Zinc	
Lamp current (mA)	6.0	7.5	
Wave length (nm)	228.8	214.0	
Slit-width (nm)	0.7	0.7	
Drying temp (˚C/ramp/hold (s)	140/15/5	Burner height (mm)	7.5
Ashing temp (˚C/ramp/hold (s)	850/10/20	Oxidant (Air) L/min	17
Atomization temp (˚C)/ramp/hold (s)	1650/0/5.0	Fuel (Acetylene) L/min	2
Cleaning temp (˚C)/ramp/hold (s)	2600/1/3		
Chemical modifier	$Mg(NO_3)_2$ + $Pd(NO_3)_2$		
Sample volume (10 µl), Cuvette = Cup, Carrier gas = (200 ml/min) Background correction (D_2 Lamp) used for all elements			

(FAAS) with an air–acetylene flame was used for the analysis of Zn. In flame absorption mode, absorbance peaks were measured as signals, while in the graphite furnace (peak area), integrated absorbance values were determined. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparation and storage of standard and solutions.

2.2. Reagents and Standard Solutions

Water system of ELGA, ELGA Lab (Bucks, UK) was used to obtain ultrapure water during the laboratory work. Strong acids, (65%) HNO_3 and (30%) H_2O_2, were purchased from Merck (Darmstadt, Germany). Standard solutions of Cd and Zn, Fluka Kamica (Buchs, Switzerland), were used by stepwise dilution of certified standard solutions of each elements of thousand ppm with 0.2 M HNO_3 before analyzing the sample solution. At 4˚C, PTFE bottles were used to stock up the solutions. Certified reference materials (CRMs) of Clincheck Control Lyophilized® human blood Recipe (Munich, Germany), Clincheck Control Lyophilized® human serum Recipe (Munich, Germany) and certified human hair BCR 397, purchased from Bureau of References of European Communities (Brussels, Belgium), were used for the accuracy of methodology. Glass apparatus and plastic materials were soaked for 24 h in 0.2 M HNO_3, were used throughout the experimental work. Deionized distilled water were used for washing the glass and plastic apparatus, then finally rinsed with Milli-Q water. After this, glass apparatus were dried and stored in a class 100 laminar flow hoods.

2.3. Diabetic Mellitus Type 1 Children and Medical Treatment Methodology

Before initial this study, the approval was obtained from ethical review committee of the University of Sindh, Jamshoro, Pakistan.

2.4. Study Population

The study conscripted T1DM children from different hospitals of Hyderabad and Jamshoro district from January 2016 to June 2016. The blood, scalp hair and serum sample of 194 control and 84 T1DM children of both genders were predominantly collected (Table 2). For all the controls and T1DM children, anthropometric parameters, comprising weight, height, BMI, WBC's, RBC's, Glucose, HbA1C, platelets, platelet distribution width, mean platelet volume, were recorded using standard protocol (Table 3). In order to collected particulars regarding health, ethnic origin, duration of diabetes, dietary habits, physical data age and consent, a questionnaire was employed. The guardians/parents of children, who were enrolled in the study, provided their written consent. They were orally explained the purpose of the study in their native language along with an approval form as most of the study subjects belong to poor uneducated families.

The selection criteria of 194 referent children of both genders belong to the same qualities with respect to age group, socio-economic status and dietary habits, not suffering from any physiological disorders. These children were generally the healthy family members of the diabetic mellitus type 1 children. Before collection of biological samples, referent children have undergone a standard routine medical examination. Questionnaire was also used to get the information related with dietary habits and socioeconomic conditions along in details, mentioned in Table 4.

2.5. Biological Samples

2.5.1. Collection of Blood & Serum Samples

Heparinized Lithium Vacutainer® tubes (B Becton Dickinson, Rutherford, USA) {10 mm} were used for the collection of venous blood samples (7 mL) between 9:30 and 11:00 a.m. About 2 mL of blood samples were sent to hospital pathological laboratories for biochemical tests using standard methods. 2 mL of venous blood samples were stored at −20˚C until elemental analysis, while the remaining 3 mL were used for separating the sera. The blood was allowed to clot at room temperature for 15 to 30 minutes. When the blood had clotted completely, it was then centrifuged for 5 - 10 minutes at 2500 rpm. The supernatant fluid was then separated using a Pasteur pipette, labeled accordingly, and stored at −20˚C until analysis.

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

Age groups	Male		Female	
	Controls	[a]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

Key: [a]Diabetic Mellitus type-1.

Table 3. Biochemical parameters in referents and diabetic mellitus type 1 children of both genders (mean ± SD).

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 ± 2.68	105 ± 2.08	151.7 ± 1.95	138.2 ± 1.35	---------
BMI (kg/m^2)	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	
RBC (mm^3)	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 (mm^3)	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 (mm^3)	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 ± 1.8	151.7 ± 1.44	144.9 ± 1.07	---------
BMI (kg/m^2)	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	
RBC (mm^3)	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 (mm^3)	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 (mm^3)	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	

Abbreviations: BMI: body mass index; Hb: hemoglobin; HbA1C: glycated hemoglobin; RBC: red blood cells; WBC: white blood cells.

Table 4. Characteristics of understudy population.

Characteristics	% of T1DM (n = 84)	% of Ref subjects (n = 194)
Ethnic origin		
Sindhi	77.3 (65)	86.6 (170)
Punjabi	11.90 (10)	6.18 (12)
Pathan	5.9 (5)	4.12 (8)
Baloch	4.76 (4)	2.06 (4)
Socioeconomic status		
Poor	29.7 (25)	41.2 (80)
Middle class	42.8 (36)	36.2 (70)
Rich	27.3 (23)	22.6 (44)
Type of Diabetes in Parents		
Type-2 Diabetes	23.8 (20)	38.6 (75)
Gestational Diabetes	5.9 (5)	6.18 (12)
No	70.2 (59)	55.5 (107)
Schooling		
Primary	35.7 (30)	23.1 (45)
Elementary	22.6 (19)	32.9 (64)
No	41.6 (35)	43.8 (85)
Diet		
Meat		
Once a week	21.4 (18)	25.77 (50)
Twice a week	41.6 (35)	18.04 (35)
Once a month	19.04 (16)	33.5 (65)
Twice a month	11.90 (10)	15.46 (30)
Rarely	5.95 (5)	7.21 (14)
Fruit		
Once a week	9.52 (10)	27.8 (54)
Twice a week	29.7 (25)	20.61 (40)
Once a month	33.3 (28)	16.49 (32)
Twice a month	16.6 (14)	20.10 (39)
Rarely	8.33 (7)	14.9 (29)

Key: [a]Diabetic Mellitus type-1.

2.5.2. Collection of Scalp Hair Samples

5 cm root of hair taken from the nape of neck were used. The scalp hair samples (approximately 0.5 g) were taken from the nape of neck. For each participant, the hair specimen were put individually in tightly sealed plastic bags and attached with identification number and questionnaire. At the time of sample pretreatment, the hair specimens were further cut into 0.2 to 0.3 cm pieces, and washed four times with 1:2 v/v dilution of Triton X-100, then rinsed three times with ultra-pure water, and two times with acetone. Then at 80˚C - 85˚C, the hair

specimens were dried in an oven.

2.6. Microwave Assisted Acid Digestion

Replicate six specimen of every certified reference materials, BCR 397 human hair (0.2 g), Clincheck® control-lyophilized human whole blood, and Clincheck control lyophilized® human serum (0.5 mL), and duplicate samples of scalp hair (0.2 g), blood and serum (0.5 mL) were taken individually in polytetrafluoroethylene (PTFE) flasks (25 mL in volume). Then added 3 mL of a freshly prepared mixture of concentrated HNO_3-H_2O_2 (2:1, v/v), kept at room temperature for ten minutes. Then put the flasks in covered PTFE container and heated at 80% of total power (900 W) for three or four minutes. 0.2 mol/L concentrated HNO_3 solution was used for dilution of digested biological samples up to 10 mL. Blank extraction (without sample) was used throughout the whole analysis.

2.7. Statistical Analysis

Software packages, Minitab 13.2 (Minitab Inc., State College, PA), Excel 2003 (Microsoft Office®) and XL State (Addinsoft, NY, USA), were used to conduct data processing and statistical analysis. The analysis of variance was used to evaluate the consequence of alterations among the concentrations of Cd 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.

2.8. Analytical Figures of Merit

Calibration curve reached from the detection limit up to 10 μg/g for the concentration range of Cd and Zn. The limit of quantification (LOQ) and detection (LOD) were found as $\mathrm{LOQ} = \frac{10\sigma}{m}$ and $\mathrm{LOD} = \frac{3\sigma}{m}$ 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 MAD requires a very short time of 2 - 3 min to digest the samples. Accuracy and efficiency of the method was checked through certified samples of blood and scalp hair (Table 5). From the certified values, the difference for the mean values of Cd and Zn were observed less than 1% - 2%. <2% of the coefficient of variation was observed and by comparing both procedures, non-significant differences ($p > 0.05$) were perceived.

3. Results

The anthropometric parameters such as weight, height, body mass index, white blood cells, and red blood cells were significantly lower in T1DM children as compared to control subjects whilst glucose level, glycated hemoglobin, platelets, platelet distribution width and mean platelet volume were higher in T1DM children as compared to control children of both genders.

Table 5. Determination of Cd and Zn in certified samples by microwave digestion method (N = 6).

Elements	Certified values	MWD Mean ± SD	(%) Recovery	Paired t-test[a] $t^{Experimental}$
Certified sample of serum (µg/l)				
Cd	4.60 ± 1.2	4.56 ± 0.18 (3.95)[b]	99.1	0.123
Zn	2.225 ± 0.334	2.75 ± 0.16 (5.81)	99.6	0.670
Certified sample of whole blood (µg/l)				
Cd	1.2 ± 0.4	1.189 ± 0.10 (8.41)	99.1	0.00636
Zn*	2.27 ± 0.06	2.19 ± 0.15 (6.61)	96.5	0.260
Certified sample of human hair (µg/g)				
Cd	0.52 ± 0.024	0.515 ± 0.042 (8.15)	99.04	0.145
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. * mg/l.

Some trace elements are nutritionally valuable minerals and required for the development and physiology of the organism. Changes in the trace elements could cause of chronic uncontrolled hyperglycemia. The current hospital-based research is performed to find out the different amount (concentrations) of Cd and Zn in all the specimen of blood, scalp hair, and serum of T1DM children (Table 6). The analyzed biological samples categorized according to T1DM, controls, and gender.

The level of Zn in scalp hair samples of T1DM children of both genders, age ranged (1 - 14) years was found to be lower than referent children. The concentration of Zn in sera samples of male control subjects, age ranged (1 - 14), was found to be higher at 95% confidence intervals (CI: 1.28 - 1.66 mg/L) than those obtained in sera samples of T1DM children (CI 0.29 - 0.67 mg/L). The same trend was observed in females. 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 ($p < 0.01$).

In scalp hair samples of male referent children, the concentrations of Cd were found to be lower {(CI: 0.93 - 1.18), (CI: 1.59 - 1.77), (CI: 2.25 - 2.57 µg /g)} in three age groups (1 - 5), (6 - 10) and (11 - 14) years, respectively than those Cd values observed in T1DM children ($p < 0.001$). The similar findings were observed in females. The concentration of Cd in serum samples of male and female control subjects age ranged 1 to 14 years was found to be lower (CI: 0.23 - 0.69

μg /L) as compared to male (CI: 0.49 - 0.93 μg/L) and female (0.44 - 0.90 μg/L) T1DM children. The Cd concentrations in the blood specimen of control children of both genders (CI: 2.28 - 4.07 μg /L) was significantly lower as compared to the Cd concentrations observed in blood samples of male and female T1DM children (CI: 3.47 - 6.11 μg/L) ($p < 0.01$). Moreover, a decrease of Zn/Cd mole ratios in biological samples of T1DM children of both genders as compared to referent children was observed (Table 7).

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

Table 6. Zinc and Cadmium concentrations in biological samples of referents and Diabetic mellitus type 1 (DM1) children of both genders (mean ± SD).

Biological samples	Age groups	Control	DM1*	Control	DM1*
		Male		Female	
Cadmium					
Scalp hair (μg/g)	1 - 5 yrs	1.05 ± 0.25	1.75 ± 0.17	0.96 ± 0.18	1.69 ± 0.23
	6 - 10 yrs	1.69 ± 0.18	2.72 ± 0.30	1.55 ± 0.16	2.59 ± 0.34
	11 - 14 yrs	2.40 ± 0.35	3.79 ± 0.59	2.29 ± 0.23	3.65 ± 0.79
Serum (μg/l)	1 - 5 yrs	0.31 ± 0.05	0.52 ± 0.05	0.27 ± 0.07	0.46 ± 0.03
	6 - 10 yrs	0.45 ± 0.08	0.69 ± 0.11	0.41 ± 0.05	0.65 ± 0.08
	11 - 14 yrs	0.62 ± 0.12	0.89 ± 0.07	0.58 ± 0.08	0.84 ± 0.14
Blood (μg/l)	1 - 5 yrs	2.63 ± 0.52	3.92 ± 0.49	2.51 ± 0.45	3.70 ± 0.51
	6 - 10 yrs	3.21 ± 0.40	4.63 ± 0.35	2.96 ± 0.52	4.50 ± 0.65
	11 - 14 yrs	3.79 ± 0.65	5.82 ± 0.72	3.54 ± 0.68	5.69 ± 0.85
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

* $p < 0.001$.

Table 7. Zn/ Cd Mole ratio in biological samples of referents and type 1vDiabetic mellitus (T1DM) children of both genders.

Specimens	Age groups	Male		Female	
		Referent	T1DM	Referent	T1DM
Scalp hair	1 - 5	270	50.3	272	52.8
	6 - 10	201	26.7	205	27.0
	11 - 14	171	15.8	164	16.2
Blood	1 - 5	7515	1513	9038	1742
	6 - 10	5006	776	5179	749
	11 - 14	3559	487	3680	447
Serum	1 - 5	7207	1950	8593	2316
	6 - 10	5729	1046	6204	1216
	11 - 14	4435	676	4504	777

4. Discussion

Type 1 diabetes mellitus (T1DM) is a chronic disorder with well-known short- and long-term consequences [28]. One of the long-term consequences is severe impairment of growth and development, the so-called Mauriac syndrome [29]. As a result of the major advances in diabetes care, this entity becomes a gem. Indeed, some studies are reporting during the last decade about the positive growth characteristics in diabetic children [30] [31]. However, growth deceleration during T1DM has been reported in various countries around the world, such as Austria, Brazil, Czech Republic, Germany, and Sudan [32] [33] [34] [35].

The prevalence of childhood overweight and obesity has risen during the last 30 years. Not only in children with type 2 diabetes but also those with type 1 are overweight and obese [36]. In children with type 1 diabetes, obesity is linked to an increased cardiovascular risk [37]. Moreover, the presence of overweight also increases insulin resistance, which can intensify complications of treatment [38]. Anemia is a prevailing symptom in type 1 diabetic patients and expresses a significant non-recognized burden. Patients were at greatest risk to be diagnosed for the presence of renal disease (albuminuria and/or renal impairment) [39]. Although low hemoglobin is generally associated with adverse events in diabetes and kidney disease [40]. Several studies showed that high hematocrit predicted in type 1 and 2 diabetes [41] [42] [43]. However, the reasons for this relationship did not explore.

Type 1 diabetes may cause hyperglycemia (high blood sugar). It is a clear sign that diabetes did not a well-control disorder. The processes of non-enzymatic glycosylation of red cell membranes and cytosol proteins are activated during hyperglycemia. An increase in glycosylated hemoglobin (HbA1c) may affect the efficiency of the oxygen-transport function in RBCs. Whereas, HbA1c enhanced the affinity for O_2 to complicate its return to cells in the microcirculation [44]. This may promote the development of tissue hypoxia. There is an enhanced level

of fetal hemoglobin (HbF) in RBCs under the condition of diabetes. Such changes are compensatory to provide a better supply of oxygen to tissues. Whereas, HbF can bind oxygen with greater affinity and return it to much less partial pressure [45].

Children with newly diagnosed type 1 diabetes had lower total white blood cell count (WBC), and fewer neutrophils, basophils, monocytes, and lymphocytes than controls. Similar, erythrocyte, eosinophil, and platelet count were also lower. This may be supporting general involvement in the innate immune system in the pathogenesis of type 1 diabetes [46]. Diabetic people have multiple abnormalities to platelet function, usually causing hyper-reactivities such as adhesiveness, activation, and aggregation with a greater extent as compared to those not affected to diabetes. These abnormalities of platelet are associated with increased clotting, impaired clot breakdown, endothelial dysfunction, and platelet hyper-reactivity. This contributes to the increased risk of atherothrombotic events in people with diabetes compared with non-diabetic individuals [47].

The homeostasis of zinc can be disturbed by DM. Zinc plays an important role to correct the function of glucose and lipid metabolism as well as regulating and forming the expression of insulin.

Concerning essential metals/elements, the major clinical attention, and most of the publication focus on the deficiencies of a one or single element or definite combination of elements [48]. In the present study, the concentrations of Zn and Cd in biological samples of T1DM children of both genders were determined and then these results were compared with referent children, belonging to different areas of Sindh, Pakistan.

The results explained that the concentration of Zn was considerably/ significantly lower in scalp hair, serum, and blood samples of T1DM children than referents ($P < 0.001$). Zn plays an important role in the physiological action of insulin as well as its contribution is also essential for glucose metabolism [49]. Zinc is necessary for many enzymes, which are involved in the biosynthesis and storage of insulin in the ß-cells. Insulin is used for the crystallization of the hormone, which is binding to Zn. A study reported lower plasma Zn levels [50]. The Zn is necessary for many enzymes, which are involved in the biosynthesis and storage of insulin in the ß-cells. Insulin is used for the crystallization of the hormone, which is binding to Zn. A study reported lower plasma Zn levels [50]. Two Zn ions with hexametric unit [51] are lying at the center of each other. Hence, after a meal, it is believed that the pancreatic β-cells release enough amount of insulin, which is stored in to permit the adequate release [51]. Animal studies showed that Zn supplements can complete by Metallothionein (MT). In the progression and development of DM, 8 Zn transporter (hZnT-8) is most concerned. It is because of its function in insulin-secretion from pancreatic vesicles [50]. Zn transporter is the islet—restricted as an applicant of the manager of insulin secretion and storage, eventually leading to DM [50].

The resulted data point out that the biological samples of T1DM children of both genders have significantly higher levels of cadmium (Cd) as compare to

control children. Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents, and refined petroleum products. Besides, acid rain and the resulting acidification of soils and surface waters have increased the geochemical mobility of Cd, and as a result, its surface-water concentrations tend to increase as lake water pH decreases [52].

Cadmium produced as an inevitable byproduct of Zn and occasionally lead refining. The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge), the disposal of industrial wastes or the deposition of atmospheric contaminants increases the total concentration of Cd in soils, and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree [53]. Low doses of Cd used in experiments mimic low or moderate levels of environmental contamination.

The antagonistic effects of Cd and Zn intensively investigated. The accumulation of Cd in the human body may be replaced by Zn in the arteries to shape the arteries brittle and inflexible [54]. The limited information is available for the impact of Cd on insulin receptors and insulin action in adipose tissue [55]. The stimulatory consequence of Cd on glucose transport was confirmed by cell culture model. However, no effects on GLUT4 protein were obvious [56]. The Cd-induced glucose transport mechanism was reported in a vitro study [57], which explained the insulin-mimetic effect of Cd on glucose lipogenesis and glucose oxidation in rat adipocytes [57].

Low Cd concentration in pancreatic islets of obese hyperglycemic mice evoked basal and glucose-stimulated insulin response [58]. In contrast, high Cd concentration significantly inhibited the secretory response of glucose [58]. In vivo rat intake of Cd resulted in lower glycemia accompanied by higher serum insulin value [58]. Further discrepancies in Cd effects on glucose homeostasis and insulin levels are results of hyperglycemia and inhibition of insulin release from rat pancreas in rats exposed to cadmium [18].

The pancreas is a glandular organ, which secretes pancreatic juice containing amylase, trypsin, lipase and other digestive enzymes to assist digestion and absorption of nutrients. Researchers found that Cd exposure decreased the activity of protease in the mouse and inhibited amylase and trypsin activity in the freshwater crab [59] [60]. Besides, ion homeostasis plays a crucial role in toxicosis and nutritional and metabolic diseases in organisms [61]. Abnormal ion homeostasis may induce the abnormal physiological structure and dysfunction of the endocrine gland [62]. Numerous studies showed that heavy metals exerted its toxicity via changing the ion homeostasis in organisms [63] [64]. However, essential elements (such as selenium, zinc) could attenuate Cd accumulation and has antagonistic effects on apoptosis triggered by Cd in the pancreas [64].

5. Conclusions

It can be concluded that impaired trace-element metabolism may have a key role in the pathogenesis and progression of Type-1 diabetes mellitus. It was observed

that the concentration of Zn in blood, scalp hair and serum samples of T1DM male and female children was found to be lower, whilst cadmium concentration was found to be higher than control subject because of insulin deficiency and glucose in tolerance, some dietary & environmental factor, family history of the T1DM patients, increased urinary excretion of essential trace elements due to poor absorption, disturbances/lack of insulin secretion or its action.

The low level of Zn was observed in type 1 diabetic mellitus children possibly due to increased urinary excretion of these essential trace elements, and its low uptake in the biological (scalp hair, serum and blood) samples of T1DM children. Higher levels of Cd with simultaneous lower contents of Zn may be correlated positively with the end results of diabetic mellitus. The mentioned trace element (zinc) is cofactor of different types of enzymes. The lower levels of zinc in biological samples of T1DM children cause a major role in the etiology of diabetes.

Replacement of zinc deficiency by cadmium, might result in abnormal physiological disorders, as well as other factors, and thus may have a role in T1DM. More studies are needed to find out if the supplementation of zinc in T1DM may help to control diabetes and prevent oxidative injuries leading to diabetic complications.

The assessment of biochemical parameters is also important aspect for confirming the severity of T1DM. It was also observed in the present study that the socioeconomic factors also play a role in higher mortality rates in T1DM children, such as poor nutrition, irregular screening, late diagnosis and unequal access to health care due to poverty, because the cost of T1DM treatment is very high.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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