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ENVIRONMENTAL CADMIUM EXPOSURE: ADDITIONAL RISK FACTOR FOR TYPE II DIABETES

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ABSTRACT

Objective: the contribution of environmental pollution to the overall risk for diabetes was assessed by evaluation to the role of inflammation and lipid peroxidation. Oxidative stress induced by accumulating cadmium in beta cells of pancreas was a suggested risk factor for type 2 diabetes. The antioxidant activities of glutathione peroxidase enzyme and sialic acid against reactive oxygen species were also investigated.

Methods: levels of cadmium in blood (S-Cd) and urine (U-Cd) were measured using the graphite furnace atomic absorption spectrophotometer. Fasting glucose level (FBS), glutathione peroxidase enzyme (GSH-Px) and serum sialic acid were measured by enzymatic colorimetric assay. The glycosylated hemoglobin (HbA1c) was determined by high performance liquid chromatography.

Results: the study was carried out on 26 patients diagnosed as type 2 diabetes for a mean duration of 4.66 ± 1.06 years, and a matched group of 31 non-diabetic subjects. Evaluating different life characteristics among the study population revealed an active role to rural versus urban residential areas and to current occupation in the development of diabetes in comparison to non-significant relation with feeding habits. The mean levels of serum (S-Cd) was 3.65 ± 1.17 μg/dl and 0.65 ± 0.42 μg/dl among type 2 diabetes and control groups, respectively, the difference was statistically highly significant, $P<0.001$. Similarly, highly statistically significant differences between the two groups were obtained for creatinine-corrected U-Cd, FBS and sialic acid, and sig-

nificant differences for GSH-Px and HbA1c. Studying the associations between the different parameters, revealed that the main response variable was disease status and potential predictors included serum cadmium, creatinine-corrected urinary cadmium, residence and farming. Highly significant associations were detected for S-Cd and creatinine-corrected urinary Cd versus the kidney functions (urea, creatine and microalbuminuria), diabetes status (FBS and HbA1c), and the enzyme glutathione peroxidase. Similar results were obtained between S-Cd and sialic acid, which associated significantly with cadmium level in urine after correction for creatinine. The correlations studied between FBS and HbA1c versus the antioxidant GSH-Px as well as sialic acid proved highly significant with sialic acid only. No significant correlation was reported between the GSH-Px and the sialic acid. The effect of age, sex, body mass index, duration and onset of diabetes were dealt with.

Conclusion: Environmental pollution by the biotoxic cadmium metal has proved to be a risk factor contributing to the high incidence of type 2 diabetes among the general population. The acute phase reactant, sialic acid appeared to give new hopes by its scavenging role against the generated hydroxyl radicals, thus guarding against the possible development of diabetes. Additionally, estimation of salivary sialic acid may be used as a predictive non-invasive bio-marker of oxidative stress induced by the accumulation of cadmium in beta cells of pancreas.

Key Words: Cadmium, environmental exposure, glutathione peroxidase enzyme, type 2 diabetes, sialic acid, HbA1c, pancreatic diseases, metallothioneins.

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by elevation of blood glucose concentration and associated with microvascular complications. Type 2 diabetes mellitus, the most common form of diabetes accounting for 90% of cases, is characterized by damage to beta cells of the pancreas ending in abnormal insulin secretion [1]. A significant association between pancreatic diseases and serum cadmium levels and farming were observed demonstrating the collective defect of the lifetime occupational, residential, environmental exposures and dietary influence [2].

Nile River water was found seriously contaminated as a result of increasing discharge of untreated industrial wastes and agricultural irrigation wastewater [3]. High concentrations of heavy metals, including cadmium, were among the pollutants in the water. Plants and fish grown in this water were also contaminated with heavy metals [4], which can in turn accumulate in humans and animals that feed on these contaminated foods [5]. Chronic exposure results in accumulation of the metal in

different tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis [6].

In the tissues of pancreas, cadmium shows different mechanisms of toxicity under different experimental conditions and in various species. The metal has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions [7]. The increased oxidative stress associated with the generation of superoxide anion and nitric oxide was suggested to promote inflammation since they can interact to produce peroxynitrite, which is a potent oxidant [8].

The role of antioxidants in reversing this oxidative stress has been of long-standing interest to basic scientists and clinicians [9]. It has been shown that various antioxidants and antioxidant defense systems protect cells from Cd-induced toxicity [10]. However, cadmium exposure was found to cause significant changes in the activity of antioxidant system enzymes. The activity of glutathione peroxide (GSH-Px) was reported to decrease as a consequence of the intracellular accumulation of reactive oxygen species with subsequent development of tissue injury. The decreased activity was explained by competition of Cd-metallothioneins and GSH-Px for sulfur containing aminoacids [6].

Recently, plasma sialic acid, which is one of the markers for acute phase response [11], was found to have an important role in the removal of hydrogen peroxide and was therefore recognized as an alternative oxidative stress marker [12]. In fact, circulating sialic acid was detected at higher levels in people with type 2 diabetes [13]. Sialic acid was reported as an essential moiety to scavenge the hydroxyl radicals [14].

Aim of the Work

This study was designed to assess the cadmium levels of type 2 diabetic patients in an attempt to evaluate the role of environmental pollution with cadmium in development of diabetes. Furthermore, the effects of inflammation and lipid peroxidation have been estimated to explore the mechanisms related to cadmium induction of diabetes. The importance of glutathione peroxidase enzyme and sialic acid as antioxidants protecting the tissues against oxidative stress was investigated.

Subjects and Methods

Study Population:

After protocol approval of the study population and by obtaining their consent,

a total of 26 diabetic outpatients, without first-degree relatives with diabetes mellitus, regularly attending the Diabetes Clinic of the Kasr Al-Aini hospital and 31 non-diabetic subjects including University staff members, hospital employees and medical students were randomly selected. The referents were matched with the exposed population by age, gender, body mass index (BMI) and socioeconomic standards. The inclusion criteria for the participants were absence of a history of any metabolic or inflammatory disorders, dyslipidemia [low-density lipoprotein-cholesterol below 130mg/dl], hypertension [systolic and diastolic blood pressure below 140 and 90mmHg, respectively), obesity [body mass index below 30kg/m^2]. Additionally, subjects with evidence of either cardiovascular or peripheral artery diseases as well as rheumatic diseases, renal, hepatic, or thyroid dysfunction, concomitant medications or drug consumption, smoking and pregnancy were all excluded from the study. All subjects underwent an interview emphasizing socio-demographic background, medical and environmental occupational histories. The study population was then offered a voluntary medical examination and gave blood samples. Further subdivision of the study population was carried out according to the level of cadmium in serum into low (0 - 1.99 µg/dl); medium (2.00 - 3.99 µg/dl) and high Cd (4.00 - 5.99 µg/dl) groups.

Anthropometric measurements

Weight and height were measured, without shoes and in light clothing, to the nearest 0.1 kg and 0.1 cm, respectively. The body mass index (BMI) was calculated as weight divided by squared height (kg/m^2). The Systolic (SBP) and diastolic (DBP) blood pressures were measured using a manual mercury sphygmomanometer. Two readings were obtained five minutes apart in the sitting position and the mean values were recorded as the blood pressure.

Laboratory Urine Analysis:

A first morning urine sample on the day of examination was collected and stored (at 30˚C) in a urine container (25ml), for urinary cadmium level (U-Cd) estimation. Fresh urine samples collected for the test were measured for microalbumin estimation in an electrochemiluminiscence analyzer (Roche, Switzerland). Direct cold-vapor atomic absorption spectrometry was used to measure the urinary cadmium. Accuracy and precision were assessed using urine reference materials. The concentrations of urinary cadmium at the time of examination were used

as indices of current exposure. The results were then corrected for urinary creatinine (creat-U-Cd).

Biochemical Parameters

Blood specimens, obtained from the cubital vein, were drawn between 08:00 and 08:30 am after a 12-hour fasting. Heparinized whole-blood samples for measurements of kidney functions were collected and stored in 10 ml tubes at -20˚C. Blood specimens for measuring cadmium, sialic acid and glutathione peroxidase enzyme (GSH-Px) were collected in 10ml tubes. After centrifugation (1500 rpm for 10 min.), the serum was frozen and stored at -20˚C in 1.8 ml tubes until analysis. Cadmium in blood (S-Cd) was measured by graphite furnace atomic absorption spectrophotometer (Perkin-Elmer model 5100PC, Norwalk, CT). Serum sialic acid was measured by a calorimetric assay using standardized chemicals and reagents. A protein precipitate of serum containing sialic acid will react with diphenylamine producing a purple color, which is quantitatively measured on a spectrometer at 540 nm. Glycosylated hemoglobin (HbA1c) was determined by high performance liquid chromatography (L-9100 Merck Hitachi, Frankfurt, Germany) (reference range: 4.5 - 6.2%). The fasting glucose level (FBS) was measured by the enzymatic colorimetric method with Olympus AU 600 auto analyzer using reagents from Olympus Diagnostics, (GmbH, Hamburg, Germany).

Glutathione Peroxidase (GSH-Px) Analysis

Following red cell lysis, deproteinization with sulphuric acid and sodium tungstate, the non-protein sulfhydryl group of erythrocytic glutathione is allowed to react with 5-5 dithiobis 2 benzoic acid (DTNB). A yellow anion is formed due to the reduction of DTNB with the sulfhydryl group of glutathione. The glutathione levels were obtained from a standard curve (R). Enzyme activities were expressed in micromol per gram of wet tissue (μmol/g tissue) [15].

Statistical analysis

The mean and standard deviation (SD) were calculated. Chi-square was used to compare the qualitative parameters among the groups. Unpaired student's t-test was used to evaluate the relation between the different indices and compare the two study groups. Pearson correlation coefficient was used to relate between age, onset and duration of disease, FBS, S-Cd, U-Cd, creat-U-Cd, sialic acid, GSH-Px, HbA1c and kidney function parameters namely urea, creatinine and microalbumin. A P value less than 0.05 was considered significant. Computer based statistical package

for social sciences (SPS) for windows 9.1 program was used.

Results

The study population consisted of a group of type 2 diabetic outpatient subjects, 17 (65.4%) males and 9 (34.6%) females, regularly attending the Diabetes Clinic of the Kasr Al-Aini hospital. The patients included in this group were chosen not to have any history of diabetes in the family in addition to other exclusion criteria. Accordingly, those cases with metabolic or inflammatory disorders; dyslipidemia, hypertension or obesity; cardiovascular or peripheral artery diseases; rheumatic diseases; renal, hepatic or thyroid dysfunction; concomitant medications or drug consumption; smoking and pregnancy were not included.

A control group, consisting of 31 non-diabetic subjects, 20 (64.5%) males and 11 (35.5%) females, was randomly selected from the University staff members, hospital employees and medical students. The referents were matched with the type 2 diabetic population by age, gender, body mass index (BMI) and socioeconomic standards. The ages of individuals in type 2 diabetes group ranged from 42 to 62 years with a mean value of 52.09 ± 6.05 years. Ages of the control group ranged from 46 to 63 years with a mean value of 56.34 ± 5.13 years, the difference between both groups was statistically non-significant.

Within the study population, different general characteristics in the form of residence, current occupation and feeding habits were evaluated. Data analysis revealed no statistically significant difference as regards the feeding habits. On the other hand, analysing the frequency distribution of residential areas and type of occupation revealed highly statistically significant differences between the type 2 diabetic and non-diabetic groups. Occupations included farming and nonfarming jobs. Farming was defined as being a farmer or a housewife who lived in a rural area. Nonfarming occupations included administrators, staff members, hospital employees, and women who lived in urban areas but reported their job title as housewife (table 1).

The study population was further subdivided into three sub-groups according to the serum level of cadmium (S-Cd) in an attempt to investigate the influence of environmental pollution with life style and status. The percentages of subjects with medium and high S-Cd were higher in rural areas, 11 (78.6%) and 6 (54.5%), respectively. However, higher percentage of those with low levels were found to live in urban areas, the difference between groups

was highly significant statistically (P<0.001). According to different occupations, farming was found to correlate to high levels of serum cadmium above 1.99 μg/dl, followed by housewives and industrial jobs and, to a lesser extent, administrative professions; the difference between diabetics and control groups was statistically significant. Contrarily, dietary habits did not reveal any significant difference between groups (table 2).

Estimation of blood urea, creatinine and urinary microalbumin was done to assess the effect of both cadmium and/or diabetes on renal functions. Though the levels were higher among type 2 diabetics than among control group, yet the difference was statistically non-significant. Measured urinary cadmium levels ranged from 0.11 to 0.59 μg/l with a mean value of 0.28 ± 0.16 μg/l among diabetic patients. As for the control group, urinary cadmium level ranged from 0.01 to 0.76 μg/l with a mean value of 0.24 ± 0.19 μg/l, the difference was statistically non-significant. Correction for urinary creatinine was done for the level of cadmium. Creatinine-corrected urinary cadmium level among the exposed population ranged from 0.03 to 1.39 μg/mg creatinine in urine with a mean value of 0.598 ± 0.46 μg/mg creat. After correction, the difference between diabetics and controls (mean 0.203 ± 0.197 μg/mg creat), proved to be highly significant (P<0.001) (table 3).

The level of cadmium in blood showed a highly statistically significant difference between type 2 diabetes and control groups with mean values of 3.65±1.17 and 0.65± 0.42 μg/dl, respectively (P<0.001). Serum glutathione peroxidase enzyme (GSH-Px) assessment revealed lower values among the diabetics with a mean of 0.39 ± 0.31 μmol/min/mg protein than among the controls (mean ± SD 0.97 ± 0.44 μmol/min/mg protein), the difference between both groups was statistically significant (table 3).

Though obesity is one of the exclusion criteria considered in the selection of subjects with type 2 diabetes, yet estimation of the body mass index (BMI) was done in order to assure the matching between groups and to correlate with cadmium levels, being a metal that stores in body tissues, skeletal muscles and bones. The body mass index, calculated as weight divided by squared height, was 25.59 ± 3.73 and 25.33 ± 4.29 kg/m^2 among type 2 diabetic and control groups, respectively; the difference was statistically non-significant. Similarly, blood pressure evaluation showed no statistical significance between groups (table 4).

As regards the group of type 2 diabetic patients, the disease was found to start at the age of 46.48 ± 5.18 years, duration of disease ranged 2.9-6.7 years (median 4.8 years; not presented). Fasting blood glucose (FBS) among this group ranged from 145.8 to 211.6 with a mean value of 185.14 ± 18.66 mg/dl and the difference from control group (mean value 81.62 ± 8.41 mg/dl) was highly significant statistically. Estimation of glycosylated hemoglobin (HbA1c) and sialic acid revealed higher levels among diabetic population than among controls, the difference proved to be significant for the former and highly significant for the latter (table 4).

Assessment of the relationships between the different variables in the study revealed positive non-significant correlations between the urinary cadmium (U-Cr) and the levels of urea, serum creatinine, urinary microalbumin, serum cadmium as well as glutathione peroxidase enzyme; and a highly significant correlation with the creatinine-corrected cadmium level in urine. Similarly, a highly significant correlation was obtained for the serum level of cadmium versus urea, serum creatinine, urinary microalbumin and glutathione peroxidase enzyme. Studying the association between GSH-Px and kidney functions revealed positive significant correlation (table 5).

Data related to the diabetic state of individuals were investigated among the study population particularly fasting blood glucose level, glycosylated hemoglobin and sialic acid, in addition to the relationship of age, body mass index, onset and duration of diabetes among type 2 individuals. According to the different associations evaluated, age showed a significant negative correlation with the level of sugar in blood, the percentage of glycosylated hemoglobin and the sialic acid. However, the body mass index was not correlating with any of the studied parameters except for the duration of diabetes. On the other hand, a significant correlation was obtained between the fasting sugar level in blood versus the onset and duration of diabetes among type 2 diabetic population and a highly significant one with the HbA1c and sialic acid, both of which are correlating together highly significantly (table 6).

In addition to the above mentioned significant associations with age, FBS and HbA1c, sialic acid was found to be significantly correlating with creatinine-corrected urinary cadmium and highly significantly with serum cadmium levels. However, the negative relation obtained between sialic acid and GSH-Px was a non-significant correlation. Evaluating the association between the cadmium levels in

urine and the creatinine-corrected urinary cadmium revealed no significant correlation with the different parametes except for the duration of diabetes. As for creatinine-corrected urinary cadmium, highly significant correlation was detected with FBS, HbA1c and duration of diabetes, while significant for sialic acid. Furthermore, highly significant positive correlation was detected for S-Cd versus FBS, HbA1c and sialic acid, significant for age, but non-significant for BMI, onset and duration of disease. Significant positive correlation was detected for the glutathione peroxidase enzyme versus duration of diabetes, however, all correlation studied with other parameters proved to be statistically non-significant (table 6).

Table (1): Frequency distribution of the different characteristics of study population (n=57)

		Type-II Diabetes Exposed Group (n = 26)		Non-diabetic Control Group (n = 31)		Chi-square	P value
		No	%	No%	%		
Residence	rural areas	18	69.23	5	16.13	16.5661	0.000*
	Urban areas	8	30.77	26	83.87		
Occupation	Housewife	5	19.23	6	19.36	18.5163	0.001*
	Farmer	16	61.54	2	6.45		
	Student	---	---	4	12.90		
	Industrial	3	11.54	5	16.13		
	Profession	2	7.69	14	45.16		
Dietary habits	Fruits	21	80.77	28	90.32	1.2432	N.S.
	Vegetables	26	100	31	100		
	Meat	19	73.08	30	96.77		
	Organ meat	8	30.77	16	51.61		

**Highly Significant P<0.005*
N.S. non-significant
Degree of freedom (df): (rows-1)(columns-1)

Table (2): Frequency distribution of the different Characteristics according to the level of cadmium in serum among the study population (n=57)

		Low Cd (n = 32) 56.14%		Medium Cd (n = 14) 24.56%		High Cd (n = 11) 19.30%		Chi-square	P value
		No	%	No	%	No	%		
Residence	rural areas	6	18.8	11	78.6	6	54.5	15.6213	0.001**
	Urban areas	26	81.2	3	21.4	5	45.5		
Occupation	Housewife	7	21.9	2	14.3	2	18.2	21.5879	0.01*
	Farmer	3	9.4	9	64.3	6	54.5		
	Student	4	12.5	---	---	---	---		
	Industrial	4	12.5	2	14.3	2	18.2		
	Profession	14	43.7	1	7.1	1	9.1		
Dietary habits	Fruits	29	90.6	11	78.6	9	81.8	1.0175	N.S.
	Vegetables	32	100	14	100	11	100		
	Meat	31	96.9	10	71.4	8	72.7		
	Organ meat	15	46.9	4	28.6	5	45.5		

Low Cd: Serum Cadmium Level (0 - 1.99 µg/dl);
Medium Cd: Serum Cadmium Level (2.00 - 3.99 µg/dl);
High Cd: Serum Cadmium Level (4.00 - 5.99 µg/dl);
**Significant P<0.05*
***Highly Significant P<0.005*
N.S. non-significant
Degree of freedom (df): (rows-1)(columns-1)

Table (3): Mean ± SD of the results of Different Laboratory Investigations among the Type-II Diabetes Group (n=26) and the Non-diabetic Control Group (n=31).

		Type-II Diabetes Exposed Group (n = 26)	Non-diabetic Control Group (n = 31)	t-test	P value
Urea	Range	32.6 - 84.2	14.7 - 50.1	1.059	N.S.
	Mean	58.258	25.419		
	SD	15.614	9.308		
S-creat	Range	0.96 - 4.16	0.56 - 1.07	1.135	N.
	Mean	2.259	0.807		S.
	SD	0.933	0.154		
U-micro-alb	Range	20.9 - 52.4	10.5 - 20.2	1.354	N.S.
	Mean	33.565	13.648		
	SD	8.735	3.339		
U-Cd	Range	0.11 - 0.59	0.01 - 0.76	0.379	N.S.
	Mean	0.279	0.238		
	SD	0.155	0.191		
Creat-U-Cd	Range	0.03 - 1.39	0.01 - 0.82	6.391	0.000**
	Mean	0.598	0.203		
	SD	0.461	0.197		
S-Cd	Range	1.23 - 5.82	0.06 - 1.52	7.340	0.000**
	Mean	3.651	0.645		
	SD	1.169	0.423		
GSH-Px	Range	0.03 - 0.99	0.12 - 1.69	2.061	0.044*
	Mean	0.388	0.971		
	SD	0.305	0.441		

Urea (mg/dl); S-creat: Serum Creatinine (g); U-micro-alb: Urinary Micro-Albumin (mg/24 hrs)
U-Cd Level of Cadmium in Urine (µg/l);
creat-U-Cd: Creatinine-corrected Urinary Cadmium Level (µg/g creatinine in urine);
S-Cd: Level of Cadmium in Serum (µg/dl); GSH-Px: Glutathione Peroxidase enzyme (µmol/min/mg protein)
** Significant P<0.05*
***Highly Significant P<0.005*
N.S. non-significant

Table (4): Mean ± SD of the results of Diabetes-related Laboratory Investigations among the Type-II Diabetes Exposed Group (n=26) and the Non-diabetic Control Group (n=31).

		Type-II Diabetes Exposed Group (n = 26)	Non-diabetic Control Group (n = 31)	t-test	P value
BMI	Range	20.9 - 29.4	17.9 - 13.4	0.811	N.S.
	Mean	25.592	25.332		
	SD	3.731	4.296		
Onset	Range	38.1- 52.8			
	Mean	46.485	---	---	---
	SD	5.176			
Duration	Range	2.9 - 6.7			
	Mean	4.662	---	---	---
	SD	1.059			
FBS	Range	145.8 - 211.6	70.8 - 102.3	5.096	0.000**
	Mean	185.138	81.616		
	SD	18.661	8.413		
HbA1c	Range	6.8 - 10.3	4.3 - 7.02	2.489	0.016*
	Mean	8.4	5.334		
	SD	1.035	0.719		
Sialic Acid	Range	70.2 - 92.3	48.1 - 74.8	4.577	0.000**
	Mean	82.8	61.684		
	SD	6.998	8.574		
Systolic BP	Range	110 - 135	110 - 125	1.738	N.S.
	Mean	121.50	118.50		
	SD	10.885	8.214		
Diastolic BP	Range	70 - 90	65 - 90	1.238	N.S.
	Mean	80.50	77.50		
	SD	6.898	6.216		

BMI: Body Mass Index (kg/m2); Onset: Onset of diabetes (years); Duration (years); Sialic acid (mg/dl) FBS: Fasting Blood Glucose Level (mg/dl); HbA1c: Glycosylated hemoglobin (% of total Hb); Systolic and Diastolic BP: Blood pressure (mmHg).

** Significant P<0.05*

***Highly Significant P<0.005*

N.S. non-significant

Table (5): Correlation Coefficient (r) of the results of Cadmium Level in Urine (U-Cr), Creatinine-corrected Urinary Cadmium Level (creat-U-Cr), Cadmium Level in Serum (S-Cr), and PGLPX versus Urea, Serum Creatinine, Urinary Micro-albumin as well as Serum, Urinary and creatinine-corrected Urinary Cadmium Levels among the Studied Population (n=57).

		Urea (n=57)	SCreat (n=57)	U-mic-Alb (n=57)	S-Cd (n=57)	U-Cd (n=57)	Creat-U-Cd (n=57)
U-Cd	r value	0.1882	0.0537	0.0737	0.2172	---	0.3876
	P value	0.1609	0.6914	0.5855	0.1046	---	0.003**
creat-U-Cd	r value	0.6319	0.6273	0.5378	0.7057	0.3876	---
	P value	0.000**	0.000**	0.000**	0.000**	0.003**	---
S-Cd	r value	0.7687	0.7588	0.7974	---	0.2172	0.7057
	P value	0.000**	0.000**	0.000**	---	0.1046	0.000**
GSH-Px	r value	0.3412	0.3438	0.3106	0.3768	0.0657	0.5158
	P value	0.009*	0.009*	0.0187*	0.004**	0.6274	0.000**

Urea (mg/dl); SCreat: Serum Creatinine (g); U-micro-Alb: Urinary Micro-Albumin (mg/24 hrs)
U-Cd Level of Cadmium in Urine (µg/l);
creat-U-Cd: Creatinine-corrected Urinary Cadmium Level (µg/g creatinine in urine);
S-Cd: Level of Cadmium in Serum (µg/dl); GSH-Px: Glutathione Peroxidase enzyme (µmol/min/mg protein)
** Significant P<0.05*
***Highly Significant P<0.005*

Table (6): Correlation Coefficient (r) of the duration and onset of diabetes among Type-II diabetes Group (n=26), Age, Body Mass Index (BMI), Fasting Blood Glucose Level (FBS), Glycosylated Hemoglobin (HbA1c) and Sialic Acid among the studied population (n=57) versus Age, BMI, FBS, HbA1c, Sialic Acid, Cadmium Level in Serum (S-Cr), and Urine (U-Cr), Creatinine-corrected Urinary Cadmium (creat-U-Cr) and PGLPX among the Diabetic and Non-Diabetic studied population (n=57).

		Duration (n=26)	Onset (n=26)	Age (n=57)	BMI (n=57)	FBS (n=57)	HbA1c (n=57)	Sialic (n=57)
Age	r value	---	0.9385	---	-0.1564	-0.2786	-0.3102	-0.2941
	P value	---	0.000**	---	0.2454	0.036*	0.019*	0.026*
BMI	r value	-0.5073	-0.1989	-0.1564	---	0.0668	0.0106	-0.2104
	P value	0.008*	0.3198	0.2454	---	0.6215	0.9377	0.1162
FBS	r value	0.4020	0.4854	-0.2786	0.0668	---	0.8688	0.7588
	P value	0.042*	0.01*	0.036*	0.6215	---	0.000**	0.000**
HbA1c	r value	0.1420	-0.0954	-0.3102	0.0106	0.8688	---	0.6928
	P value	0.4888	0.6361	0.019*	0.9377	0.000**	---	0.000**
Sialic acid	r value	-0.3020	0.015	-0.2941	-0.2104	0.7588	0.6928	---
	P value	0.1337	0.9403	0.026*	0.1162	0.000**	0.000**	---
U-Cd	r value	0.4576	0.1679	-0.0305	-0.071	0.1294	0.0615	0.0459
	P value	0.018*	0.4024	0.8217	0.6049	0.3375	0.6497	0.7346
Cr-U-Cd	r value	0.7122	0.0745	-0.0589	-0.1668	0.5383	0.5694	0.2865
	P value	0.000**	0.7121	0.6631	0.2149	0.000**	0.000**	0.031*
S-Cd	r value	0.3469	-0.1869	-0.3497	-0.0675	0.8203	0.7544	0.6183
	P value	0.0824	0.3504	0.008*	0.6176	0.000**	0.000**	0.000**
GSH-Px	r value	0.4319	-0.1873	-0.0136	-0.1331	0.0463	0.0834	-0.1649
	P value	0.028*	0.3496	0.9203	0.3236	0.7322	0.5375	0.2203

Duration (years); Age (years); BMI: Body Mass Index (kg/m2); Onset: Onset of diabetes (years); Sialic acid (mg/dl); FBS: Fasting Blood Glucose Level (mg/dl);
HbA1c: Glycosylated hemoglobin (% of total Hb);
Urea (mg/dl); SCreat: Serum Creatinine (g); U-micro-Alb: Urinary Micro-Albumin (mg/24 hrs)
U-Cd Level of Cadmium in Urine (µg/l);
cr-U-Cd: Creatinine-corrected Urinary Cadmium Level (µg/g creatinine in urine);
S-Cd: Level of Cadmium in Serum (µg/dl); GSH-Px: Glutathione Peroxidase enzyme (µmol/min/mg protein)
** Significant P<0.05*
***Highly Significant P<0.005*

Discussion

Cadmium is a biotoxic heavy metal that has been recognized as one of the most toxic environmental pollutants in agricultural soils because of the potential adverse effects it may pose to food quality, soil health, human health and the environment [16]. In the cell, cadmium (Cd) mainly accumulates in the cytosol followed by the nucleus and is lowest in the mitochondria and endoplasmic reticulum of hepatocytes[17], cells of proximal and distal renal tubules and glandular cells of the pancreas [18].

It has been suggested that Cd may induce oxidative stress by producing hydroxyl radicals [19], superoxide anions, nitric oxide and hydrogen peroxide [6]. Moreover, cadmium has been recognized as an ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in the tissues [20,21]. Studies have shown that Cd inhibits the activity of majority of enzymes involved in the antioxidant system [7] by binding to their sulfhydryl groups or by inhibiting the protein synthesis [6]. Therefore, inhibition of GSH-Px enzyme, which has an important role in detoxification of xenobiotics, exposes the cells to redox cycling and oxidative stress [9,22].

Oxidative stress has been shown to play an important role in the pathogenesis of type 2 diabetes, being an important pathogenic constituent in diabetic endothelial dysfunction [23]. Pancreatic beta-cell function continuously deteriorates in type 2 diabetes despite optimal treatment regimens, which has been attributed to hyperglycemia itself and formation of excess levels of reactive oxygen species (ROS). Extreme decrease in the level of glutathione peroxidase in beta cells removes the protection against oxidative stress [24]. Recently, an acute phase reactant named sialic acid detected in serum of diabetics, was found to play an important role in the removal of reactive oxygen species [12]. However, it is still discussed whether oxidative stress precedes or merely reflects diabetic complication [23].

The present study was carried out to investigate the possibility for development of type 2 diabetes as a result of environmental exposure to cadmium with special emphasis on the role of the inflammatory response and oxidative stress. The importance of sialic acid was considered as an early indicator of cadmium-induced serious effects on the pancreas. Accordingly, a group of 26 previously diagnosed type 2 diabetic patients, without a family history of the disease to avoid genetic predisposi-

tion, were selected. Another group of non-diabetic subjects including University staff members, hospital employees and medical students was considered as a control group matched with the exposed population by age, gender, body mass index (BMI) and socioeconomic standards.

Smoking has been considered as one of the exclusion criteria in this study because smoking has been identified as a strong risk factor for pancreatic disease [25], and in addition cadmium is a by-product of cigarette smoke, and adjusting for serum cadmium levels would have been required [26]. Additionally, ex-smokers were not included as cadmium accumulates in the body, and even after exposure ceases, the concentration in the blood never returns to pre-exposure levels but continues to influence blood levels [27].

Cadmium (Cd) is a known environmental and industrial pollutant that affects various organs in humans and animals [28]. Liver, kidney, lung, testes, and heart are the target organs, with the severity of intoxication dependent on the route, dose, and duration of the exposure to the metal [17]. Only a few studies are available regarding Cd-induced oxidative stress in animals, but no reports are available regarding the effects of Cd on oxidative stress during occupational exposure [29]. Accordingly, studying the characteristics that influence the incidence of tissue affection concentrated on the role of residence, current occupation and dietary habits on beta cells of the pancreas causing diabetes mellitus.

Data analysis revealed a highly significant statistical difference in the incidence of type 2 diabetes between rural and urban areas, 69.23% (18) and 30.77% (8), respectively. However, certain epidemiologic studies failed to prove any significant association between the risk of beta cell injury and residence [30], though they expected rural areas to show increased risk of pancreatic affection because of pesticides, fertilizers and heavy metal exposure. A recent study reported an association with farming and other farming-related occupations reflecting intense exposure to cadmium [2]. These data are consistent with the results obtained in the current study that showed 61.54% (16) of patients with type 2 diabetes to be involved in farming jobs.

Hence, the study population was further divided depending on serum cadmium level. Previous investigations showed that, although about 10% of whole-blood cadmium is circulating in serum, serum levels correlate with blood levels [31]. Cadmium in whole blood has been used as a biologi-

cal indicator reflecting recent exposure. The groups formed consisted of low (0-1.99 µg/dl); medium (2.00-3.99 µg/dl) and high Cd levels ranging from 4.00 to 5.99 µg/dl,, thus exceeding the toxic level. OSHA recently considered 5 µg/l of whole blood cadmium as a toxic level at which further medical surveillance is required [32]. Analysis of life characteristics accordingly revealed significant associations, not only with farming but also with residence in rural areas. However, another study carried out in Egypt on markers of environmental pollution showed significantly high incidence of pancreatic diseases in urban population. They related the results to important differences that distinguish urban from rural regions as regards cadmium level in air and diet [33].

Reports from urban areas in Egypt have shown high levels of cadmium in organ meats that are frequently consumed by the local population. The high dietary intake of rice and fish grown in polluted soils and water were other additional risk factors [34]. Analysis of present data showed no significant difference between type 2 diabetes and non-diabetics in relation to different cadmium levels as regards the dietary habits in the study population. Unfortunately, neither detailed environmental exposures nor precise dietary intake was considered.

Cadmium has been recognized as one of the most toxic environmental pollutants [17], that interfere with the use of essential metals such as calcium, zinc, selenium, and iron. Deficiencies of these essential metals, in conjunction with protein and vitamin deficiencies, exaggerate cadmium toxicity by increasing absorption through the gut and enhancing retention in different organs [35]. Though cadmium in blood increases in correspondance to the intensity of exposure, yet nutritional deficiencies may be major factor in its accumulation in body organs [2].

Accumulation of cadmium in the body influences blood levels; it starts to decrease with an initial half-time of 2 to 3 months after exposure stops. Accordingly, blood cadmium has been proposed as one of the more accurate estimators of accumulated body burden [27]. As a perfect dose estimator for cadmium is not available, cadmium levels were estimated both in serum and in urine in the present study. Analysis of detected values revealed a highly significant statistical difference between type 2 diabetic group and non-diabetics in serum cadmium (S-Cd), but on the contrary, the difference in urinary cadmium (U-Cd) appeared non-significant statistically. Urinary cadmium levels, however, are still often used, though studies in

animals and humans have shown that renal damage may lead to higher than normal cadmium excretion [26]. Adjusting for serum creatinine in this study was able to control for the effect of kidney affection and it revealed a difference that proved to be highly significant. For this reason, S-Cd was reported to provide a better dose estimate than urinary cadmium concentrations, especially when tubular proteinuria is present [37].

Microalbuminuria was recently considered a marker of early renal effects from cadmium exposure [38]. Conventional indicators of renal function such as total urinary protein, serum urea and creatinine are considered insensitive indicators of early renal dysfunction, but may indicate the progression of cadmium-related damage [39]. In the current study, however, a highly significant positive correlation was obtained, not only with micro-albuminuria but also with urea and creatinine versus serum and urinary cadmium corrected for creatinine. Estimating the urinary microalbumin besides urea and creatinine among groups revealed non-significant difference between the groups indicating the absence of any renal impairement. These results could be attributed to short duration of diabetes following the diagnosis of disease ranging from 2.9 to 6.7 years with a median of 4.8 years. Epidemiologic reports about type 2 diabetes indicated that despite modern diabetes treatment and self-monitoring of blood glucose, some patients still might develop signs of nephropathy during the first 10 years of diabetes but with a median of 9 years [38].

Analysis of current results in relation to diabetes, revealed elevation above normal levels in fasting blood sugar among type 2 diabetes population showing a statistically significant difference compared to the controls. These patients had worse glycemic control as evidenced by the mean weighted HbA1c (8.4 ± 1.04 vs 5.3 ± 0.72%; P=0.016). The studied cases with type 2 diabetes were found to be within the highest quartile of HbA1c values described in literature (range from 8.2-11.2 %) [38]. As in previous studies, the present work indicates that insufficient glycemic control is an important risk factor for development of microalbuminuria as evidenced by the highly significant positive association for both FBS, r =0.7672 and HbA1c, r =0.8211 (P <0.001).

Therefore, microalbuminuria might be considered the earliest sign for the development of renal complications associated with diabetes. However, certain patients with type 2 diabetes developed microalbuminuria rapidly despite reasonable gly-

cemic control, indicating the importance of other risk factors [38]. The development of diabetes and its complications, attributed to the nonenzymic glycation of tissue proteins, has recently been attributed to the possible role of free radicals [40]. Evidently, updated researches described continuous deterioration of pancreatic beta-cell function in type 2 diabetes because of excess reactive oxygen species as a result of hyperglycemia [24]. Additionally, cadmium was found to augment reactive oxygen species levels in beta cells of pancreas and to interfere with the antioxidant defense system, although cadmium does not directly induce oxidative stress [41,42]. Increased serum amylase level was a suggested bioindicator for pancreatic function in cadmium exposure [43].

The suspicion of an association between cadmium exposure and diabetes was proved by the significant statistical correlation obtained between serum cadmium versus fasting blood sugar and HbA1c, thus indicating the possible role of environmental pollution in the pathogenesis of diabetes. A highly significant positive association was also detected between hyperglycemia and the level of urinary cadmium after correction for creatinine. In fact, early experimental studies showed that cadmium accumulation in beta cells of pancreas increase the pancreatic metallothionein levels [44]. The Cd-metallothioneins compete with GSH-Px for sulfhydryl groups in aminoacids, thus decreasing their activity [6].

The level of glutathione peroxidase, which is by virtue of its ability to catabolize both H^2O^2 and lipid peroxides to protect tissues from reactive oxygen species, appeared extremely low [24]. Therefore, data are accumulating to link alteration and abnormality of GPH-Px expression to etiology of diabetes [45]. In fact, extracellular glutathione peroxidase (GSH-Px) was found to exhibit progressive reduction with microalbuminuria, and was even considered a potential biomarker for the diagnosis of type 2 diabetics [46]. Currently, the level of glutathione peroxidase enzyme appeared to be significantly lower in the type 2 diabetes patients than the controls and correlated significantly with microalbuminuria and diabetes duration ($P < 0.05$).

Prospective studies have reported associations among various markers of inflammation and incidence of diabetes and it has been proposed that inflammation has a causal role in the development of diabetes [47]. To date, a clear role for inflammation in the development of type 2 diabetes has been proved. Circulating inflammatory

markers such as C-reactive protein, interleukin-6 and sialic acid have been recognized as significant independent predictors of the disease [48].

Plasma sialic acid, one of the markers of acute phase response, is a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids [11]. Studying the normal physiology of subjects and the relation of sialic acid levels to age revealed that plasma sialic acid is higher among the younger than older generations [19]. However, no significant relation could be detected either with the body mass index or with the onset and duration of diabetes. This is similar to other researches that considered sialic acid independent of diabetes duration [1]. Other studies, though, were able to utilize the percentage of body fat as a prediction for plasma sialic acid concentration in diabetes [13].

Sialic acids were found to be vital biomarkers for some diseases particularly diabetes [12]. This was supported by the present study via detection of highly significant elevation of plasma sialic acid among type 2 diabetics compared to controls. Additionally, the data obtained appeared to correlate significantly with fasting blood sugar and degree of metabolic control as estimated by the glycosylated hemoglobin (HbA1c). Other research findings suggested the association between sialic acid and diabetic complications through the acute phase inflammatory response induced by oxidative stress [1].

This study was able to detect a significant positive association between sialic acid and urinary cadmium after being corrected for creatinine and a highly significant correlation with cadmium levels in serum. To our knowledge, no available researches have studied the relationship and effects of cadmium exposure on sialic acid. Recently, sialic acid was found to be over-excreted in saliva and to have an important role in the removal of hydrogen peroxide. Besides, the increase in sialic acid has been found to be in accordance with the increase in antioxidant enzymes. Accordingly, the sialic acid was considered an alternative oxidative stress marker [12], and was recently reported to be an essential moiety to scavenge hydroxyl radical [14].

Surprisingly, a negative association was detected between glutathione peroxidase and sialic acid. This non-significant correlation might be attributed to the decreased activity of glutathione by the accumulated cadmium, evidenced by lower GSH-Px levels among type 2 diabetes group than controls, which is not the case

with sialic acid. These observations suggest, in accordance with other studies [12], that sialic acid is more efficient than GSH-Px in the decomposition of free radicals and as a bio-indicator in cases of diabetes mellitus induced by cadmium exposure.

Worth mentioning is the fact that, in updated research, glutathione and lipid peroxidation levels did not significantly change in response to cadmium, and the overall redox balance remained stable. This stability suggested that an increased formation of superoxide anions upon Cd-induced mitochondrial free radical generation, was not apparent. Additionally, a second defense activation was observed because of increased glutathione peroxidase providing evidence of a biphasic defence activation that might lead to adaptation and survival [50].

In conclusion, oxidative stress that is characterized by the generation of reactive oxygen species is nowadays considered one of the many risk factors for the type 2 diabetes mellitus. Oxidative stress is therefore an important pathogenic constituent in diabetic endothelial dysfunction. Among the most important causes appears the inflammatory response and lipid peroxidation affecting the beta cells of the pancreas, induced by the accumulation of heavy metals mostly cadmium. The oxidative stress induced by chronic cadmium exposure was found to be evaluated by changes in the amount of lipid peroxides and changes in antioxidant enzyme activities of glutathione peroxidase levels, though results are inconsistent. On the other hand, estimation of the more valuable sialic acid in saliva might be used as non-invasive specific diagnostic index in those patients with diabetes exposed to cadmium.

Unfortunately, cadmium seems to be seriously contaminating the soils and water in the region, therefore accounting for the high incidence of type 2 diabetes among the general population. Other contributing factors include residence, occupation and above all the nutritional deficiencies that favor the accumulation of the metal in the pancreatic cells. However, much work remains to be done to explain how minerals are involved in physiological changes. The specific reasons for this association are still unclear and the relatively small sample size may limit the generalization of results. Therefore, studies should further investigate the etiologic relationship between cadmium exposure and type 2 diabetes. Future research should focus on the mechanism of glutathione peroxidase in the pathogenesis and potential applications of GSH-Px manipulation in the treatment of diabetes. Additionally,

further studies are required to quantify the precise role of sialic acid as a vital biologic marker for cadmium-induced diabetes.

Recommendations

1- Prevention is the key to managing cadmium exposure as no effective treatment for cadmium toxicity exists. A national pollutant discharge and elimination system and general pretreatment regulations should be considered overseeing regulations and guidelines applicable to cadmium. Protection of human health is to be undertaken through air quality planning and through programs applying the standards of drinking water, solid waste and cadmium-containing pesticides.

2- At the work place, the permissible exposure limits for occupational exposures to cadmium set by the Occupational Safety and Health Administration should be adopted. Cadmium waste should be properly managed and recycled on-site and off-site.

3- It is important that once potential exposures are identified, an exposure assessment is conducted. During the preemployment, periodic and preretirement examinations of workers at risk of cadmium exposure, analysis of the metal in blood is indicated.

4- Estimation of essential elements such as calcium, zinc, selenium and iron is also recommended as deficiencies of these essential metals in conjunction with protein and vitamin deficiencies exaggerate toxicity. Therefore, health promotion and nutritional education of general population should be considered to guard against environmental pollution and metal toxicity.

5- A better understanding of the molecular mechanisms by which cadmium may influence pancreatic cells is recommended to study genetic susceptibility and markers of genetic predisposition and to clarify the role of such exposures in diabetes.

6- Additionally, estimation of sialic acid in saliva is indicated for cadmium-exposed workers and for patients diagnosed as type 2 diabetes, being as an efficient oxidative stress marker. Increased serum amylase level could be used as an additional bio-indicator for pancreatic function in cadmium exposure.

7- As glutathione peroxidase reduction was observed as a result of cadmium exposure, glutathione peroxidase mimetics [24], recently suggested may represent a valuable ancillary treatment

that could add a novel layer of protection for the beta cell of pancreas against xenobiotics.

8- Selenium supplementation should be considered as a major antioxidant trace element since it is the co-factor of glutathione peroxidase with some protective role from the toxic actions of cadmium and other heavy metals. More light is to be shed on the relationship between selenium, with its insulin-mimic properties [51], and cadmium-induced type 2 diabetes.

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