ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Lead Toxicity in Relation to Diabetes Mellitus and Chronic Renal Failure

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Manuscript Info

Manuscript History:

Received: 12 November 2013
Final Accepted: 29 November 2013
Published Online: December 2013

Key words:
Lead, Diabetes Mellitus (DM),Chronicrenal failure(CRF), Antioxidant Enzymes, Super oxide dismutase (SOD), Nitric Oxide (NO).

Abstract

The present study was designed to investigate the toxic effects of lead and diabetes mellitus on chronic renal failure. . Exposure to lead can have deleterious effects on multiple organ systems, including the nervous, hematopoietic, renal, endocrine, and reproductive systems. Chronic kidney disease (CKD) is an increasing public health problem worldwide. We have determined the levels of lead in blood samples of DM and CRF patients. We also have estimated the levels of HBA1c, glucose, urea, creatinine, creatinineclearance and antioxidant enzymes (SOD, catalase, NO, Gluthathione peroxidase) by using kit method.The result shows that lead levels are higher in DM and CRF patients as compared to control. Urea, creatinine and creatinineclearance levels are high in DM patient with CRF. Glucose and HBA1c levels are higher in DM and DM with CRF patients as compared to control. The activities of antioxidant enzymes were decreased in DM and DM with CRF patients. The results indicate that lead exposure with DM can be the cause of CRF.

Introduction

More than 7000 different industries are working in three industrial zones of Karachi region discharging their effluents up to 75-100 thousand m^3 daily through Lyari River and Malir River across the city to the Arabian Sea damaging the quality of underground water of the city and the coastal area. Although there are few small treatment plants which are totally insufficient (Khan and Ahmed, 2001). Heavy metals get into water from many sources, including industries, automobile exhaust, mines, wastes, and even natural soil. Like pesticides, heavy metals become more concentrated as animals feed on plants. When they reach high levels in the body, heavy metals can be immediately poisonous, or can result in long-term health problems (Khan et al., 2004).

Lead is heavy metal with a specific gravity of 11.34 and atomic weight of 207.21. It is bluish in color that tarnishes to dull gray. It is used in making bullets due to its high density and minimum air resistance, in construction because it resists corrosion, in chemical industry due to its immunity to acids and as protective shielding of x-rays machines. Normal daily intake of lead is 0.3mg but daily intake of 3.5 mg taken for a few months results in toxicity (Ahmed et al., 2013). Drinking water supplied to the distribution system is essentially free of lead up to and through the main lines, which typically run down the middle of city streets under the pavement. Lead is subject to leach under the certain condition of corrosivity (Guidotti et al., 2007). Lead-containing particles>2.5µm in diameter are deposited in the ciliated regions of the nasopharyngeal and tracheo-bronchial airways, where they ~~are~~ passed to the gastrointestinal tract then subject to intestinal absorption. Lead circulates widely and is found in all organs and tissues; it also crosses the blood- brain barrier and placenta, making the brain and developing fetus among the targets of concern (Hu et al., 2006).

Exposure to lead can have deleterious effects on multiple organ systems, including the nervous, hematopoietic, renal, endocrine, and reproductive systems (Morales et al., 2005). The incidence of kidney failure is increasing, and

will nearly double over the next decade as the population ages (Xue et al., 2001). Chronic kidney disease (CKD) is an increasing public health problem worldwide, resulting in substantial morbidity and mortality related to cardiovascular disease and end-stage renal disease (Coresh et al., 2007). Established risk factors for CKD include diabetes, hypertension, and metabolic syndrome (Ryu et al., 2009).Many previous studies have documented that individuals with type 1 diabetes have an increased age-adjusted mortality risk of death compaired to the general population. This excess mortality risk in large part results from the development of chronic complications, particularly renal disease (RD) (Orchard et al., 2010). Diabetic nephropathy, followed by various forms of ischemic renal disease and primary and secondary glomerulopathy, chronic tubulointerstitial nephritis and autosomal dominant polycystic kidney disease are the leading causes of CRF (Zadrazil, 2011).

Type II diabetes and diabetic nephropathy are clearly chronic progressive diseases that are associated with a combination of genetic, lifestyle and environmental factors (Russo et al., 2010). Late complications of DM are the leading cause of adult blindness and end-stage renal disease in the western world, and a major contributor to cardiovascular, cerebrovascular and peripheral vascular disease (PVD). These complications are mainly vascular in nature and may be loosely classified as either microvascular or macro-vascular complications include diabetic retinopathy (DR), nephropathy (DN) and neuropathy, while macrovascular complications are represented by coronary artery disease (CAD), PVD and cerebrovascular disease (Szafranek et al., 2002).The global burden of DM is rising; the prevalence is estimated to reach 438 million by 2030, and more than 80% of the adult cases will be in newly developed or developing countries (Baker et al., 2011).

Currently, the susceptibility to toxic hazards in populations at high risk is of increasing concern. In such high-risk groups, diabetes is one condition that can be suspected of increasing the susceptibility to toxicants (Chen et al., 2006). Multifactorial interventions have been effective in reducing the risk of non-fatal and fatal CVD,CKD among diabetic patients through therapy targeting hyperglycaemia, hypertension and hypercholesterolaemia (Chamnan et al., 2009). There are twoincretin hormones known as glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). In type 2 DM, GIP no longer modulates glucose-dependent insulin secretion and there are modest but significant reductions in meal-stimulated circulating levels of GLP-1. EXE is a functional analog of human GLP-1 that binds to and stimulates GLP-1 receptors, thus increasing insulin secretion. Mechanisms by which EXE improves glycemic control include the regulation of glucose-dependent insulin secretion, the suppression of inappropriately high glucagon secretion, the slowing of gastric emptying (which reduces the rate at which meal-delivered glucose appears in the circulation) and the reduction of food intake (Robles and Franco, 2009).

Experimental

This is a prospective, cross sectional and comparative study which has been conducted on patients with diabetes mellitus type 2, diabetes mellitus with CRF and controls. A total of 150 adult (age > 40 years) who were taken as subjects, 50 were diagnosed patients of diabetes type 2, 50 were diagnosed patients of diabetes type 2 with chronic renal failure and 50 were normal healthy individuals. For this study patients would be selected from the areas under heavy water pollution by the toxic metals.The blood samples will be collected with the collaboration of Jinnah post graduate medical centre and Kidney centre Karachi. .

The subjects were selected after taking written consent, detailed history and examination. Patients suffering from other endocrinal disorders, hepatic disease, alcoholism or other drug abuse and in case of female patient, having pregnancy and using oral contraceptive pills were excluded.

Strictly predefined protocol was used for specimen preparation. Blood was collected in a Gel Barrier Silicone coated Neotube from Nipro Japan. The additive free tubes were kept at room temperature until clotting was complete. Those samples which showed signs of haemolysis were discarded. Samples were centrifuged at 3000 rpm for 10 minutes within one hour of collection; serum was separated and stored in aliquots in deep freezer at -20°C until assayed. Each bottle was labelled with subject identification number. Samples were analysed in one run at the end of the study. Before analysis, the serum samples were allowed to reach room temperature.

Plasma glucose was assayed using the glucose oxidase method (Tu et al., 2013).Diazyme Direct Enzymatic Hemoglobin A1c (HbA1c) use in the quantitative determination of HbA1c in human whole blood samples. Measurement of hemoglobin A1c is a valuable indicator for long term diabetic control (Hoelzel et al., 2004). For the HbA1c assay used, high correlation of results is reported when compared to clinical standard methods. The urea was assayed enzymatically by the improved Jung method utilizes a chromogenic reagent that forms a coloured complex

specifically with urea. The intensity of the colour, measured at 520nm, is directly proportional to the urea concentration in the sample (Owiredu et al., 2013).Creatinine test is most widely used to assess kidney function. Creatinine concentration is estimated by jaffe method. The improved Jaffe method utilizes picrate that forms a red colour complex with creatinine. The intensity of the colour, measured at 510nm, is directly proportional to creatinineconcentration in the sample (Owiredu et al., 2013). Creatinine clearance (CCr) is calculated from the creatinine concentration in the collected urine sample (UCr), urine flow rate (V), and the plasma concentration (PCr). Creatinine clearance is calculated as removal rate per min (UCr×V) divided by the plasma creatinine concentration (Owiredu et al., 2013). Atomic Absorption Spectrophotometer of Perkin Elmer model AAnalyst 700 2003, at recommended wavelengths for metal ion was used as per standard procedure published by the American Public Health Association for the examination of blood samples (Liua et al., 2012; Alves et al., 2011). The SOD (Gecit et al., 2012), catalse (Besten et al., 2013), NO (Gecit et al., 2012)and Glutathione peroxidase (Raffa et al., 2012; Staimer et al., 2012) activities were analysed with Randox kits and were evaluated with a Shimadzu Spectro Photometer machine.

Statistical Analysis:

The data entry and statistical analysis was done on SPSS (Statistical Packages of Social Sciences) version 11.0. A descriptive analysis of continuous variables was performed. Data on continuous variables i.e. glycemic status [fasting blood sugar (mg/dl), HbA1c (%)], renal function [urea (mg%), creatinine (mg%), creatinine clearance (ml/min)], toxic metals in serum like lead (ug/dl) and antioxidant enzymes [superoxide dismutase (U/ml), catalse (U/ml), glutathione peroxidase (Ug/Hb), Nitric acid (umol/L)] was presented as Mean ± SD. Statistical comparison was performed by using ANOVA with tukey's test for continuous variables. In all statistical analysis only p-value <0.05 will be considered significant.

Results

During the study, we examined 150 blood samples obtained from different age and gender.

Fig 4 shows that many blood samples had lead concentration higher than the maximum acceptable limit (MAC) in blood, established by W.H.O (1.5ug/dl). DM and DM with CRF patients have significantly higher ($p<0.001$) lead levels as compared to controls.

Fig – 1(a):Correlation between lead and creatinine in diabetes patients

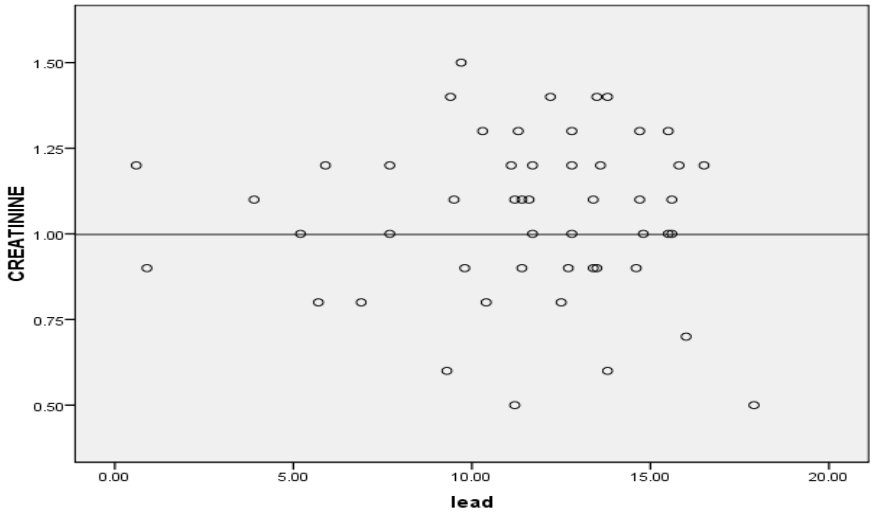

Fig – 1(b)Correlation between lead and creatinine in diabeteswith CRF patients

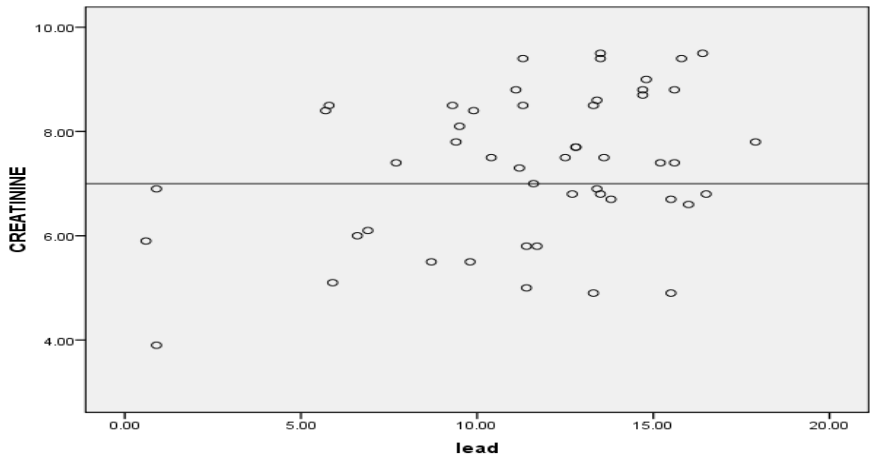

Fig – 2(a) Correlation between lead and HbA1C in diabetes patients

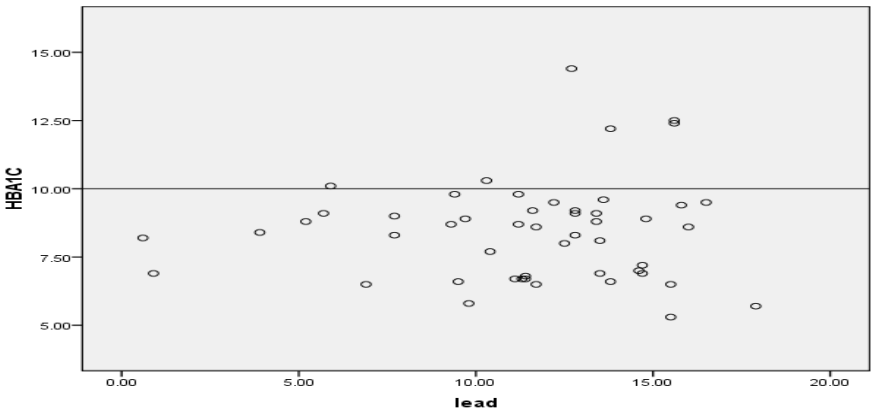

Fig – 2(b) Correlation between lead and HbA1C in diabetes with CRF patients

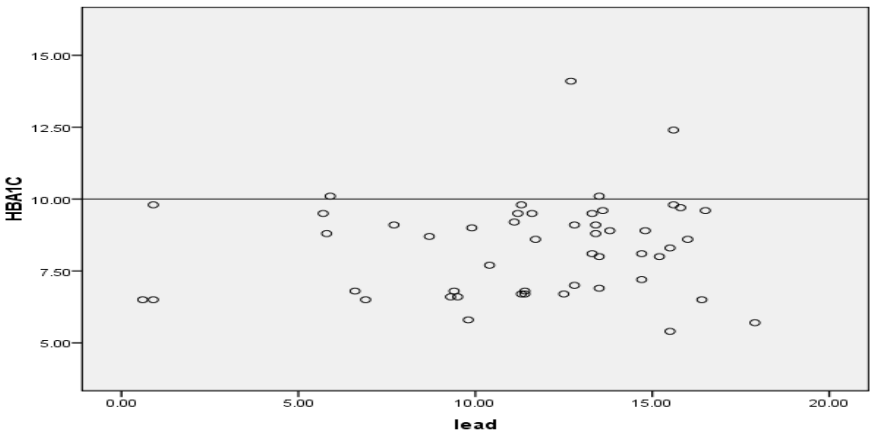

Fig. 3 . Levels of Lead in Tap and Bore Sources of Water in different regions of Karachi

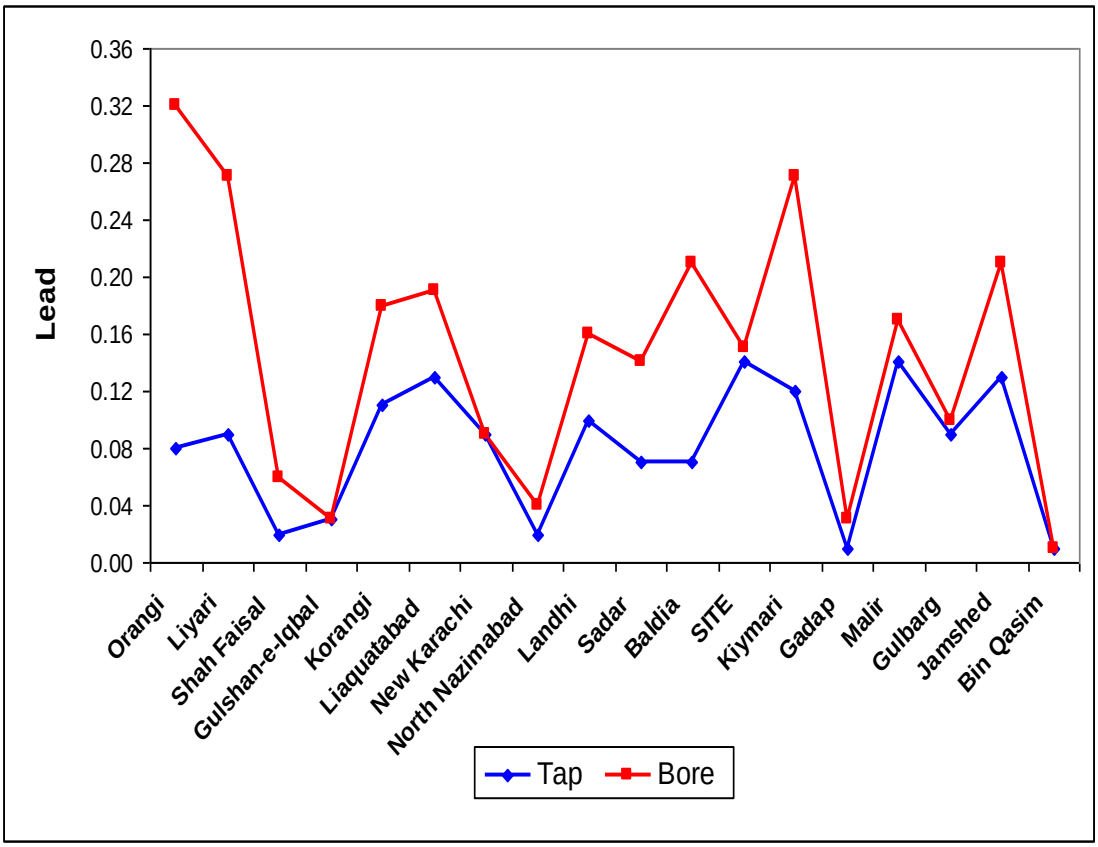

Fig. 4 Biochemial Level of Lead (µg/dl) in patients and controls

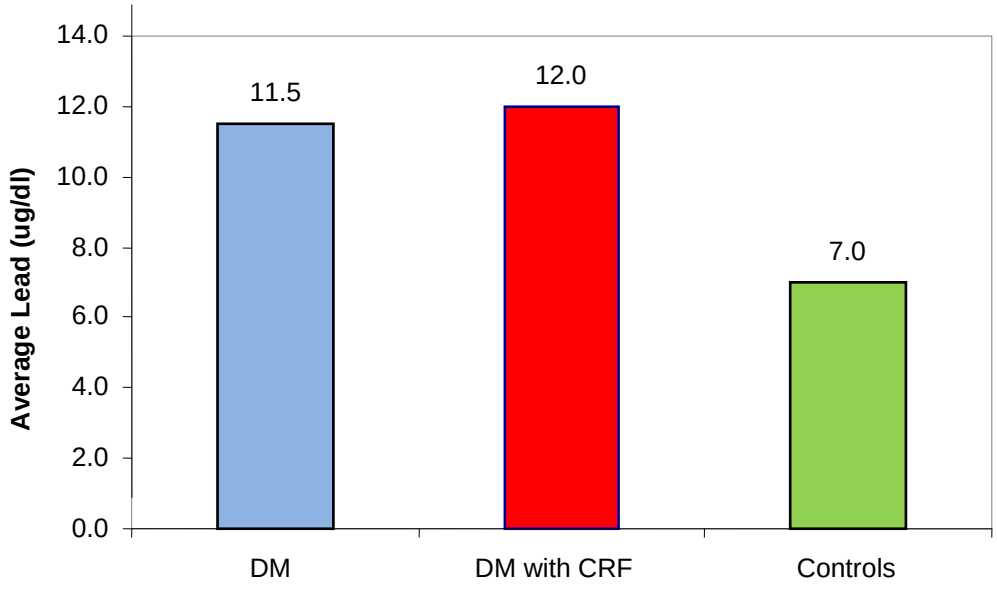

Table 1 show that levels of fasting blood sugar (197.4 ± 51.93) and HbA1c (8.3 ± 1.69) in DM with CRF patients were almost similar to Type II diabetic patients. Fasting blood sugar and HbA1c in Type II diabetic with CRF patients were significantly ($p<0.01$) high as compared to controls.

Table 2 shows that levels of urea (125.2 ± 32.49) and creatinine (7.3 ± 1.41) in DM with CRF patients were significantly high ($p<0.01$) as compared to hypertensive and controls. Creatinine clearance (55.1 ± 9.61) in DM with CRF patients were significantly less ($p<0.01$) as compared to hypertensive and controls. Lead were significantly high ($p<0.01$) (11.5 ± 4.06) in DM with CRF and (11.4 ± 3.82) in type II diabetic patients as compared to controls.

Table 3 shows that levels of antioxidant enzymes such as superoxide dismutase were significantly ($p<0.01$) less (120.9±16.28) in DM with CRF and (122.9±16.32)in hypertensive as compared to controls, Catalase were significantly ($p<0.01$) less (3.7±1.29) in DM with CRF as compared to type II diabetic and controls, Glutathione peroxidase were significantly less ($p<0.01$)in (39.0 ± 11.21) DM with CRF and (42.7 ± 11.48) in type II diabetic as compared to controls. Nitric oxide were significantly ($p<0.01$) less (10.8 ± 3.07) in DM with CRF as compared to hypertensive and controls.

Table 4 shows the correlation between Lead and glycemic index (FBS, HBA1c) and renal parameters (Urea, Creatinine and Creatinineclearance) in DM and DM with CRF patients. Analysis have shown that lead has significant correlation with glycemic index ($p<0.01$) in both DM and DM with CRF, while correlation with renal failure was significant ($p<0.01$) only in DM with CRF patients. Correlation between lead and creatinine and HBA1c were presented respectively in figure 1 (a,b) and figure 2(a,b) in DM patients (fig 1a and 2a) and in DM with CRF patients (fig 1b and 2b).

Table 5 shows the correlation between Lead and antioxidant enzymes in DM and DM with CRF patients. Pearson correlation analysis shows that lead has significant ($p<0.01$) correlation with SOD in both DM and DM with CRF patients while catalase ($p<0.05$), Glutathione peroxidase ($p<0.05$),nitric oxide ($p<0.01$) have significant correlation with lead in DM with CRF patients but in DM patients it is insignificant.

Tab. 1. Comparison of glycemic status in DM and DM with CRF and controls

GLYCEMICSTATUS	TYPE II DIABETIC (n=50)	DM WITH CRF (n=50)	CONTROLS (n=50)	p-VALUE
FASTING BLOOD SUGAR (mg/dl)	201.4 ± 58.50 *	197.4 ± 51.93 *	100.8 ± 15.47	0.001
HbA1c (%)	8.4 ± 1.84 *	8.3 ± 1.69 *	5.5 ± 0.94	0.001

* As compared to controls $p<0.01$

Tab. 2. Comparison of renal function in DM and DM with CRF and controls

RENAL FUNCTION AND LEAD	TYPE II DIABETIC (n=50)	DM WITH CRF (n=50)	CONTROLS (n=50)	p-VALUE
UREA (mg%)	23.4 ± 9.32	125.2 ± 32.49 ◊	22.3 ± 8.49	0.001
CREATININE(mg%)	1.05 ± 0.24	7.3 ± 1.41 ◊	1.12 ± 0.23	0.001
CREATININE CLEARANCE (ml/min)	111.9 ± 14.59	55.1 ± 9.61◊	108.8 ± 14.36	0.001
LEAD (ug/dl)	11.4 ± 3.82 *	11.5 ± 4.06 *	7.0 ± 5.11	0.001

* As compared to controls p<0.01, ◊ As compared to Type II diabetic p<0.01

Tab. 3. Comparison of antioxidant enzymes activity in DM and DM with CRF and controls

ANTIOXIDANT ENZYMES	TYPE II DIABETIC (n=50)	DM WITH CRF (n=50)	CONTROLS (n=50)	p-VALUE
SUPEROXIDE DISMUTASE (U/ml)	122.9 ± 16.32 *	120.9 ± 16.28 *	204.5 ± 22.68	0.001
CATALSE (U/ml)	5.5 ± 1.56*	3.7 ± 1.29 *◊	7.1 ± 1.98	0.001
GLUTATHIONE PEROXIDASE (Ug/Hb)	42.7 ± 11.48	39.0 ± 11.21*	52.1 ± 5.27	0.001
NITRIC OXIDE (umol/L)	16.7 ± 2.98	10.8 ± 3.07 *◊	18.6 ± 2.98	0.001

* As compared to controls p<0.01, ◊ As compared to Type II diabetic p<0.01

Tab. 4. Correlation between Lead, FBS, HbA1c, Urea, Creatinine and CreatinineClearence in Control, Diabetes and Diabetes with CRF patients

			FBS	HBA1C	UREA	CREATININE	CREATNINECLEARENCE
LEAD	Control & DM	Pearson Correlation	.400**	.361**	.113	-.154	.076
	Control & DM With CRF	Pearson Correlation	.408**	.460**	.377**	.512**	-.456**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Tab. 5. Correlation between Lead and Antioxidant enzymes in Control, Diabetes and Diabetes with CRF patients

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

			SUPEROXIDE DISMUTASE	CATALASE	GLUTITHIONEPEROXIDASE	NITRICOXIDE
LEAD	Control &DM	Pearson Correlation	-.449**	-.081	-.191	-.141
	Control &DM With CRF	Pearson Correlation	-.438**	-.232*	-.224*	-.395**

Discussion

The present study determines the consequences of lead exposure through water. We have found that lead exposure may be the cause of diseases like renal failure and DM. Lead has long been known to be a renal toxicant. Some recent studies focused on patients with chronic kidney disease, with some evaluating whether greater lead exposure is associated with the loss of kidney function. A large study of incident chronic kidney disease (CKD) cases among lead workers in Sweden (926 cases, 998 controls) failed to find an increased risk of CKD or faster rate of decline in GFR over a 7–9 year follow-up interval (Evans et al., 2010). A disproportionate share of the morbidity associated with lead exposure is borne by developing countries. The World Health Organization noted that, in 2000, approximately 10% of children had a blood lead level of 20 µg/dL or higher, but that 99% of these

children lived in developing countries and that lead exposure accounted for nearly 1% of the global burden of disease (Fewtrell et al., 2003).

Previously we have estimated the lead levels in the tap and bore water samples obtained from different areas of Karachi, Pakistan (Ul-Haq et al., 2011). We have found that bore water of have significantly high lead levels in the areas of Kiymari, Baldia, SITE,Lyari, Gulberg, New- Karachi, Shah-Faisal, Sadar, Orangi, Liaquatabad, Jamshed, Korangi, Landhi and Malir towns as compared with WHO recommended level and as compared to tap water in the areas of Orangi, Korangi, Lyari, Shah Faisal, Baldia, Jamshed and Kiymari towns shown in the fig 3(Ul-Haq et al., 2011). This suggests that peoples of these areas using bore water have more risk of lead exposure and are more prone to diseases caused by lead. So, in the present study we have selected the population from these areas for determining the consequences of lead exposure. Several factors have been reported to modify the association between blood lead level and kidney function, although the evidence is inconsistent. These include certain genetic polymorphisms, including ALAD, the vitamin D receptor and nitric oxide synthase (De Burbure et al., 2006). One study investigated the association between lead exposure and change over time in renal function. In the NAS cohort, Tsaih et al. (2004) found that the lead-related decline in renal function over a 6-year follow-up interval, specifically the rate of rise in serum creatinine level, was greater in individuals who, at baseline, had diabetes. The few data available on lead exposure and renal function in even younger children suggest that higher blood lead levels are associated with increased GFR (as estimated by serum creatinine or cystatin C levels), suggesting a paradoxical effect that, perhaps, reflects a hyperfiltration phenomenon(De Burbure et al., 2006).

The results of this study revealed that most of the blood samples of diabetic and chronic renal failure patients have unacceptably high levels of lead particularly in industrial areas of the city. Earlier reports have demonstrated that some medical and socioeconomic factors like hypertension, diabetes, and hypercholesterolemia are also associated with chronic kidney disease (CKD). Lead poisoning may remain asymptomatic for many years. Major symptoms of lead poisoning are hypertension and neuropathy. Furthermore, chronic lead poisoning may be a cause of chronic renal failure. The design of our study does not enable us to discuss role of lead in the onset of CRF. Determination of such a relationship would require epidemiological studies conducted according to rigorous methodology.

In our study, the blood lead levels were significantly higher, almost twice that of the control group in diabetics and diabetes with CRF patients. This suggested that diabetes mellitus can be a major risk factor for CKD and can induce several other risk factors. In our study 97% of blood samples of diabetic patients with chronic renal failure had lead levels above the levels recommended by W.H.O. Considering our results, we have estimated that diabetics might well represent cases of chronic Pb intoxication, and that this might explain a certain proportion of those diabetic persons who develop CRF despite of good dietary control, proper medication and follow-up. We have also estimated the other significant parameters to confirm the presence of CRF and DM in these peoples. We have found that DM patients have significantly high blood glucose level as compared to control and DM with CRF patients and HbA1c levels as compared to control. While the parameters of renal function including urea, creatinine and creatinine clearance rate, are significantly high in DM with CRF patients as compared to DM patients and controls. Substantial experimental evidence implicates oxidative stress via oxidation-reduction–inactive metal pathways for lead, resulting in increased reactive oxygen species. Recent studies show that oxidative stress takes part in the etiopathogenesis of many illnesses. Here, two types of damage may come to play. First, the free oxygen radicals may increase. Second, one of the defense mechanisms of the body may be malfunctioning due to the lack of SOD (Tabakoglu et al., 2004). In our study the levels of antioxidant enzymes were altered in DM and DM with CRF patients. Levels of SOD, catalase, glutathione peroxidase and nitric oxide were decreased significantly in DM and DM with CRF patients but this decrease is more in DM with CRF patients.

Our study suggests that CRF does not lead to Pb accumulation by itself. If CRF patients exist with chronic Pb poisoning then it is reasonable to think that Pb is the cause of the CRF in those patients even though the source of Pb exposure remains unclear. Many studies contain groups of patients with mixed etiologies of CRF, or groups of patients with and without previous history of Pb exposure. The present study was designed to answer, how many patients with CRF of unknown etiology have an excessive Pb levels as the possible cause for their renal disease. To try to find an answer to the question it is important to compare a group of patients with CRF and DM with a group of patients with DM only. Unfortunately blood Pb levels are not a suitable basis for the diagnosis of chronic Pbintoxication as they are only an indicator of recent exposure. Thus from the present results it can be concluded that lead poisoning may be pronounced risk factor for CRF.

Acknowledgement

I acknowledge the academic support of Dr.TabasumMehboob, Professor at Department of Biochemistry, Karachi University. The authors are thankful to the JPMC and Kidney Center for helping in this project.

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