

Effect of heavy metals on Creatinine level in Human blood samples of inhabitants living in the vicinity of Hudiara Drain, Lahore, Pakistan

ABSTRACT

Aims: Modern industrialization, anthropogenic and industrial activities have increased the risk of human exposure to heavy metals and in turn effecting human health badly. Hundreds of millions of people are globally affected by heavy metal toxicity through contaminated water in one way or- another. In order to find a correlation between the heavy metal concentrations and serum creatinine level, a study was conducted on the inhabitants living near the Hudiara drain suffering from kidney diseases.

Study design: The subjects (n=498) were selected from the vicinity of polluted Hudiara drain to conduct this matched case-control study in 2019-2020. Instrumental techniques were used for the determination of heavy metals and creatinine was determined by using Kit. The correlation of between heavy metals and creatinine was checked.

Place and Duration of Study: To determine heavy metals and creatinine in blood, samples were collected from the habitants of Hudiara drain and the control group 100Km far from Hudiara drain.

Methodology: Blood samples of male subjects with and tested for heavy metals in the blood using atomic absorption spectrophotometer and creatinine level by using Creatinine Elisa Kit. The study was conducted in 2020 and included n= 498 healthy volunteers (control) and n=498 effected (suffering from chronic kidney disease).

Results: The ANOVA shows the significant difference between two groups i.e. control group and affected group in all traits of the respondents (weight, age, heavy metal values and serum creatinine level). Pearson's correlation coefficient was calculated for heavy metals and creatinine subjects' blood samples. It was found that heavy metal levels in subjects have a significant correlation with serum creatinine. The study shows that serum creatinine level has no significant correlation with age, so is independent of age.

Conclusion: The concentration of heavy metal contaminants (Cd, Hg, Pb) in the blood of the inhabitants of Hudiara drain flowing in Lahore city, Pakistan is highly polluted by industrial effluents. The effects of higher concentration of heavy metals in the inhabitants of Hudiara drain have been determined in serum creatinine levels. It was observed that high serum creatinine values are found in subjects suffering from CKD.

Keywords: Biomarker, creatinine, chronic kidney disease, heavy metals.

1. INTRODUCTION

Heavy metals contamination of water is of environmental concern throughout the world and affects millions of people globally[1]. Balali-Mood has tried to find an association between heavy metals concentration in food, water and air, and human health. The metals like chromium, iron, copper, zinc, cobalt, selenium, magnesium, molybdenum, chromium, and manganese are essential for life, whereas arsenic, cadmium, mercury, nickel, lead and palladium are toxic for humans at diverse levels[2]. Earlier researchers found cumulative effects by exposure. Simultaneously to more than one metals [3,4,5,6]. The complications of high-dose exposure of heavy metals like mercury and lead were studied by [7] and severely induced kidney failure, bloody diarrhea and abdominal colic pain were observed. Few epidemiological studies have indicated the association between chronic kidney disease and exposure to heavy metals [8]. The proximal part of the nephron tubule in kidneys can be harmed more specifically by heavy metals and it was observed that exposure to heavy

metal like As and Cd causes chronic kidney disease [9]. It has been proved experimentally that Cd causes damage to kidney and diabetic nephropathy by lowering the rate of glomerular filtration [10]. It has been found that accidental exposure to Hg intoxication exerts harmful effects on the proximal tubules as the kidney is the most favorable storehouse of mercury [11]. Two studies conducted in extremely mercury polluted zones of China to evaluate the highly raised serum creatinine levels in Hg exposed inhabitants compared to residents living in controlled areas. Hg-induced renal function impairment was revealed to be higher in females and the older people [12, 13]. Impaired renal function can be caused by maternal exposure, as shown by a study conducted by Zhang et al., 2020 in Hg polluted areas. Evans and Elinder described that lead could cause the swelling of mitochondrial membrane in renal tubules, which in turn affect the energy formation [14].

Kidneys being sensitive organs are highly affected by the toxicity of heavy metals. The Kidney is a complex organ comprising of nephrons highly specialized functional units. One million nephrons are present in human kidneys, which perform plasma filtration approximately 150–180 L/day [15]. The creatinine is produced by the breakdown of muscles and is filtered as a by-product from the body through the kidneys [16]. The creatinine in the blood can be estimated by glomerular filtration rate. High levels of creatinine seen in blood serum may indicate glomerular dysfunction, where is related to chronic kidney disease. Serum creatinine indicates the improper function of the kidney. Tubular injury causes significant fluctuations in serum creatinine levels [2]. Chronic kidney disease (CKD) is widely recognized as an epidemic in developing and developed countries. It is intensifying with every passing day, having a ratio between 11% and 13% deaths are caused worldwide [17,18].

Overview of the universal problem of chronic kidney disease, the following study was designed. The purpose of the present study was to determine whether significant relation exists between heavy metal toxicity and serum creatinine levels in CKD patients. The serum creatinine level varies with age, sex, weight, or race and can be calculated by estimating glomerular filtration rate (eGFR) [19]. The present study provides data on the existence of heavy metal levels in patients suffering from CKD living near Hudiana drain in Lahore city (Pakistan). The authors also looked at creatinine levels in both patients and healthy patients as controls. The study included 498 non-smokers subjects (male) and suffering with CKD in vicinity of Hudiana drain. The heavy metals (Pb, Cd, Hg) were determined in their blood samples. Blood samples of controlled subjects (n=498) were also collected from people living 100km far from Hudiana drain.

2. MATERIAL AND METHODS

Sample digestion was performed using a domestic microwave oven (Pel PMO23, Japan) that can be programmed for time having a micro wave power of 900 W. Heavy metals (Pb, Cd, Hg) were measured by using atomic absorption spectrophotometer model 700 equipped with deuterium lamp and graphite furnace HGA- 400, pyrocoated graphite tube with an integrated platform. The certified standard solutions of Pb, Hg and Cd were prepared by dilution of certified standard solutions (1000ppm) of FlukaKamica (Buchs, Switzerland). Acid-washed plastic (polypropylene) vessels were used for preparing and storing solutions. The ELGA pure Lab water system (Buck, UK) was used for obtaining ultra-pure water. Analytical grade reagents like hydrogen peroxide and nitric acid Merck (Germany) were used. Before use, all glassware and plastic materials dipped in 5M nitric acid and rinsed with and distilled water is used. For required analysis, samples were stored at 4°C prior to analysis. For human blood Clincheck control-lyophilized were (Recipe, Munich Germany) used as reference. Creatinine was determined by Jaffe Method using serum creatinine biomarker.

2.1. SAMPLE LOCATION

For the determination of heavy metals and creatinine in blood, samples were collected from the habitants of Hudiana drain and the control group 100 Km far from Hudiana drain. The origin of Hudiana Drain (earlier a channel of natural storm water) in India (City: Batalas, District: Gurdaspur) and runs through Laloo village (city; Lahore, province: Punjab, Pakistan) [20]. Hudiana Drain is 98.6 km long and in Pakistan it flows for 54.4 km and then it fuses with Ravi River. Wastewaters from Industrial and domestic use are directly discharged into the drain throughout its their way in India and Pakistan [21]. This Industrial pollution can badly affect public health as toxic elements and pathogens present in Wastewater are high sources of diseases [22].

2.2. SAMPLE COLLECTION AND PRETREATMENT

Adult subjects (n=498) were selected from the vicinity of the polluted Hudiana drain to conduct this matched control-case study in 2019-2020. Hudiana drain wastewater is used for irrigation purposes and then the same crops and vegetables are used as food by these people. The meat used by these people is also of the same animals grazed near Hudiana drain. Although people living near Hudiana drain suffer from different diseases most people have kidney illnesses. The institutional ethics committee approved the study. Each subject gave a written, informed consent to participate in this study. All participants filled a questionnaire including details like living conditions, educational and occupational status, and personal behavior such as the use of alcohol and smoking habits. Venous blood (10mL) of affected and healthy subjects was collected in tubes of Becton Dickinson. All blood samples were transported to the laboratory immediately in a cold chain and processed over there. The microwave-assisted acid digestion method was used to prepare samples for heavy metals determination as it needs 5-minutes for complete biological digestion of blood [23]. Jaffe's method was used to analyze Serum creatinine using standards and reagents [2, 24]. The same blood sample was used for the determination of heavy metals (Pb, Hg and Cd) and creatinine.

2.3. STATISTICAL ANALYSIS

Many samples were collected and analyzed both for subjects and controls and statistical analysis was performed to validate the data. Mean values and their standard deviations were used to present baseline data. Groups were labelled as mean to describe the comparative data. The SPSS21 ANOVA paired sample t-test was applied to check the significant level with regression analysis. The values measured under detectable limits were expurgated to analyze means and standard deviation to determine heavy metals. Pearson's correlation was determined to assess the correlation between heavy metals and serum creatinine.

4. RESULTS AND DISCUSSION

Table 1: Shows the descriptive analysis of weight, age and level of Cd, Hg, Pb (mg/L) and creatinine in the blood.

Control/ Affected group		Weight in Kg	Age in Years	Cd mg/L	Hg mg/L	Pb mg/L	Creatinine mg/dl
Control Group	Mean	49.93	42.74	.0317114	.0004253	.0497371	.6317871
	Minimum	37	25	.00100	.00004	.00100	.30000
	Maximum	64	60	.32000	.00510	.19000	.87000
	Range	27	35	.31900	.00506	.18900	.57000
	Std. Deviation	7.047	8.490	.02944439	.00030401	.03113676	.11475553
Affected Group	Mean	54.84	43.78	.3449337	.0043986	1.5922169	1.7826506
	Minimum	31	14	.01000	.00100	.09300	1.03000
	Maximum	97	84	.99000	.00910	206.00000	2.90000
	Range	66	70	.98000	.00810	205.90700	1.87000
	Std. Deviation	11.704	13.891	.32222380	.00191583	9.21519523	.30044736
Total	Mean	52.38	43.26	.1883226	.0024119	.8209770	1.2072189
	Minimum	31	14	.00100	.00004	.00100	.30000
	Maximum	97	84	.99000	.00910	206.00000	2.90000
	Range	66	70	.98900	.00906	205.99900	2.60000
	Std. Deviation	9.963	11.518	.27721231	.00241460	6.55843970	.61896785

This Table1 shows the descriptive statistics of the control group, affected group and combined group. The mean value of weight, age, Cd, Hg, Pb and creatinine level in the control group is 49.93, 42.74, 0.0317, 0.0004, 0.0497 and 0.6317, respectively. Similarly, the mean value of weight, age, Cd, Hg, Pb and creatinine level in the affected group is 54.84, 43.78, 0.3449, 0.0043, 1.5822 and 1.7826, respectively. The mean value of weight, age, Cd, Hg, Pb and creatinine level in the combined group is 52.38, 43.26, 0.1883, 0.0024, 0.8209 and 1.2072, respectively.

Table 2: Shows analysis of variance in control and affected group of parameters.

			Sum of Squares	df	Mean Square	F	Sig.
Weight in Kg * Control/ Affected group	Between Groups	(Combined)	6006.944	1	6006.944	64.369	.000
	Within Groups		92760.546	994	93.320		
	Total		98767.490	995			
Age in Years * Control/ Affected group	Between Groups	(Combined)	269.402	1	269.402	2.033	.154
	Within Groups		131728.727	994	132.524		
	Total		131998.129	995			
Cd mg/L * Control/ Affected group	Between Groups	(Combined)	24.429	1	24.429	466.668	.000
	Within Groups		52.033	994	.052		
	Total		76.462	995			
Hg mg/L * Control/ Affected group	Between Groups	(Combined)	.004	1	.004	2089.397	.000
	Within Groups		.002	994	.000		
	Total		.006	995			
Pb mg/L * Control/ Affected group	Between Groups	(Combined)	592.432	1	592.432	13.953	.000
	Within Groups		42205.634	994	42.460		
	Total		42798.066	995			
Creatinine mg/dl * Control/ Affected group	Between Groups	(Combined)	329.797	1	329.797	6376.746	.000
	Within Groups		51.408	994	.052		
	Total		381.206	995			

The ANOVA table shows the significant difference between the two groups, i.e., the control group and affected group, in all traits of the respondents (weight, age, heavy metal presence and creatinine level). The F-value with a 1 % level of significance shows that there is a significant difference in all traits like weight, Cd, Hg, Pb and creatinine level of the subjects except the age of the individuals. Hence it is concluded that both the groups are significantly different from one other in all traits except age.

Table 3: Shows correlation between weight (Kg), age(years), Cd, Hg, Pb and creatinine level of blood serum in human beings.

		Weight in Kg	Age in Years	Cd mg/L	Hg mg/L	Pb mg/L	Creatinine mg/dl
Weight in Kg	Pearson Correlation	1	.091**	.094**	.233**	.057	.205**
	Sig. (2-tailed)		.004	.003	.000	.070	.000
	N	996	996	996	996	996	996
Age in Years	Pearson Correlation	.091**	1	.007	.010	.031	.043
	Sig. (2-tailed)	.004		.830	.746	.330	.171
	N	996	996	996	996	996	996
Cd mg/L	Pearson Correlation	.094**	.007	1	.469**	.035	.549**
	Sig. (2-tailed)	.003	.830		.000	.268	.000
	N	996	996	996	996	996	996
Hg mg/L	Pearson Correlation	.233**	.010	.469**	1	.122**	.764**
	Sig. (2-tailed)	.000	.746	.000		.000	.000
	N	996	996	996	996	996	996
Pb mg/L	Pearson Correlation	.057	.031	.035	.122**	1	.115**
	Sig. (2-tailed)	.070	.330	.268	.000		.000
	N	996	996	996	996	996	996

Creatinine mg/dl	Pearson Correlation	.205**	.043	.549**	.764**	.115**	1
	Sig. (2-tailed)	.000	.171	.000	.000	.000	
	N	996	996	996	996	996	996
** . Correlation is significant at the 0.01 level (2-tailed).							

The correlation table results show a significant correlation of weight, Cd, Hg, and Pb with creatinine level at 1% level of significance. At the same time there is no significant correlation of age with creatinine level.

The pollution from factories and industries contaminated our environment. The toxicity of heavy metals contaminates air, soil and water. Cd accumulates in the renal cortex and Pb in bones [25]. Cd has a long half-life and very minute concentration absorb in specific tissues and the main target is kidney and disturb the physiological function of the human body. Pb enters in the human body by exogenous sources and absorb in bones [26]. Pb, Hg and Cd in the human body cause renal damage, diabetes and hypertension. The Cd harms kidneys as a result of diabetes- induced effect. The renal damage has relations hip with heavy metals. The exposure of environmental heavy metals in human blood causes kidney diseases. The level of Pb and Hg is not associated with CKD. The low level of Cd exposure damages the kidney, chronic diseases with hypertension or diabetes [27].

The heavy metals like Ni, Pb and As damage human body. The poisoning of Hg compounds cause neuronal damage. The Hg toxicity causes inflammation, psychiatrics, tremor and peripheral neuropathy [28]. There is strong positive correlation between creatinine and heavy metals. The metal in the ionic form is attached with to cell membranes and helps to circulate the blood protein [29]. The ionic form of Hg attach with SH group of the cell membrane, protein, enzyme and oxidized nervous tissue. Heavy metals moderate the formation of free radicals and damage the cell membrane, and inhibit the enzymes and bind to mitochondria [30]. To screen kidney diseases serum creatinine biomarker is used. This biomarker has high specificity in the treatment of kidney diseases [31].

Owing to the large variation of heavy metals concentration prevailing among subjects and controls as well as on age groups, large number of samples from effected and controlled areas were collected to achieve significant data that was statistically analyzed. The study included 498 non-smokers subjects (male) suffering from CKD with an equal number of controls. The anthropometric characteristics of cases and controls are given in table 1. The mean values (\pm SD) of age for subjects and controlled were 43.78 (\pm 13.89) and 42.74 (\pm 8.49) respectively, while for weight, the values were 54.84 (\pm 11.7) for subjects and 49.93 (\pm 7.04) for controlled (Ttable 1). The Clinical properties of subjects and controls are given in table 2. A significant difference was observed between variables both for subjects and controls.

The subjects had an average value of Cd 0.34s(\pm .03) mg/L, Hg 0.0043(\pm .002) and Pb (\pm .002) mg/L in blood serum with a mean level of serum creatinine as 1.8(\pm 0.3) mg/L. The average value of heavy metals in healthy males (controls) was as follows: Cd 0.31 (\pm .029) mg/L, Hg 0.0004(\pm .0001) and Pb 0.5 (\pm .03) mg/L having a mean level of serum creatinine as 0.06314 (\pm .0.1) mg/L. The mean value of weight, age, Cd, Hg, Pb and creatinine level in the combined group is 52.38, 43.26, 0.1883, 0.0024, 0.8209 and 1.2072, respectively. The level of serum creatinine is higher in inhabitants of Hudiara drain vicinity as compared to controls. This higher level of serum creatinine can be attributed to higher levels of Cd, Hg and Pb in people suffering from CKD than healthy ones.

A higher concentration of Cd can be attributed to it being primarily distributed in the liver and kidneys [31]. Kidneys take up toxic protein complex (Cd+ metallothionein) [32] and this Cd complex causes nephrotoxicity. The higher Serum creatinine levels measured in CKD patients in the present study can be related to higher concentration of Mercury as it supported by a study conducted by [11]. Who found that mercury badly effects kidneys' function by accumulating over there. This fact was also supported by many other studies conducted in China[34,12, 13, 29] where higher serum creatinine levels (upto 88.5 μ g/g) were found in people exposed to high Hg concentration. The higher values of serum creatinine in present study can also be related to Higher Pb concentration as it is supported by other studies[35, 36].

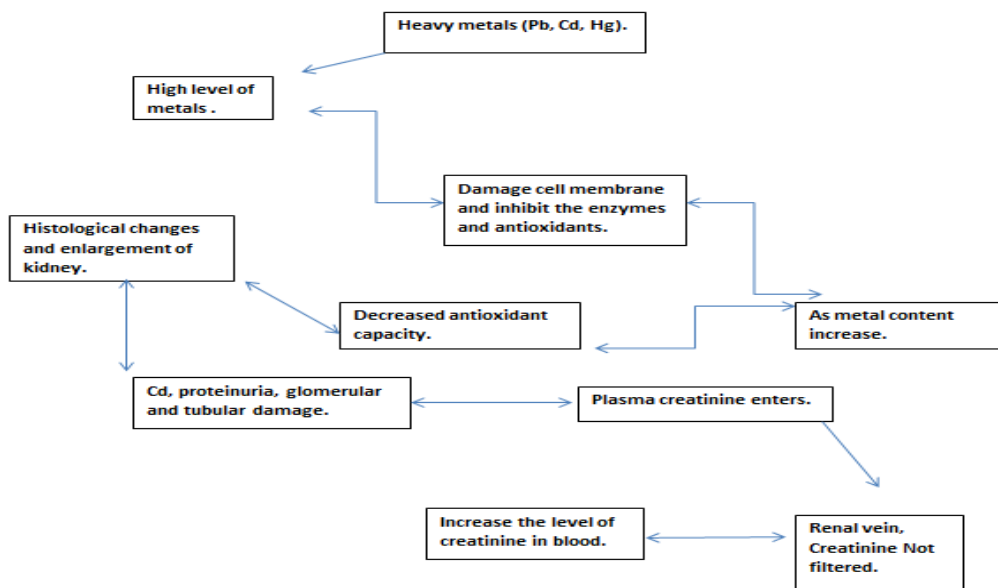
The ANOVA shows the significant difference between the two groups i.e., the Control group and the affected group, in all traits of the respondents (weight, age, heavy metal presence and creatinine level). The F-value with 1 % level of significance shows a significant difference in all traits like weight, Cd, Hg, Pb and creatinine level of the subjects except age of the individuals. Hence it is concluded that both the groups are significantly different from one other in all traits except age (table 2).

For correlation analysis, Pearson's correlation coefficient was calculated for heavy metals and creatinine in subject's blood samples (table 3). The most positive correlation was observed between weight -serum creatinine ($r=0.205$), Cd - serum creatinine ($r=0.549$), Hg -serum creatinine ($r=0.764$) and Pb-serum creatinine ($r=0.115$). The highly positive correlation of all the three heavy metals in the present study can be proved by studies conducted by other researchers as

cumulative effects is produced by two or more heavy metals when exposed to them Simultaneously[37, 4, 5, 7]. The study shows a significant correlation of weight, Cd, Hg, and Pb with creatinine level at 1% level of significance, at the same time there is no significant correlation between age and creatinine level.

The results of the present study are in accordance to with the epidemiological studies conducted to find an association between CKD and exposure to heavy metals [38, 1] but these results are not in compliance with the studies conducted in India [39].

Figure 1: Shows the mechanism of increasing Creatinine level in blood.



Heavy metals (Pb, Hg, Cd) increasing level reduce the antioxidant activity and damage the renal vein of kidney. It damages the kidney functions and badly effect on glomerular filtration rate. Kidney diseases enhance the risk of cancer. The high level of creatinine in blood indicates the failure of kidney.

4.CONCLUSION

The present study talks about the concentration of heavy metal contaminants (Cd, Hg, Pb) in the blood of the inhabitants of Hudiara drain flowing in Lahore city, Pakistan and highly polluted by industrial effluents. The study also contained an equal number of controls to make it clearer. A considerable variation was observed in heavy metal levels both for the subjects and controls. The concentration of heavy metals followed the trend Cd>Pb>Hg. Higher values of contaminants were observed in subjects than in controls. The effects of higher concentration of heavy metals in the inhabitants of Hudiara drain have have been determined in serum creatinine levels. It was observed that high serum creatinine values are found in subjects suffering from CKD. The study shows a significant correlation of weight, Cd, Hg, and Pb with creatinine level while no significant correlation exists between age and creatinine levels. The study also reveals that the heavy metal effects determined in the form of renal dysfunction by using creatinine biomarker are similar to the international studies carried out in different polluted regions of the world by using different biomarkers and serum creatinine.

Based on our results, we can conclude that Hudiara drain is a major source of pollution for the individuals (suffering from renal dysfunction) residing in its vicinity, as they are dependent directly or indirectly on its water to grow vegetables and graze their animals. It can also be concluded from the present study that the use of serum creatinine as a biomarker to diagnose renal dysfunction is far more efficient than other biomarkers as it is easy to determine creatinine concentration in serum. In the present study, simultaneous exposure to cadmium, lead and mercury in the present study is a strong

determining factor of CKD. These data have significant public health implications owing to the extensive exposure to the heavy metals and the growing global burden of CKD.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used in our research area and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company; rather, it was funded by the personal efforts of the authors.

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