International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Heavy Metal Induced Toxicity and Glutathione Enzyme Antioxidants Status in Diabetic Nephropathy: A Case-Control Study in Western U. P., India

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Abstract

Heavy metals (e.g., lead, cadmium, arsenic, etc.) are the serious risk factors for impairment in pancreas and insulin resistant that can lead to hormonal imbalance and metabolism. While the enzyme antioxidants play a defense mechanism against heavy metal toxicity. Furthermore, heavy metals have binding capacity with essential elements (e.g., zinc, copper, selenium, etc.) which disturb their normal functioning. Total no of 120 subjects (40 subjects in 3 groups) were registered as controls and cases of matched age and sex. Cases were divided into two groups: diabetes mellitus and diabetic nephropathy. For screening of diabetes mellitus and diabetic nephropathy, history and biochemical investigations were carried out for control and cases to rule out the other causes of nephropathy. Males and females of age group 30-75 years were included in the study. Endogenous enzyme antioxidants, *glutathione peroxidase (GPx)* and *glutathione reductase (GR)* were analyzed in hemolysate. Heavy metal analysis was performed using whole blood on ICP-OES. The results observed were presented in mean ± SD format. Various circulatory parameters of controls and cases were compared using independent "t-tests" and ANOVA. Bivariate correlations were determined by Pearson's correlation. Heavy metals such as lead and Cadmium were found significant among the cases as compared to healthy controls (**p = 0.00**). The concentration of heavy metals and *GPx* were found higher in DN group (vis-à-vis DM and healthy control groups). There is a compensatory decrease in *GR* in DN group as compared to DM and healthy control groups.

Keywords: Heavy metals, Glutathione reductase, Glutathione peroxidase, and Diabetic nephropathy

Full-length article

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1. Introduction

Nephropathy or renal disease is a serious health concern that affects ~ 16% of the population worldwide. The prevalence of renal failure is correlated with diabetes and hypertension. As per International diabetes federation report-2022, the prevalence of T2DM worldwide in population of age group 20-79 year was estimated to be ~ 536.6 million cases (10.5%) ~ () which is expected to increase upto ~783.2 million (12.2% by 2045. The prevalence of T2DM was reported to be similar in men and women and highest in the population of the age-group of 75-79 years. [1]. Approximately 30% of T2DM cases progress to diabetic nephropathy (DN) [2]. DM and DN are correlated to several factors such as heavy metals, essential elements, endogenous enzyme antioxidants, and so forth. In particular, heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), are the serious risk factors for impairment in pancreas and insulin resistant that can lead to hormonal imbalance and metabolism [3]. While the enzyme antioxidant plays a defense mechanism against heavy metal toxicity. Furthermore, the heavy metals have binding capacity with essential elements (e.g., zinc, cooper, selenium, etc.) which disturb their normal functioning.

Alteration in the level of a particular element in body fluids and tissues may influence normal levels of other element and vice-versa [4]. The human body essentially requires minerals in the form of food for metabolic/cellular functions and homeostasis e.g., sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), , iron (Fe^{2+} or Fe^{3+}) ions, and so forth. On the other hand, minerals having high density (e.g., cadmium (Cd), and lead (Pb)) referred as heavy metals are hazardous to human health [5]. These can be accumulated in the liver, kidney, and pancreas, once they entered the human body. Being hazardous, heavy metals affect the glucose metabolism and insulin action that may contribute to T2DM [6]. Exposure to heavy metals in high doses can cause enzyme inactivation, suppression of the antioxidant defense [7], [8]. An experimental investigation on rats by Riaz et al. (2019) revealed that rats suffering from diabetes are more deleterious towards heavy metals (i.e., Pb Cd, etc.) and are therefore, more prone towards renal damage [9].

In addition, beside insulin resistance, suppression of the antioxidant defense mechanism, aforementioned heavy metals play a detrimental role to cause tubular insufficiency in kidneys of diabetic subjects. [6, 10, 11] Wang et al. (2022) reported that the exposure to multiple metals may also cause renal disease in T2DM patients diabetes due to potential interaction of heavy metals (e.g., Pb, Cd, and As) with essential trace element (Zn). [6] Wan et al. (2021) reported that lead exposure is a vulnerable factor for diabetic nephropathy. Therefore, avoiding its exposure is a primary or even secondary interventions. Their findings revealed association between the Pb level in blood and macro- and microvascular complications. [10] Recently published article by Yimthiang et. al. (2023) reports that cadmium exposure via environmental routes is one of the deleterious factors to tubular proteinuria in people with and without diabetes. Furthermore, prolonged intake of low levels of environmental cadmium causes proteinuria in subjects of diabetic nephropathy [11].

The above discussion clearly suggest that the heavy metal toxicity is potential cause for the kidney dysfunction. Heavy metals chelate with essential trace elements to reduces their activity which, inturns, causes the loss of several essential trace elements [4]. The other crucial factors of diabetic nephropathy are endogenous antioxidants that are enzymes such as: catalase, glutathione reductase (GR), glutathione peroxidase (GPx), and superoxide dismutase (SOD). Glutathione (GSH), vitamins A, C and E etc. are the examples of a few nonenzymatic antioxidants. Albumin has good antioxidant capacity in the body [2].The major antioxidant enzymes defense GPx reduces oxidative stress and requires GSH as a donor of hydrogen ion which is found in almost all living cells [2].

The information available in existing literature revealed that red blood cells (RBC) GSH, GPx and GR are significantly reduced in uncontrolled diabetic patients without nephropathy, compared with age and sex matched group of healthy volunteers. The decrease in these enzymes is associated to a reduction in GSH concentration, thereby, leading to an increase in oxidative stress. It is important to note that oxidative stress is generated due to the increase of glucose level in blood This could contribute to an increase in glutathione utilization. The deficiency of most potent antioxidant GSH, and GPx, GR enzymes leads to increase free radical (e.g., H_2O_2, superoxide, and NO) which are major oxidant in in raising oxidative stress which results in derangement of cell functioning [12] Specifically GR maintains glutathione in its reduced form and thereby, protect red blood cells (RBC), hemoglobin, and cell membranes against oxidative stress and metal toxicity [13].

There are several studies on heavy metal induced toxicity in T2DM and its related complications in India and very less in the state of Uttar Pradesh and rare in Western Uttar Pradesh. In this line, current study aims to find the association of heavy metal-induced toxicity and glutathione enzyme antioxidants status in DN. Objectives of the study are (i) to estimate the level of heavy metals in patients of T2DM and DN, (ii) to estimate the level of glutathione enzymes in patients of T2DM and DN. This study was designed to know the exposure of humans with heavy metals and its effect on glutathione antioxidant defense in diabetes mellitus patients with or without nephropathy, that will be helpful in the monitoring and prevention of early renal injury.

2. Materials and Methods

Total no of 120 subjects (40 subjects in 3 groups) were registered as controls and cases of matched age and sex. Cases were divided into two groups: diabetes mellitus and diabetic nephropathy. Physical examination (such as age, gender, blood pressure), history (disease other than DMT2 and medication) taken from all the patients and control as well as biochemical investigations for screening of diabetes mellitus and diabetic nephropathy were carried out to rule out the other causes of nephropathy. Males and females of age group 30-75 years were included. Screening of diabetes mellitus and diabetic nephropathy carried out as per the guidelines of the American Diabetes Association (ADA) [14]. Diabetes mellitus subjects were diagnosed based on the following criteria: (i) normo-albuminuria less than 30 mg/24 h, (ii) microalbuminuria when albumin excretion is 30 to 300 mg/24 h, and (iii) macroalbuminuria or nephropathy more than 300 mg/24 h along with fasting plasma glucose: ≥ 126 mg/dL), (iv) Random/Post prandial glucose: ≥200 mg/dL and HbA1c less than 6% in controls, 6-7 % in the diabetes mellitus group, and more than 7 % in the diabetic nephropathy group. Pregnant/lactating women, polycystic ovarian syndrome, hypertension, non-diabetic kidney disease, or any cause of nephropathy other than diabetes mellitus were excluded. The present study was done after ethical approval from the institutional ethical committee of Lala Lajpat Rai Memorial Medical (LLRM) College, Meerut, Uttar Pradesh (Reference No. S-1/2019/9263 Dated: 16.12.2019), and formal consent was taken from each subject.

2.1. Laboratory Investigation

A total of 5 mL of peripheral blood samples was collected from the patients as well as controls. From which 2.5 mL blood was collected in an EDTA vial for HbA1c, plasma/lysate preparation, and analysis of heavy metals, rest 2.5 mL blood in a plain vial for biochemical parameters. Estimations of blood glucose & Kidney function tests (serum urea and serum creatinine). Urinary albumin excretion (UAE) of 24 h Urine sample has been performed using Selectra Pro M/Selectra XL Autoanalyzer (ELITECH Group). HbA1c was performed on a D-10 HbA1c Analyzer (Bio-Rad). Estimated Glomerular filtration rate (eGFR) was calculated by CKD-EPI Creatinine equation (2021) given by National Kidney Foundation is as follows the classification of CKD as per Table 1 given below [15] :

$$Estimated\ GFR\ (creatinine) = 142 * \min(Scr/\kappa, 1)^{\alpha} * max\ (Scr/\kappa, 1)^{-1.200} * 0.9938^{Age}\ (if\ female, * 1.012)$$

Where, the terms *Scr* and *age* (written in superscript) stand for standardized serum creatinine measured in mg/dL and the age of the subjects in years, respectively. Further, the terms κ and α are the constants and correspond to a value of 0.7, and -0.241 for females, respectively. Whereas the value of κ and α for males are 0.9 and -0.302, respectively. The terms *min (Scr/κ, 1)* and *max (Scr/κ, 1)* correspond to minimum of Scr/κ or 1.0 and maximum of Scr/κ or 1.0, respectively. Classification of estimated Glomerular Filtration Rate (eGFR) as per National Kidney Foundation is in 5 groups, eGFR (mL/min/1.73 m^2): Grade 1 ≥ 90, Grade 2: 60-89, Grade 3: 30-59, Grade 4: 15-29 and Grade 5: < 15; interpreted as normal or high, mildly decreased, mildly to moderately decreased and severely decreased, respectively.

2.2. Plasma and Lysate separation

Whole blood was collected in an EDTA vial for plasma and lysate preparation. 1 mL of whole blood from EDTA vial is transferred to a 2 mL Eppendorf tube and centrifuged at 2,500 rpm for 15 minutes at 25-30 ºC for plasma separation. The RBC pellet which remained intact in the bottom of the tube was used for lysate preparation. Endogenous enzyme antioxidant, glutathione peroxidase (GPx) and glutathione reductase (GR) were analyzed in lysate by Paglia & Valentine (1967) method [16] and Chang et. al. (1978) [13], respectively on MULTISCAN GO with SkanIt Software (Installation code: 73617) (Cat. No.51119300, Thermo-fisher Scientific, Japan). Heavy metal analysis was performed using whole blood using microwave digestion method. Initially, 0.5 mL whole blood sample is transferred in vessel followed by addition of 1.0 mL of Milli-Q-water, 1.0 mL of hydrogen peroxide, 1.5 mL nitric acid, 0.5 mL of perchloric acid, and digested in a Multiwave Reaction System equipped with the 16HF100 Rotor (100 mL PFA vessels, pressure 40 bar) and pressure/Temperature (p/T) sensor. The clear solution obtained after Microwave digestion was analyzed by Inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 8000, Perkin Elmer) for heavy metal analysis. The statistical analysis was done employing online software Statistical Package for the Social Sciences (SPSS) 16.0 version (SPSS Inc., Chicago, Illinois, USA). The results observed were presented in mean ± SD. various circulatory parameters of controls and cases were compared using independent sample "t-tests" and ANOVA for continuous variables. The bivariate correlations between quantitative variables were determined by the Pearson's correlation. A "p" value less than 0.05 & 0.01 was considered statistically significant.

3. Results and Discussion

For comparative analysis of various parameters such as age, sex, HbA1c, eGFR, urinary albumin excretion, heavy metals, glutathione enzymes for the subjects of non-diabetic healthy control, diabetes mellitus type 2, and diabetic nephropathy (DN) are shown in Table 1. The as-mentioned data suggest that there was a significant difference in the duration of diabetes between diabetes mellitus and diabetic nephropathy groups at **p-value < 0.001**. The mean of glycated haemoglobin (HbA1c), serum urea, serum creatinine, estimated glomerular filtration rate (eGFR), and Urinary albumin excretion (UAE) were found significantly higher in diabetic nephropathy group (vis-à-vis DM and healthy control groups) **at p < 0.001.** Both Pb and Cd were separately found significant among the DN cases as compared to healthy controls (**p < 0.001**). Likewise, a significant difference was found for Pb and Cd between DM vs DN case groups for Pb (**p < 0.001)** and Cd (**p = 0.02),** respectively. No major differences were found between diabetic and non-diabetic healthy control groups for both *GPx & GR* enzymatic antioxidants. Furthermore, activities of *GPx* were found statistically significant between two groups viz., DN vs DM (**p = 0.02)** and DN vs non-diabetic healthy controls (**p < 0.001),** and in combination of all three groups **(p =0.02)**. Whereas activities of *GR were not found significantly different between any comparison groups except diabetes mellitus vs* non-diabetic healthy controls **p = 0.01**. Duration of diabetes in cases of DM has shown a negative association with HbA1c while a positive association in cases of DN for **r- values = -0.317* and 0.321*,** respectively. It has been found that a significant negative association of eGFR with UAE in DM as well as in DN group for **r- values = -0.410** and -0.446**, respectively (Table 2)**. As shown in **Table 3**, duration of diabetes has shown a positive association with GPx in DM group (**r = 0.425****) only while for DN group (**r = -0.323***) it has a negative association with lead (Pb). We could not find any significant association of eGFR, UAE, and Pb with either of parameters like Pb, Cd, GPx, or GR. Additionally, we have found a significant negative association of Cd with GPx (**r = -0.367***) and a positive association of GPx with GR in diabetic nephropathy subjects (**r = 0.411**).** In the present study age and sex-matched subjects were included in 3 groups such as diabetic nephropathy (DN), diabetes mellitus (DM) without nephropathy, and healthy control groups. Significant difference was found between the duration of diabetes between DN and DM. All clinical parameters such as glycated haemoglobin (HbA1c), estimated glomerular filtration rate (eGFR), serum urea, serum creatinine, and Urinary albumin excretion (UAE) were found to be higher in DN as compared to DM as well as healthy control. Similarly, the concentration of heavy metals (Pb and Cd), and antioxidant enzyme *GPx* were found higher in DN group (vis-à-vis DM and healthy control groups). There is a compensatory decrease in antioxidant enzyme *GR* in DN group as compared to DM and healthy control groups. Based on Pearson correlation analysis (r- value), there is a strong negative correlation with severity of diabetes (i.e., duration of diabetes) with HbA1c parameter in DM group without nephropathy and a positive association with HbA1c as well as urinary albumin excretion (UAE) in DN group. Estimated GFR has been found in strong negative association with UAE in patients of both the DN and DM without nephropathy group. In cases of DM patients without nephropathy, duration of diabetes with *GPx* and HbA1c with Cadmium has shown a positive association. On the other hand, for diabetic nephropathy cases, a negative correlation was found between the two set of parameters, i.e., (i) extent of diabetes with Pb and (ii) concentration of Cd with *GPx*. Whereas *GPx* with *GR* was found to be positively correlated.

Table 1: Comparative analysis of circulatory parameters between 3 study groups

S. No.	Parameters	Nondiabetic healthy Controls (Group I) Mean ± SD	Diabetes mellitus (Group II) Mean ± SD	Diabetic Nephropathy (Group III) Mean ± SD	Comparison between groups (P-Value)			
					I & II##	I & III ##	II & III ##	I, II, & III #
1	**Age (in years)**	50.13 ±9.55	52.60±7.27	53.05±10.11	0.09	0.09	0.40	0.30
2	**Sex (male) n**	25	20	20	0.25	0.25	1.00	0.43
3	**(females) n**	15	20	20				
4	**Duration of Diabetes (in years)**	Nil	3.21±1.91	10.50±3.61	-	-	**<0.001*** **	-
5	**HbA1c (%)**	5.01±0.50	7.98 ±0.96	9.59±2.83	**<0.001*** **	**<0.001*** **	**0.001*** **	**0.001** **
6	**Serum Urea (mg/dL)**	29.26±8.64	32.31±8.02	74.43±40.30	**0.05***	**<0.001** **	**<0.001** **	**<0.001** **
7	**Serum Creatinine (mg/dL)**	0.81±0.10	0.94±0.11	1.95±0.81	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **
8	**e GFR (CKD-EPI 2021) (mL/min/1.73m^2)**	100.85±11.42	85.43±12.18	41.05±16.38	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **
9	**Urine albumin excretion (UAE) (mg/24 h)**	16.82±2.93	20.11±3.11	427.19±207.84	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **
10	**Pb (µg/dL)**	3.57±0.49	9.26±3.90	18.95±4.63	**<0.001*** **	**<0.001*** **	**0.001*** **	**<0.001*** **
11	**Cd (µg/dL)**	0.09±0.04	0.07±0.03	0.22±0.11	**0.02***	**<0.001*** **	**<0.001*** **	**<0.001*** **
12	**GPx (U/mg/mL protein)**	1.72±0.90	1.55±1.00	2.13±0.92	0.20	**0.02***	**<0.001*** **	**0.02***
13	**GR (U/min/mg/mL protein)**	0.18±0.12	0.15±0.12	0.12±0.12	0.09	**0.01** **	0.17	0.07

*#ANOVA, ##Independent t-test, *p<0.05, **p<0.01, ***p < 0.001, considered statistically significant, HbA1c: Glycosylated Haemoglobin, Pb: Lead, Cd: Cadmium, GPx: Glutathione peroxidase, GR: Glutathione reductase. The significant p-values are represented in bold.*

Table 2: Association of Clinical Parameters among Diabetes mellitus and diabetic nephropathy groups (r- values given in the table)

S. No.	Parameters	Association of biochemical parameters					
		Diabetes mellitus			Diabetic nephropathy		
		HbA1c	eGFR CKD EPI 2021	UAE	HbA1c	eGFR CKD EPI 2021	UAE
1	**Duration of diabetes**	**-0.317***	-0.159	0.211	**0.321***	-0.169	**0.453****
2	**HbA1c**	1	0.242	0.196	1	0.096	-0.012
3	**eGFR CKD EPI 2021**		1	**-0.410****		1	**-0.446****

**p<0.05, and **p<0.01, considered statistically significant, HbA1c: Glycosylated Haemoglobin, eGFR: estimated glomerular filtration rate, UAE: Urinary albumin excretion. The significant r & p-values are represented in bold.*

Table 3: Association of Clinical Parameters among Diabetes mellitus and diabetic nephropathy groups (r- values given in the table)

S. No.	Parameters	Association of toxic and non-toxic parameters									
		Diabetes mellitus					Diabetic nephropathy				
		MDA	Pb	Cd	GPx	GR	MDA	Pb	Cd	GPx	GR
1	**Duration of diabetes**	-0.038	-0.067	-0.213	**0.425****	0.044	0.092	**-0.323***	-0.160	0.216	-0.145
2	**HbA1c**	-0.062	-0.031	**0.323***	-0.226	0.099	0.061	-0.140	-0.054	0.027	-0.034
3	**eGFR CKD EPI 2021**	0.028	-0.176	0.152	-0.063	0.278	0.208	0.011	0.082	-0.020	-0.289
4	**UAE**	-0.127	0.061	0.019	0.034	-0.150	0.076	-0.180	-0.24	0.199	0.170
5	**Pb**		1	0.180	0.082	0.138		1	0.107	0.112	0.148
6	**Cd**			1	-0.276	0.129			1	**-0.367***	-0.069
7	**GPx**				1	-0.123				1	**0.411****

**p<0.05, and **p<0.01, considered statistically significant, HbA1c: Glycosylated Haemoglobin, Pb: Lead, Cd: Cadmium, GPx: Glutathione peroxidase, GR: Glutathione reductase. The significant r & p-values are represented in bold.*

Lead (Pb) is usually ingested by drinking water or food

Lead (Pb) is usually ingested by drinking water or food and absorbed by the intestines as well as inhaled from air by lungs through environmental exposure. Circulating lead in body fluid is transported into various tissues and organs including kidneys and liver. In particular, kidney cells which are particularly susceptible to lead-induced damage such as proximal tubular cells [17], [18]. Few studies observed a decline in functioning of kidneys in middle-aged and elderly peoples who depend upon both on lead stores and their circulation in blood among diabetic individuals. [19], [20]. Heavy metals get deposited in kidney tubules resulting in their higher localized concentrations in tubular cells as compared to that in other body tissues. Such a state of overloaded heavy metals causes deterioration of the tubular cells leading to tubular proteinuria [21].

In a cross-sectional study, it was found that high cadmium level in blood is associated with DM and its complications [22]. In an experimental study, severe consequences of cadmium (Cd) toxicity caused damage to the kidneys and its functions [23]. Cadmium forms a complex with metal transporting protein metallothionein and is transported to glomeruli and subsequent filtration. Due to excessive transport and release of free cadmium ions from metallothionein-Cd^{2+} complex tubular cells are overloaded, thereby inducing oxidative stress, and downregulating mitochondrial enzymes causing kidney damage [24], [25], [26]. Biomolecules, such as albumin, metallothionein, and glutathione (GSH) contains thiol (-SH) group that exhibit good affinity to cadmium and lead ions present in blood and are therefore intracellularly detoxified by GSH. [60]. [17]. Although, heavy metal- SH molecules binding via intracellular pathways provide a protective mechanism, however, such a binding might contribute to intracellular retention of the heavy metals as well. that the above discussion clearly shows that GSH is utilized as a complexing and/or protecting agent during the exposure [27].

Using glutathione (GSH), GPx catalyzes the reduction of hydrogen peroxide or lipid peroxides to form water or lipid alcohols [28]. The role of GPx in cellular defense against oxidant attack has been discussed for many years. [29], [30] It is anticipated that the GPx protects the erythrocyte against peroxides that are generated intracellularly or exogenously. The relative importance of GPx in defending the cell has been debated since the discovery of glutathione peroxidase [31].

Chronic ingestion to toxic metals such as Pb and Cd can be hazardous to the kidneys. This will affect individuals GFR getting reduced. Patients with deranged kidney function, either associated to disease, aging, and/or a combination of both, are susceptible to these toxicants [27]. The heavy metals may be hastened out of the body by gastric lavage, activated charcoal, and skin decontamination. Supportive care can be in the form of intravenous fluids, oxygen, ventilatory, and circulatory support as needed. In severe cases, hemodialysis, and plasma exchanges. Specific therapy to remove the HM is by the administration of chelating agents, which are metal-binding ligands specific for each metal forming a ring-structure called a chelate. It is more effective when used in combination with antioxidants [32]. However, no such ideal chelator exists, and the search is on. Due to several drawbacks of chelating agents, research has focused on plant products to serve such functions. These naturally occurring phytochelatins may offer a cheaper and safer alternative, especially in economically downtrodden nations where the problem is manifold [33]. In treating lead (Pb) toxicity, DMSA (meso-2,3-dimercaptosuccinic acid) was superior to dimercaprol, also called British anti-Lewisite (BAL) plus calcium ethylene diamine tetra acetic acid. This resulted in increased Pb accumulation in various organs, including the brain. BAL was ineffective, but a combination of deferasirox and deferiprone was extremely effective in Cd toxicity [34].

4. Conclusions

Diabetic nephropathy is one of the most common health problems worldwide. Taking this serious issue into consideration, we observed heavy metal exposure in individuals and derangement in glutathione enzyme antioxidant system. In the present study, we have found that heavy metals were comparatively higher in diabetic nephropathy subjects. Glutathione peroxidase activity is negatively associated with cadmium level in blood. As the cadmium toxicity progresses glutathione peroxidase activity increases which uses substrate glutathione. A positive correlation was found between glutathione peroxidase activity and glutathione reductase. The reduced glutathione (GSH) converts itself into oxidized glutathione (GSSG) by the enzyme glutathione peroxidase and oxidized glutathione reduces itself by the enzyme glutathione reductase. Heavy metal detoxification uses glutathione and glutathione peroxidase compensatory increase in oxidized glutathione, which is normally detoxified by glutathione reductase. In case of excess oxidized glutathione, glutathione reductase might be unable to reduce all the oxidized glutathione.

Note: Authors declare that no competing interests that influenced for doing the work reported here.

Acknowledgements

This study was not funded by any funding agency or institution. I acknowledge to Dean, Doctoral Studies, and Integral University for their continuous support (MCN ref. no. IU/R&D/2023-MCN0002128). I acknowledge to Dr Jamal Akhtar Ansari, Assistant Professor, Department of Chemistry, Integral University, Lucknow, U. P. India for helping and guiding me to perform experiments. I acknowledge all authors of this study, colleagues, and lab staff for their co-operation.

References

[1] H. Sun , P. Saeedi , S. Karuranga , M. Pinkepank , K. Ogurtsova , B. B. Duncan , C. Stein , A. Basit , J. C. N. Chan , J. Claude Mbanya. et al., (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Research and Clinical Practice. 183: 109119.2.

[2] N. Vodošek Hojs, S. Bevc, R. Ekart, R. Hojs. (2020). Oxidative stress markers in chronic kidney disease with emphasis on diabetic nephropathy. Antioxidants. 9(10): 925.

[3] N. Speck and T. Stacy. (1995). Critical reviews in eukaryotic gene expression. Begell House New York. Critical Reviews™ in Eukaryotic Gene Expression. 5 (3-4).

[4] A.R. Khan and F.R. Awan. (2014). Metals in the pathogenesis of type 2 diabetes. Journal of Diabetes & Metabolic Disorders. 13(1): 16.

[5] D. Witkowska, J. Słowik, and K. Chilicka. (2021). Heavy metals and human health: Possible exposure pathways and the competition for protein binding sites. Molecules. 26(19): 6060.

[6] R. Wang, T. Long, J. He, Y. Xu, Y. Wei, Y. Zhang. (2022). Associations of multiple plasma metals with chronic kidney disease in patients with diabetes. Ecotoxicology and Environmental Safety. 244: 114048.

[7] M. Balali-Mood, K. Naseri, Z. Tahergorabi, M.R. Khazdair, M. Sadeghi. (2021). Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. Frontiers in Pharmacology. 227.

[8] A Javaid , I Akbar , H Javed , U Khan , H Iftikhar , D Zahra , F Rashid , U. A. Ashfaq. (2021). Role of Heavy Metals in Diabetes: Mechanisms and Treatment Strategies. Critical Reviews in Eukaryotic Gene Expression. 31(3): 65-80.

[9] M A Riaz, Z U Nisa , M Anjum , H Butt , A Mehmood, A Riaz 4, A B T Akhtar. (2020). Assessment of metals induced histopathological and gene expression changes in different organs of non-diabetic and diabetic rats. Scientific Reports. 10(1): 5897.

[10] H Wan, S Chen, Y Cai, Y Chen 1, Yuying Wang 1, Wen Zhang 1, Chi Chen, N Wang, Y Guo, Y Lu. (2021). Lead exposure and its association with cardiovascular disease and diabetic kidney disease in middle-aged and elderly diabetic patients. International Journal of Hygiene and Environmental Health. 231: 113663.

[11] S Yimthiang, D A Vesey, P Pouyfung, T Khamphaya, G C Gobe, S Satarug. (2023). Chronic Kidney Disease Induced by Cadmium and Diabetes: A Quantitative Case-Control Study. International Journal of Molecular Sciences. 24(10): 9050.

[12] H. Waggiallah and M. Alzohairy. (2011). The effect of oxidative stress on human red cells glutathione peroxidase, glutathione reductase level, and prevalence of anemia among diabetics. North American journal of medical sciences. 3(7): 344.

[13] J C Chang, L H van der Hoeven, C H Haddox. (1978). Glutathione reductase in the red blood cells. Annals of Clinical and Laboratory Science. 8(1): 23-29.

[14] American Diabetes Association Professional Practice Committee, & American Diabetes Association Professional Practice Committee:. (2022). 16. Diabetes care in the hospital: Standards of Medical Care in Diabetes—2022. Diabetes care, 45(Supplement_1). S244-S253.

[15] T Wang, Z Lv, X Fu, S Zheng, Z Yang, X Zou, Y Liu, Y Zhang, Y Wen, Qi Lu., et al. (2022). Associations between plasma metal levels and mild renal impairment in the general population of Southern China. Ecotoxicology and Environmental Safety. 247: 114209.

[16] D.E. Paglia, W.N. Valentine. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine. 70(1): p. 158-169.

[17] J L Reyes, E Molina-Jijón, R Rodríguez-Muñoz, P Bautista-García, Y Debray-García, M D Carmen Namorado. (2013). Tight junction proteins and oxidative stress in heavy metals-induced nephrotoxicity.

[18] H Wang, Z K Wang, P Jiao, X P Zhou, D B Yang, Z Y Wang, L Wang. (2015). Redistribution of subcellular calcium and its effect on apoptosis in primary cultures of rat proximal tubular cells exposed to lead. Toxicology. 333: 137-146.

[19] S W Tsaih, S Korrick, J Schwartz, C Amarasiriwardena, A Aro, D Sparrow, H Hu. (2004). Lead, Diabetes, Hypertension, and Renal Function: The Normative Aging Study. Environmental Health Perspectives. 112(11): 1178-1182.

[20] F Harari, G Sallsten, A Christensson, M Petkovic, B Hedblad, N Forsgard, O Melander, P M Nilsson, Y Borné, G Engström., et al. (2018). Blood lead levels and decreased kidney function in a population-based cohort. American Journal of Kidney Diseases. 72(3): 381-389.

[21] T Ruge, A C Carlsson, T E Larsson, J J Carrero, A Larsson, L Lind, J Ärnlöv. (2014). Endostatin level is associated with kidney injury in the elderly: findings from two community-based cohorts. American Journal of Nephrology. 40(5): 417-424.

[22] P Gong, P Wang, S Pi, Y Guo, S Pei, W Yang, X Chang, L Wang, and F Chen. (2022). Proanthocyanidins protect against cadmium-induced diabetic nephropathy through p38 MAPK and Keap1/Nrf2 signaling pathways. Frontiers in Pharmacology. 12: 801048.

[23] N.F. Nordberg. (2009). Historical perspectives on cadmium toxicology. 238(3): 192-200.

[24] V. Eybl, D. Kotyzova, and J.J.T. Koutensky. (2006). Comparative study of natural antioxidants–curcumin, resveratrol and melatonin–in cadmium-induced oxidative damage in mice. Toxicology. 225(2-3): 150-156.

[25] T K Dua, S Dewanjee, R Khanra, N Bhattacharya, B Bhaskar, M Zia-Ul-Haq, V D Feo. (2015). The effects of two common edible herbs, Ipomoea aquatica and Enhydra fluctuans, on cadmium-induced pathophysiology: a focus on oxidative defence and anti-apoptotic mechanism. Journal of Translational Medicine. 2015. 13(1): 1-19.

[26] Y Liang, L Lei, J Nilsson, H Li, M Nordberg, A Bernard, G F. Nordberg, I A. Bergdahl, and T Jin. (2012). Renal function after reduction in cadmium exposure: an 8-year follow-up of residents in cadmium-polluted areas. Environmental Health Perspectives. 120(2): 223-228.

[27] J Aaseth, J Alexander, U Alehagen, A Tinkov, A Skalny, A Larsson, G Crisponi, and V M Nurch. (2021). The aging kidney—As influenced by heavy metal exposure and selenium supplementation. Biomolecules. 11(8): 1078.

[28] K Mohammedi, T A Patente, N Bellili-Muñoz, F Driss, H Le Nagard, F Fumeron, R Roussel, S Hadjadj, M L Corrêa-Giannella, M Marre. et al., (2016). Glutathione peroxidase-1 gene (GPX1) variants, oxidative stress and risk of kidney

complications in people with type 1 diabetes. Metabolism. 65(2): 12-19.

[29] L D Vega, R P Férnandez, M C M Mateo, J B Bustamante, A M Herrero, E B Munguira. (2002). Glutathione determination and a study of the activity of glutathione-peroxidase, glutathione-transferase, and glutathione-reductase in renal transplants. Renal Failure. 24(4): 421-432.

[30] M Robin L. V Dianne, M Brenda S, C James P. (2002). Oxidant stress and reduced antioxidant enzyme protection in polycystic kidney disease. Journal of the American Society of Nephrology. 13(4): 991-999.

[31] R M Johnson, G Goyette Jr, Y Ravindranath, Y S Ho. (2000). Red cells from glutathione peroxidase-1–deficient mice have nearly normal defenses against exogenous peroxides. The Journal of the American Society of Hematology. 96(5): 1985-1988.

[32] J J Kim 1, Y S Kim 1, V Kumar. (2019). Heavy metal toxicity: An update of chelating therapeutic strategies. Journal of Trace elements in Medicine and Biology. 54: 226-231.

[33] C N Amadi, S J Offor, C Frazzoli, O E Orisakwe.(2019). Natural antidotes and management of metal toxicity. Environmental Science and Pollution Research. 26: 18032-18052.

[34] M R Rahimzadeh, M R Rahimzadeh, S Kazemi, A Moghadamnia. (2017). Cadmium toxicity and treatment: An update. Caspian Journal of Internal Medicine 8(3): 135.