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Lead Toxicity & Potential Health Hazards

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Lead has been recognized as one of the most serious environmental poisons amongst the toxic heavy metals and has recently been the focus of public health regulations in most of the developed world. Lead intoxications particularly nephrotoxicity, hepatotoxicity and neurotoxicity are now widely known. Health risks are increasingly associated with occupational exposures to lead and its derivatives. Occupational lead exposures occur during lead smelting processes, manufacture of batteries, painting, printing and pottery glazing. Exposure occurs mainly through the respiratory and gastrointestinal systems. The ingested or absorbed lead is conjugated in liver and passes to the kidney, where a small quantity is excreted in urine and rest accumulates primarily in soft tissues and bones. Lead is a divalent cation with a propensity to settle in the proximal tubule of nephron leading to hyperuricaemia and gout by inhibiting uric acid secretion and diminished glomerular filtration rate (GFR). Acute, high dose lead-induced impairment of proximal tubular function manifests in aminoaciduria, glycosuria and hyperphosphaturia (a Fanconi-like syndrome). These effects appear to be reversible, however, continued or repetitive exposures can cause a toxic stress on the kidney that, if unrelieved, may develop into chronic and often irreversible lead nephropathy (interstitial nephritis). Recent studies demonstrated that lead like other toxic metals (cadmium, mercury and arsenic) act as catalyst in the oxidative reactions of biological macromolecules, hence the toxicity associated with this metal might be due to oxidative tissue damage. Lead depletes cell's major antioxidants, particularly thiol containing antioxidants and enzymes by generating reactive oxygen species (ROS) such as hydroxyl radicals (HO^{*}), superoxide radicals (O_2^{*-}) and hydrogen peroxide (H_2O_2). Enhanced generation of ROS can overwhelm cells intrinsic antioxidant defenses, results in ''oxidative stress''. Cells under oxidative stress display various dysfunctions due to reaction of ROS to lipids, proteins and DNA. Therefore it is concluded that lead-induced oxidative stress in cells can be particularly responsible for its deleterious toxic effects. This article also discusses that supplementation of antioxidants along with a chelating agent prove to be better in earlier improvements. Therefore keeping in view current concerns and literature available we have selected lead for this article.

Sources associated with lead intoxication

In developing countries like India lead water pipes, lead-acid batteries, lead food cans, lead containing cosmetics, paints, lead as anti-knocking agent in petroleum and lead oaring, mining are the constant sources of lead intoxication (Sharma and Singh, 2014). Lead compounds are used in petrol to increase the efficiency of car engines and to prolong engine life by reducing knock. Accelerated growth in vehicle population has led to increased release of lead in the environment. Occupational exposure of lead to factory workers is a problem for both developing and developed countries. Its unique properties like softness, ductibility, malleability, low melting point and corrosion resistance, have made lead useful in different industries associated with the manufacturing of storage batteries, packing material, food cans and beverages, lead pipes, paints and protective coatings and explosive industry. Prolonged exposure of workers in these factories makes them prone to lead intoxication. Lead toys and suckling lead paints are well-established cause of lead poisoning and elevated blood lead concentrations in children, in developing countries. In New Zealand, the blood lead concentrations fell by 42% between 1978 and 1985 after lead-based paints and varnishes were abandoned and lead-soldered food and drink cans were replaced by seamless-welded containers. Cigarettes contain 2.4μg of lead and 5% of this occurs in ash and side stream smoke (Mussalo-Rauhmaa et al., 1986).

Some nutritional factors associated with susceptibility to lead are total food intake, sources of calories, calcium phosphorus, iron, zinc and various vitamins (thiamine, ascorbic acid and vitamin E). Many studies have revealed that lead absorption has been shown to be greatly increased during fasting and with diets rich in fat or poor in calcium (3). Low calcium dietary intake also produces an increase in lead toxicity and deposition in bone. Iron deficiency is a widespread problem and results in an increased susceptibility to lead toxicity (3, 11, 12). Generally, calcium and iron are the most important of these factors, and during pregnancy their importance is enhanced due to changes in calcium and iron metabolism and increased demand (10). In addition, Pb can also be removed from storage sites in bone in response to pregnancy (2, 13).

Effect of lead on absolute body and kidney weight

Chronic lead exposure leads to gradual loss of body weight. This might be due to nausea, vomiting anorexia. Buck et al (1996) explained that loss of body weight may be due to decreased muscle mass and cachexia due to oxidative stress induced by lead. Another explanation for decrease body weight was due to reduced food consumption via lead effects on the satiety set-up. The anorectic effect exercised by

this toxic metal used to justify its involvement in the nerve transmission system (catecholaminergic, glutamatergic and serotonin). In addition to anorectic effect water consumption decreased significantly in lead treated animals compared to normal (Fromentin, 2005). This decreased water intake would be related to dose, which explained the fall in the body weight of treaties.

Although there was not a significant change in the kidney weight after low-dose lead exposure for one week, however relative weight of the kidney was significantly higher in lead treated mice groups exposed to 2-3 weeks. Possible mechanisms for increase in the kidney weight may be the formation of nuclear inclusion bodies, initial DNA replication and proximal tubular proliferation induced by lead as explained by Choie and Richter (29). Other possible explanation for initial increase in absolute kidney weight may be due to the accumulation of lead in the form of nuclear inclusion bodies in the proximal tubular cells. Although absolute kidney weight was markedly reduced due to chronic lead exposures (Afonne et al., 2000).

Effect of lead on renal tissue histology

The presence of eosinophilic proximal tubular nuclear inclusion bodies once thought to be manifestations of lead nephropathy, during the early years of lead exposure. However the chronic sub-toxic lead exposure resulted in granular, contracted kidney appearance, progressive tubular, glomerular and interstitial alterations. Some of these findings are in agreement with results of previous investigators (Sushma Sharma and Balbinder singh, 2014). The varieties in the alterations of the renal tissue histology due to lead intoxication by different investigators could be due to the variations in the level of lead exposure, duration, route of administration and animal species used in experiments (ceruti et al., 2002). The data of previous research demonstrated that cortex is more affected than medulla due to long term treatment with lead. This could be due to uneven distribution of lead in the kidney tissue, where about 90% of the total renal blood flow enters the cortex via blood stream. Therefore, a relative high lead concentration might reach the cortex. The results also demonstrated that tubular changes occur earlier than glomerular and interstitial changes. Moreover, tubular damages were more prominent in proximal convoluted tubules (PCT) than distal convoluted tubules (DCT). This could be due to the reason that the PCT's are the primary sites of reabsorption and active transport leading to higher concentration of lead in the epithelial lining of these tubules (Jarrar et al., 2007).

Necrosis, tubular vacuolization and tubular dilation were also reported by the workers (Diamond, 2005). These tubular alterations due to lead toxicity might be a result of hydraulic changes in renal tissue and suggest that lead intoxication yields to

a partial failure in the ion pump transport of tubular cells, which in turn produces tubular swelling and causes necrosis and vacuolization of tubules(Senapati et al., 2001; Bellinger, 2008; Sharma et al., 2011). These findings also indicate incapability of renal cells to deal with accumulated residues resulting from metabolic structural disturbances caused by lead.

There is experimental evidence to indicate that the incidence and severity of renal histopathological changes increased with increasing dose, where the kidney of high dose treated mice showed extensively atrophied glomerulus with increased perivascular space, MNC's infiltration in interstitial spaces, hemorrhages and cell debris in tubular lumen (Sushma Sharma and Balbinder Singh, 2014. The histopathological alterations described above confirm to the generally accepted classification of tubulo-interstial nephritis. The pattern which emerges in the lead exposed animals is a progressive nephritic syndrome affecting first tubules and surrounding blood vessels with secondary to glomerular lesions involving the epithelial cells and capillary loops. In conclusion, lead toxicity results in alterations in the renal tubular and glomerular cells, which could play an important role in renal dysfunction.

Effect of lead on renal function

Impaired kidney functions have been reported as one of the most silent feature of heavy metal toxicity. Mostly renal dysfunction occurs at high levels of lead exposure (>60μg/dL) but alteration at lower levels (~10μg/dL) has also been reported (Grant, 2008). Moreover, many studies have shown a strong association between lead exposure and renal effects. Chronic, high dose lead-induced impairment of proximal tubular function manifests in aminoaciduria, glycosuria, hyperphosphaturia (Toni-Debre-Fanconi like syndrome), β-2-microglobinuria and proteinuria. Proteinuria due to lead induced kidney function impairment may be a cause of protein loss among the animals because lead has inhibitory role in RNA and DNA synthesis. Protein loss during lead intoxication might decrease the level of specific proteins such as albumin, hormones, hormone binding proteins, metal binding proteins, drug binding proteins and enzymes and therefore disturb the homeostasis and rate of metabolic activities. These effects appear to be reversible; however, continued or repetitive exposures can cause a toxic stress on kidney.

Urinary chemistry is a marker factor to provide a good indication of kidney impair functions. Urinary volume is markedly increased in lead treated groups. Missoun et al, (2010) reported increased urine volume in lead treated mice groups. This increased urine volume might be due to diuretic effect of lead or high concentration of calcium in plasma. However no variation in urinary pH and specific gravity was

observed. Ghorbe (2001) showed that lead induces a decrease of urinary pH observed after 45th day of experiment.

Lemann et al, (1991) reported that the presence of crystals is a risk factor in the pathogenesis of hypercalciuria than renal failure. The amount of crystalluria depends on the level of super saturation above the formation product, but also is influenced by the Ca/Ox ratio. Increased urinary calcium concentration is a key factor favouring the nucleation and precipitation of calcium oxalate or apatite from urine and subsequent crystal growth. Crystal growth is induced by the presence of proteins in urine. Super saturation of urinary colloids results in precipitation as a crystal initiation particle which when trapped, acts as nidus, leading to subsequent crystal growth (Grover and Ryall, 1997). Cantarow et al, (1962) reported that the presence of leucocytes and cylinders in urine of treated rats may be due to the inflammation in urinary tract caused by lead. Staessen et al (1990) reported that increase of calcium and phosphorus ion in urine and significant increase of calcium in blood due to lead may be as a result of impairment of renal function or inhibitory action of lead on cation transport in tissues of rats.

Lead also has an inhibitory effect on depolarization induced Ca^{2+} uptake and the inhibition was found to be a competitive one. These results suggest that lead calcium messenger system which would have serious consequences on the concentration of calcium in blood, this high concentration may induced hypercalciuria and polyurie. Which is beginning of dehydration of rats and alteration of glomerular filteration rate (GFR) as reported by Staessen et al (1990). This gradual increase in urinary phosphorus and calcium may lead to the formation of calcium phosphate crystals (Lemman et al., 1991). Cameron and Greger, (1998) observed decrease in urine and elevation in the serum of creatinine caused by lead suggest that renal function impairment which might result from intrinsic renal lesions, decreased perfusion of the kidney obstruction of lower urinary tract or due to deranged metabolic process caused by this metal. Rats treated with lead had significantly decreased urine urea and slight increase in serum urea. Serum urea has been reported to increase in acute and chronic intrinsic renal diseases and also when there is decreased effective circulating blood volume with decreased renal perfusion (Cameron and Greger, 1998).

Ghorbe et al (2001) reported that oral lead acetate administration caused significant increase in the blood urea and serum creatinine. The reduction in serum glucose concentration following the administration of lead may be due to the inhibitition of the uptake and transport of glucose by lead (Fowler et al., 1980).

In conclusion increased fractional excretion of calcium and phosphorus, the increase of uremia and creatinemia, the increase of serum calcium concentration and the decrease of creatinuria and glycemia, clinically signifies renal impair functions.

Biomarkers used in assessing renal function

The clinical diagnosis of lead nephropathy is complex because the symptoms of lead-intoxication are variable and non-specific as a result of sub- clinical nephrotoxicity from low level lead exposure. The assessment of renal function in lead nephropathy involvs the evalution and assessment of tubular and glomerular parameters (103,104). The assessment of tubular function is important in the evaluation of lead nephropathy as chronic lead nephropathy can be missed in its early stages because changes induced by chronic low level lead exposure are subtle and not reflected by changes in routine renal function tests (104). Lead induced renal disfuntion can be detected in its early stages through the measurement of proteins and enzymes from the tubuls and glomeruli, excreted in urine which reflect the functional integrity of these portions of kidneys (103, 105). Various markers of proximal tubular integrity, such as α1-microglobulin, β-microglobulin, retinol binding protein and enzymes such as lysozyme, ribonuclease, N-acetyl-β-D-glucosaminidase (NAG), alanime aminopeptidase (AAP), alkaline phosphate (ALP) and γ-glutamyltransferase (GGT) and distal Tamm-Horsfall glycoprotein, enzyme kallikrein have been used and tried. Glomerular urinary markers are the high molecular weight proteins, transferrin, immunoglobulin G (IgG) and albumin. The urinary levels of these markers are usually not significantly altered in lead nephropathy as observed in various studies (84, 104, 106). The tubular urinary markers have been used the subject of various studies attempting to identify sensitive and specific markers of lead nephropathy, useful in early diagnosis. Various studies have shown that urinary NAG is a sensitive biomarker in detecting early tubular impairments (54,80, 81,82). Pergande et al () observed increased excretion of urinary α1-microglobulin, ribonuclease and Tamm- Horsfall protein and recommended that the combined determination of NAG and α1-microglobulin in urine may be useful in early diagnosis on lead induced damages in the kidney. Jung et al.108 proposed the use of urine α1-microglobulin as an early and reliable indicator of lead nephropathy.

Sr. No.	Renal Functional markers	Renal Biochemical markers	Renal Cytotoxic markers
1	Creatinine in urine (Crt-U)	Fibronectin	Brush border tubular antigens (BBA, BB_{50} and HF_5)
2	Creatinine in serum	Thromboxane (TXB_2)	β-galactosidase (β-g)

	(Crt-S)		
3	Urinary proteins of low and high molecular weight (3.1) Low molecular weight proteins: retinol binding proteins (RBP), β-microglobulin (β_2-m), urinary α-1-microglobulin (Uα-1-m). (3.2) High molecular weight proteins: Albumin, transferrin and immunoglobulin G	Eicosanoids-6-keto-prostaglandin $F_1\alpha$ (6-keto-$PGF_1\alpha$), Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and Prostaglandin E_2 (PGE_2)	
4	Urinary N-acetylglucosaminidase (NAG)	Urinary Sialic acid activity, Sialic acid in plasma or in RBCs	
5	Alkaline phosphate (ALP)	Urine Kallikrein activity	
6	γ-glutamyltransferase (GGT)	Urinary glycosaminoglycans (GAG)	

Earlier effects of lead intoxication are manifested by decrease in PGE_2, $PGF_2\alpha$ and enhanced excretion of TXB_2, because lead interefers with renal synthesis of eicosanoids, resulting in lower excretion of 6- keto-PGFα and enhanced excretion of TXB_2. It is generally considered that urinary 6-keto-$PGF_1\alpha$ and TXB_2 mainly affect the glomerular synthesis of prostacyclin and TXA_2. Which in turn decrease PGE2 and $PGF_2\alpha$ by renal medulla (29,30). The effect of lead on urinary excretion of 6-keto-PGFα and TXB_2 demonstrated that earlier changes due to Pb might also involve yhe vasculature and glomeruli and are not exclusively restricted to localized tubulointerstitial compartment (32).

Increase excretion of Tamm-Horsfall glycoprotein (THG) is also a sensitive biomarker of early renal effect induced by lead. This increase may be due to the injury to the epithelial cells os ascending limb of loop of Henle and proximal part of distal convoluted tubule where THG is located. One of the most important function of THG is to make the ascending limb of loop of Henle impermeable to water, transport to sodium and defense aganst infection and immunoregulation of several cytokines (22,32). Urinary Kallikrein is a serine protease synthesized by distal tubular cells. As most of the kallikrein is associated with the membranes that face urinary

compartment, increased urinary Kallikrein excretion could result from lead induced damage in the distal tubular cells. GAGs are polysaccharides composed of repetitive disaccharide units is located abundantly in renal papilla. They are also found in glomeruli and tubules, their leakage into urine has been diagnosed to be a marker of nephron injury(24).

Pathophysiology of lead nephropathy

Lead storage and excretion

Lead nephropathy results primarily because the kidney is a major route for the elimination of lead via glomerular filtration and tubular secretion in addition to the kidney also being a minor storage site for absorbed lead following prolonged excessive absorption (3, 7, 12). The tubule-interstitial structure of kidney are particularly susceptible to the toxic effects of lead due to the high reabsorptive activity of the tubule which results in high concentration of lead in the already poorly perfused medulla (123). Following its uptake, lead is absorbed by the proximal tubular cells where it binds to specific lead binding proteins forming lead-protein complexes. The lead binding proteins which are postulated to facilitate the movement of lead across the mitochondrial membranes of renal tubular cells possess genetic variability in their expression which is thought to determine or modulate the individual differences in the susceptibility of lead nephropathy (3, 47).

Renal mitochondria structural and functional alterations

Following its movement into the mitochondria of renal tubular cells, lead accumulates in the mitochondria resulting in both structural and functional alterations (123, 7, 12). These alterations result in mitochondrial swelling and inhibition of respiratory function and energy [adenosine triphosphate (ATP)] production. This disruption of energy production impairs energy dependent processes including tubular transport leading to the proximal tubular reabsorptive defects which leads to the features of Fanconi syndrome and hyperuricemia (3, 15, 17). The impairment of mitochondrial enzyme function is another important pathogenetic process of lead toxicities (2, 3, 7.12) as the activity of mitochondrial enzymes such as aminolevulinic, ferrochelatase and vit-D hydroxylase are inhibited.

Effect of lead on antioxidant defense system

The mechanism of lead induced oxidative stress is not fully understood, data is accumulating to support the evidences indicating that multiple mechanism balance between reactive oxygen metabolites and antioxidant defense results in" oxidative stress" (). Participation of iron in fenton reaction in vivo, leading to production of

more reactive hydroxyl radicals from superoxide radicals and H_2O_2 (Halliwell, 1994) results in increased lipid peroxidation. This might be one of the reasons for significant alteration in lipid peroxidation (LPO) and significant changes in the activity of antioxidant enzymes. Usually the deleterious effects of oxidative stress are counteracted by endogenous antioxidant enzymes, mainly, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). The binding activity of lead compounds with oxidative stress factors and the gene erythropoiesis ratio of reactive oxygen species, such as hydrogen peroxide and its interaction with different metals and also toxic activity of delta-aminolevulinic acid (ALA) are reported earlier (Ariza et al., 1998; Ding et al., 2000).

SOD and CAT are considered primary enzymes since they are involved in direct elimination of reactive oxygen species (ROS). SOD plays an important role in protecting the cells against the toxic effects of O_2^- by catalyzing its dismutation reactions. The enzyme requires copper and zinc for its activity. Copper ions appear to have a functional role in the reaction by undergoing alternate oxidation and reduction, where zinc ions seem to stabilize the enzyme instead of having a role in the catalytic cycle (Halliwell and Gutteridge, 1989). SOD keeps the concentration of superoxide radicals at low levels and therefore plays an important role in the defense against oxidative stress (Fridovich, 1997). Various findings demonstrated that lead has inhibitory effects on SOD and CAT, also found to inhibit antioxidant enzymes involved in the prevention of lipid peroxidation such as SOD and CAT (Soltanianejad et al., 2003; Vaziri et al., 2003). The biological role of SOD is to dismutase superoxide ion, hydrogen peroxide (H2O2) produced in this reaction is eliminated by catalase, one of the most active enzymes in the human beings.

Catalase is a heme protein, which catalyzes the reduction of hydrogen peroxides to oxygen and water and protects tissues from highly reactive hydroxyl radicals. Various reports regarding influence of lead on SOD and CAT activities have given divergent results. Some studies showed decreased activities of SOD and CAT (Ramstoeck et al., 1980) and others showed increased activities (Adler et al., 1993; Ahmed et al., 2006). Superoxide anions (O^{2-}) itself directly affects the activity of catalase by affecting intracellular enzymes (Ghosh and Myers, 1998), creatine phosphokinase (Lee et al., 1998). SOD was found to be decreased in the treated animal's tissues particularly in liver, kidney and testis (Sharma et al., 2011). Decrease in SOD was explained by direct blocking action of the metal on –SH group of the enzyme (Kasperczyk et al., 2004). Lower activities of CAT and SOD may be explained by the interaction between lead and essential metals such as copper, zinc and iron. Copper and zinc are essential cofactors for SOD, where as CAT also has heme as the prosthetic group, the biosynthesis of which is inhibited by lead (Patil et

al., 2006). Several studies reported alterations in antioxidant enzyme activities such as SOD and CAT and glutathione peroxidise (GPX) in lead exposed animals (Sugawara et al., 1991; Solliway et al., 1996; Gayathri et al., 2007). These findings suggest a possible involvement of oxidative stress in the pathophysiology of lead toxicity.

One of the effects of lead exposure is on glutathione metabolism. GSH is very helpful in the detoxification and excretion of heavy metals. Glutathione is a cysteine-based molecule produced in the interior compartment of the lymphocyte. More than 90 percent of non tissue sulphur in the human body is found in the tripeptide glutathione (Meister and Anderson, 1983). In addition to acting as an important antioxidant for quenching free radicals, glutathione is a substrate responsible for the metabolism of specific drugs and toxins through glutathione conjugation in the liver (Meister and Anderson, 1983). The sulfhydryl complex of glutathione also directly binds to toxic metals that have a high affinity for sulfhydryl groups.

Cervello et al. (1992) demonstrated that GST enzyme catalyzes the reaction via the thiol (-SH) group of glutathione, thereby neutralizing and rendering the products more water soluble. Taking into account mutual relations GST and GSH in the redox system, the simultaneous decrease in both GST activity and GSH concentration may suggest that the decrease in renal GSH concentration might result, at least partly, from the decrease in GST activity (Newairy and Abdou, 2009). The decrease in GST activity after exposure to lead could be caused by lead-induced changes in the enzyme structure as well as by the lack or insufficient amount of GSH, being a substrate for this enzyme (Neal et al., 1999). Glutathione reductase, the enzyme responsible for recycling of glutathione from the oxidized form (glutathione disulfide; GSSG) to the reduced form (reduced glutathione; GSH) was also deactivated by lead.

Sugawara et al. (1991) have reported a significant decrease in GSH contents of erythrocytes from workers exposed to lead. Indirect depletion of GSH may occur when lead inhibits enzymes and aminolevulinic acid dehydratase (ALAD) before it catalyzes the condensation of two molecules of d-aminolevulinic acid (δ) to porphobilinogen (Haeger-Arosen et al., 1971). When the activity of ALAD is inhibited an effect of lead exposure which has been confirmed experimentally by several authors, the amount of δ-ALA increased (Ribarov and Bochev, 1982; Gibbs et al., 1991). Since δ-ALA itself is known to be a potent inducer of lipid peroxidation (LPO) and ROI formation both in vivo and in vitro, its accumulation may facilitate the depletion of GSH from lead burdened cells (Monteiro et al., 1986). The involvement of ROS in Pb poisoning has been addressed by Schwartz et al. (2000) who found a decreased in GSH and an increase in oxidized glutathione (GSSH) concentration in

lead acetate treated rats. In addition, they also found that the effect was reduced by treatment with N-acetyl cystein, a precursor of GSH. This proved a possibility of antioxidant therapy for individuals who were exposed to lead. GSH/GSSH ratio is an important component of anti oxidant defense system in mammalian cells, which was considered a sensitive indicator of oxidative stress (Wilson et al., 2000).

Kidney enzymes such as ALT, AST, ACP and ALP are the marker enzymes for kidney function evaluation. The levels of these enzymes are generally raised in acute nephropathy or due to mild renal cellular injury, however tend to decreased with prolonged intoxication due to sever damage to renal tissue. The possible mechanism behind lead induced decreased level of these enzymes is attributed to its binding with sulfydryl groups of enzymes containing cysteine and also found to form complexes with amino acids and proteins. Lead alters the level of ALT in the tissues by altering their membrane leading to discharge of cell contents.

Alkaline phosphatase (ALP) is a marker enzyme for any damage to endoplasmic reticulum and plasma membrane (Shahjahan et al., 2004). It is oftenly used to check the membrane integrity (Akanji MA, Olayoke, 1993) and needed in certain amounts for proper functioning of organs (Brain and Kay, 1927). Any increase in ALP level following lead exposure illustrates disruption of lipid bilayer of membrane structure of affected organs. In kidney rich sources of ALP are proximal tubules (Verley, 1967). Any damage to these tubules would leads to elevated level of ALP in serum. It is also investigated that lead induces the biosynthesis of ALP in kidney before the disruption of cellular integrity, if any, usually occurs by the way of lipid peroxidation (Sarkar et al., 1995).

Lipid peroxidation, a basic cellular deteriorative change, is one of the primary effects induced by oxidative stress and occurs readily in the tissues due to presence of membrane rich in polyunsaturated highly oxidizable fatty acids (Cini et al., 1994). Lead, being a heavy metal and potent environmental pollutant in elicits variety of toxic manifestations in the living systems (perlstein and Attala, 1996). The toxic effect of lead in various tissues/organs have hardly been believed due to some peroxidative activities, expect in few tissues (Quinlan et al., 1988; Acharya et al., 1994). Lead binds to plasmic protein, where it causes alterations in high number of enzymes. Georinge (1993) found that lead can also disturb protein synthesis in hepatocytes. The decreased in protein content of mice may be due to hepatic DNA and RNA (Shalan et al ., 2005). B-2-microglbinuria and enzymuria were reported in lead toxicity in children (Gourrier et al., 1991). Hassanin (1994) observed decreased in hepatic total protein content in response to lead intoxication. This may be because Pb^{2+} disturbs intracellular Ca^{2+} homeostasis (simons, 1993) and damages the endoplasmic reticulum which inturn results in reduction protein synthesis. In

addition, lead has been shown to enter in cells through voltage dependent Ca^{2+} channels at a higher rate than Ca^{2+} as an intracellular secondary messenger. Interaction between lead and two second messenger mediators of Ca^{2+} signals (Calmodulin and protein kinase C) have been studied extensively (Goldstein, 1993). Calmodulin exhibits a higher affinity for lead than it does for Ca^{2+} , leading to an up regulation of the enzymes (Habermann et al., 1983).

The generation of reactive oxygen species such as superoxide ions, hydrogen peroxides and hydroxyl radicals or by products of lipid peroxidation such as lipid hydroperoxides and lipid aldehyde (1, 5) have implicated in lead toxicity. These lipid hydroperoxides are formed due to oxidation of lipids and cholesterol containing cellular molecules like cell membrane phospholipids, lipoproteins, glycolipids and other lipid containing molecules. The oxidation is usually caused by ROS like oxy radicals, peroxy radicals and hydroxyl radicals. The balance between the production of oxidants and their scavenging by antioxidants determines the extent of lipid peroxidation (). Increased lipid hydroperoxides could be explained by lead-induced inhibition of free radicals scavenging enzymes, leading to the accumulation of ROS to accumulate and cause increased oxidation in various body organs(). Other reason for LPO increase may be due to the combined inhibitory effects of the various antioxidants enzymes (SOD, CAT and GSH).

Guidelines and possible treatments for lead toxicity

Although various occupational and public health measures have been undertaken in order to control lead exposure, cases of lead poisoning are still reported. Possible measures of lead toxicity depend upon the blood lead levels and age of intoxicated population. Researchers have proved that children are more prone to lead toxicity and are more severely affected than adults; therefore timely diagnosis and treatment of these children produce significant alteration in their clinical manifestations and neuropsychological impairment. Several therapies used chelators and dietary antioxidant supplements to remove toxic metal burden from the body. These involve oral or and parenteral administration of chelating agents which form complexes with divalent cations including lead, zinc, magnesium and calcium and enhance their excretion by kidneys (28). Several chelating compounds such as Dimercaptosuccinic acid (DMSA) and Sodium 2, 3-dimercapto-1-propanesulfonate (DMPS) have been used to manage lead toxicity. After world war-II, D-penicillamine and BAL were clinically used against lead and mercury intoxications. However China and Soviet Union used chelating compounds (DMSA and DMPS) during 1950s. Interestingly these drugs have been made available in western world since 1970s. Penicillamine, DMPS, DMSA and water soluble derivatives of BAL (BAL) are oral chelating agents. It is important to note that D-Penicillamine can be used only when

the treaties have been totally remove from lead exposure sources, because it can enhance gastrointestinal absorption of ingested lead (132). Moreover DMSA has been approved for treating children with lead intoxication. However DMSA and DMPS are efficiently used against several divalent metal insults besides lead and mercury. Although chelating therapy is the main stay of treatment, therapy also has very high incidence of side effects and a considerable fraction of individual experience nausea, vomiting, sweating, high fever, hypertension and tachycardia. BAL is more toxic than DMPS as its administration increasing the deposition of arsenite and organic mercury compounds in brain and increased the toxicity of cadmium and lead as shown in various animal experiments (32). Moreover, these chelators often fail to remove Pb burden from all body tissues hence are potentially toxic (Gilman et al., 1991). DMSA and DMPS induced adverse effects include gastrointestinal disorders, skin reactions and elevated renal enzymes. Mostly the people prone to allergy may develop hypersensitivity to DMPS (33). In addition to these side effects of chelation therapy, deaths may occur due to hypocalcaemia because of excessive removal of calcium ions from the body along with other divalent cations (34). As mentioned earlier chelation therapy has high incidence of side effects, research has been carried out for exploring other means to minimize the toxicity of lead by interfering with the generation of ROS, caused by lead insults. Many studies have revealed that antioxidant supplements has a role in ameliorating lead and other heavy metals induced oxidative stress in the body.

Researchers are being evaluating effects of dietary supplements such as vitamin C, vitamin E, methionine, N-acetylcysteine, alpha lipoic acid, selenium, pyridoxine (vitamin B_6) and taurine on different parameters of lead intoxication (35). Many studies have demonstrated that combination therapy, a new trend, resulted in significant decline in tissue Pb burden and provided protection against lead induced oxidative stress. This combination therapy uses to different chelators, which act differently. General mechanism of combination therapy is based on the assumption that various chelating agents are likely to mobilize toxic metals from different tissue compartments. Example of such chelator combination is DMSA and $CaNa_2$ EDTA against chronic lead intoxication provides a more pronounced elimination of lead and better recoveries in various biochemical parameters (147).

In addition, the use of two different chelators for the combined therapies, experimental studies have been conducted where a co-administration of vitamins (thiamine, vitamin C and vitamin E), essential metal (zinc) and amino acids (methione and cysteine) with different chelating agents provide better clinical recoveries. Co-administration of vitamin E or vitamin C with chelators like DMSA or MiADMSA is more beneficial in restoration of altered by chemical variables due to

lead intoxication. Although several metal chelators have been used to manage lead toxicity but none are suitable in reducing lead body burden (Osweiler, 1999). Moreover these chelators in turn are potentially toxic (Aposhian, 1983) and often failed to remove Pb burden from whole body tissues (Cory-Slechta et al 1987). Thus there has been an increased interest in the therapeutic potential of plant products or medicinal plants having antioxidant properties in reducing free radical-induced tissue injury (Gupta and Flora, 2006).

Preventive control of lead intoxication

Prevention is regarded as best approach. People should be educated about the health risks of lead and sources that may cause lead poisoning. This is a very important step in preventing further exposure to lead and its adverse health effects. Secondly prevention measures which involve the early detection and treatment of lead poisoning should be instituted and involve targeted screening of people with high risk of environmental and especially occupational lead exposure. Avoid purchasing or using products known to contain lead. Avoid consuming food or beverages or putting items in the mouth, in the areas where lead-based compounds or materials are in use. Washing hands with soap after handling lead. Developing new legislative approaches. Imposing taxes on lead containing items.

Conclusion

Lead poisoning has been known to mankind since antiquity, although the situation got aggravated since the 18^{th} century during the industrial revolution. It was the period when various important qualities of lead were discovered that made it one the most widely used industrial metals. Lead has no known biological function in the body and once it entered the body, it is known to cause severe health effects that might be irreversible. It almost affects almost all the major organ system of the body, kidney is one of them. Various molecular, cellular and intracellular mechanisms have been propose to the toxicological profile of lead that includes generation of oxidative stress, ionic mechanism and apoptosis. Of these oxidative stress has been found to be more pronounced and much more severe. Lead causes generation of ROS which results critical damage to various bio-molecules such as DNA, enzymes, proteins and membrane based lipids, while simultaneously it impairs the antioxidant defense system. Chelation therapy has so far been used as the mainstay of the treatment that involves quenching of lead from different sites of the body and expels it through urine. Prevention is regarded as the best approach. Various naturally occurring antioxidants like vitamins, flavonoids and herbal antioxidants have been reported for the prevention and treatment of lead induced oxidative stress. These antioxidants were also reported to provide an elevated therapeutic impact when

administered with chelating agents like DMSA and DMPS. Beneficial effects of these antioxidants (antagonists) be carefully considered as an antioxidant may become a pro-oxidant in the presence of certain other molecules.

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