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Long Term Exposure to Some Heavy Metals Induce Oxidative Stress in Humans

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ABSTRACT

Heavy metals are leading toxic agents detected in the environment. These have carcinogenic and cell-transforming potential. Occupational and environmental exposure to heavy metals causes toxicity in living organisms. Interaction between air pollutants and living tissues may cause disturbance of pro-oxidants and anti-oxidants in body. The red blood cells are exposed to damage induced by ROS leading to inhibition of heme biosynthesis. The body is protected against oxidant injury by antioxidant enzymes such as glutathione peroxidases (GPx), superoxide dismutases (SODs), and catalase as well as other antioxidants. Industrialization is fulfilling human demands at the cost of human health thus causing adverse effects on human population and ecosystem. Keeping this in view human serums were used as bioindicators of metal toxicity. Serum of group of workers from electroplating industries (25) and glass bangle making industry (25) were analysed for their antioxidant levels. Serum of healthy subjects served as control. GPx activity was measured by method of Paglia and Valentine (1967). SOD was assayed by the method of Beauchamp and Fridovich (1971). Serum levels of Alpha -1 antitrypsin (an enzymatic biomarker of oxidative stress) were also analysed in control and exposed group. Alpha-1 antitryptic activity was measured by method of Waheed and Salahuddin (1975). The results indicated that prolonged exposure to heavy metals such as nickel, cadmium and lead caused adverse health effects. This was due to induction of oxidative stress. The level of Alpha-1-antitrypsin was slightly raised in diseased subjects as compared to healthy controls.

Key words: Heavy Metal Toxicity, Reactive Species, Antioxidant Enzymes and Alpha-1-antitrypsin.

INTRODUCTION

Occupational and environmental exposure to hazardous heavy metals causes toxicity in living organisms. The air in the work environment usually contains a number of chemicals, which when inhaled and absorbed by the body, pose a potential risk for worker's health. Industrialization, besides fulfilling the demands of people, is also polluting the environment to certain extent (Wasowicz et al., 2001) thus causing adverse effects on population and ecosystem. Toxic exposure can be differentiated by acute exposure versus chronic exposure, with each type further affecting symptom presentation and outcomes. Heavy metal exposure is often encountered in industrial workplace environments, where chronic, prolonged exposure is more likely. In such chronic exposure, neurodegeneration and psychiatric manifestations are more prevalent. Cadmium, lead, nickel and other heavy metals have a high affinity for sulfhydryl (-SH) groups, inactivating numerous enzymatic reactions, amino acids, and sulfur-containing antioxidants with subsequent decreased oxidant defence and increased oxidative stress (Houston 2007). These metals generate reactive oxygen and nitrogen species which lead to toxicity and carcinogenicity. Various studies have confirmed that metals activate signalling pathways and the carcinogenic effect of metals has been related to activation of mainly redox-sensitive transcription factors, involving NF-kappa B, AP-*1* (activating protein-1 and p53. Antioxidants (both enzymatic and non-enzymatic) provide protection against deleterious metal-mediated free radical attacks (Valko 2005). Oxidative attack of essential cell components by ROS (Reactive Oxygen Species) is a mechanism generally recognized as that responsible for the pathogenesis of several human diseases (Devasagayam et al., 2004). The red blood cells are exposed to damage induced by ROS leading to inhibition of heme biosynthesis and imbalance in pro-oxidants and antioxidants balance in human system. Lead directly affects the hematopoietic system through restraining the synthesis of haemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes leads to anaemia. Our body is protected against oxidant injury by enzymes such as glutathione peroxidases (GPx), superoxide dismutase (SOD) and catalase as well as other antioxidants. Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Flora 2011). Pro-oxidants are by definition free radicals, atoms or clusters of atoms with single unpaired electrons. External sources of free radicals include exposures to environmental toxins such as ionizing radiation, ozone and nitrous oxide, cigarette smoke (including passive inhalation) and heavy metals, as well as dietary intake of excess alcohol, unsaturated fat, and other chemicals and compounds present in food and water (Afridi 2010). Antioxidants are chemical compounds that can bind to free radicals and thus prevent them from damaging healthy cells. Antioxidants can be divided into enzymatic and non-enzymatic subtypes.

Several antioxidant enzymes are produced by the body, with the three major classes being catalase, the glutathione peroxidases, and the superoxide dismutase (SODs). Non-enzymatic antioxidants include the innate compound glutathione as well as antioxidant

vitamins obtained through the diet, such as α-tocopherol (vitamin E), ascorbic acid (vitamin C) and β-carotene. "Pollution" has become a major threat to the very existence of mankind on the planet earth. Heavy metal pollution affects flora, fauna and other abiotic components of the ecosystem. Metal leads to various metabolic alterations and undesirable changes, which in many cases may cause severe injury and health hazards. Keeping this in view, human serums were used as bioindicators of metal toxicity. Serums of a group of workers electroplating industries and glass industries like bangles making, were analyzed for their antioxidant levels (enzymatic markers of oxidative stress). Serums of healthy subjects were used as control.

Nickel: Nickel can enter body via inhalation, ingestion and dermal absorption. Daily intake: 35-300 microgram per day. NF-κB is an important transcription factor in both apoptosis and the inflammatory process and was found to be activated by nickel. For example nickel-induced allergic response and skin hypersensitivity are connected with activation of NF-κB (Goebeler et al., 1993). Also the ATF-I (activating transcription factor-1) was found to be activated in nickel treated cells. The ATF-I transcription factor belongs to an ATF/CREB family that was originally identified as a target of the cAMP signalling pathway (Shaywitz and Greenberg 1999). Nickel produces rather low, but measurable levels of free radicals in cells (Bal and Kasprzak 2002). Fluorescent methods revealed that both, soluble $NiCl_2$ and insoluble Ni_3S_2 evoked formation of free radicals (Salnikow et al., 2000). Many studies also revealed depletion of glutathione (GSH), due to chronic nickel exposure, representing another marker of oxidative stress.

Cadmium: The deleterious health effects of cadmium (Cd) were first described in the mid 19th century. As part of industrial developments, increasing usage of Cd has led to widespread contamination of the environment that threatens human health, particularly today. Increased cadmium concentrations in blood have been linked to leading diseases like cancer, diabetes, and cardiovascular diseases (Thévenod and Lee 2013).

Cadmium can cause osteoporosis, anaemia, non-hypertrophic emphysema, irreversible renal tubular injury, eosinophilia, anosmia and chronic rhinitis. Cadmium is a potent human carcinogen and has been associated with cancers of the lung, prostate, pancreas, and kidney. Because of its carcinogenic properties, cadmium has been classified as a #l category human carcinogen by the International Agency for Research on Cancer of USA. Cadmium itself is unable to generate free radicals directly; however, indirect generation of various radicals involving the superoxide radical, hydroxyl radical and nitric oxide has been reported (Galán et al., 2001). Some experiments also confirmed the generation of non-radical hydrogen peroxide which itself in turn may be a significant source of radicals via Fenton chemistry (Watanabe et al., 2003).

Lead: Among heavy metal exposures, lead exposure is one of the most common exposures that can lead to significant neuropsychological and functional decline in humans. Plumbum (Pb), a chemical element in the carbon group otherwise known as lead, is a soft and malleable metal, which is considered a heavy metal. Lead introduced into the bloodstream is excreted in urine and bile at a clearance rate of 1 to 3 mL/min, with a half-life of roughly 30 days. The remaining lead binds to red blood cells, is distributed throughout the soft tissues of the body, and eventually accumulates in bone. The

half-life of bone-deposited lead ranges from 20 to 30 years. Turnover of bone tissue releases lead back into the bloodstream, and such processes as pregnancy, menopause, or lactation may increase blood lead levels by speeding bone tissue turnover. Lead in the body is measured with both blood and bone levels. Blood lead levels are more reflective of acute exposure, whereas bone lead levels better reflect cumulative exposure over time (ATSDR, Toxicological Profile for Lead USA, 2007.). Acute high-dose exposure to lead is not the only source of lead-based neurotoxicity. Acute low-dose exposure also appears to produce measurable, if less dramatic, effects on nervous system function. Epidemiological studies have failed to find evidence of a threshold for neurological effects; recent large-scale, prospective studies suggest that blood lead levels below 10 µg/dL significantly worsen intellectual functioning in children and that the strength of association is stronger at the low range of exposure (Surkan et al.,2007).

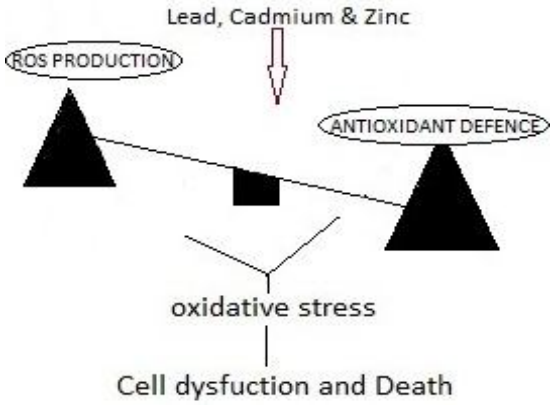

Figure 1. Presence of some heavy metals such as Lead, Cadmum and Mercury leads to oxidative stress generation.

MATERIALS AND METHODS

Bovine serum albumin, Alpha1-1 antitrypsin and N-Alpha-Benzoyl-DL-Arginine P Nitroanilide (BAPNA) was purchased from Sigma Chemical Co., St. Louis, USA. Trypsin was obtained from SRL. All other chemicals such as Folin Phenol Reagent DMSO, TRIS, salts of phosphoric acid, sodium chloride, acetic acid, copper sulphate, sodium carbonate, sodium potassium tartarate, calcium chloride and hydrochloric acid (HCl) were purchased from Qualigens Fine Chemicals, Glaxo India Ltd., Mumbai, India.

Samples: Four ml of venous blood was collected from the exposed group (males and females) working in electroplating industries and from those working in glass industries like bangles making. Blood from each case was collected in sterilized plain vial. The blood was allowed to clot. The serum was separated with the help of a centrifuge. The serum thus obtained was stored at -4°C. All the haemolysed samples were discarded. The serum was taken out from refrigerator only at the time of experiment.

Atomic absorption spectrophotometry was used for determining the concentration of toxic metals (cadmium, lead and nickel) in serum samples from standard curve of Bovine serum albumin (BSA) using the method of Lowry et al. (1951). The linear curve obtained by the method of least square

Absorbance at 680 nm = {0.018 × (µg of protein)} – 0.0019

Glutathione peroxidase activity

It was measured by the method of Paglia and Valentine (1967). Reaction mixture

contains 0.1M phosphate buffer, 1mM EDTA, 10 mM Glutathione (GSH), NaN_3, 1 unit glutathione reductase, 1.5mM NADPH, and 0.1 ml of cell lysate. Following incubation H_2O_2 was added. GP_X activity was measured at the rate of NADPH oxidation at 340 nm.

Superoxide Dismutase (SOD) activity

It was assayed by the method of Beauchamp and Fridovich (1971). Nitroblue tetrazoium (NBT) is the key chemical used. NBT is reduced to blue formazan by superoxide anion which has a strong absorbance at 560 nm. Presence of SOD inhibits this reaction.

Determination of trypsin inhibitory activity

The serum trypsin inhibitory activity (TIA) of all samples was determined by measuring the inhibition of trypsin by the sera according to the method of Waheed and Salahuddin (1975) using BAPNA as substrate (Waheed and Salhuddin 1975). Trypsin was allowed to react with BAPNA resulting in the formation of P-nitroanilide which gives yellow colour. The intensity of colour was measured at 410 nm and was used for checking the activity of trypsin in the presence and absence of serum.

Calculations: The TIA of the serum was calculated as follows:

Suppose the absorbance of the test tube containing serum and the standard tube is **T** and **S**, respectively. The specific activity of trypsin is calculated by the formula: $\frac{\text{Absorbance}}{\text{Enzyme concentration}}$. The specific activity of trypsin in the standard tube is considered 100%, as the trypsin here was not inhibited. Hence, $\frac{\mathbf{S}}{\text{Enzyme concentration}}$ denotes 100% specific activity of trypsin on substrate. The specific activity of trypsin in the tubes containing serum is $\frac{\mathbf{T}}{\text{Enzyme concentration}}$

So, the percentage inhibition of trypsin by 0.02 ml of serum can be calculated as:

$$= \frac{\text{Specific activity of the standard} - \text{Specific activity of the test}}{\text{Specific activity of the standard}} \times 100$$

$$= \frac{\mathbf{S} - \mathbf{T}}{\mathbf{S}} \times \mathbf{100}$$

The percent inhibition of trypsin was also calculated using different concentrations of commercial alpha-1 antitrypsin solution (instead of sera), and a graph of "alpha-1 antitrypsin concentration" versus "% inhibition of trypsin" was plotted. The linear curve obtained by least square analysis was found to fit the equation:

AAT conc. (mg/ml) = {0.0479× (% inhibition of trypsin)} + 0.0531

This equation was used to calculate the concentration of alpha-1 antitrypsin in the serum.

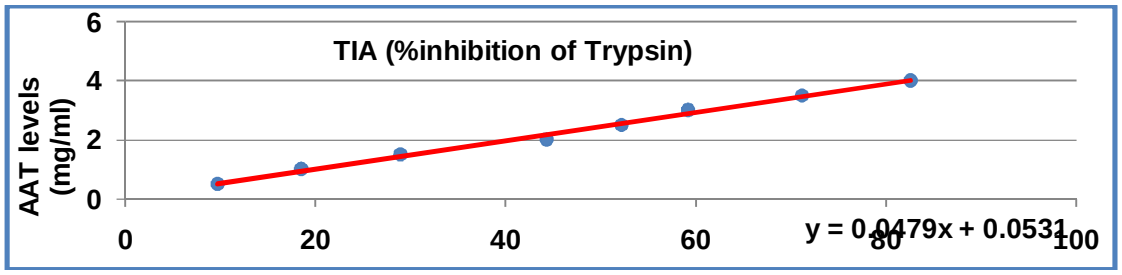

Figure 2.Calibration curve for the estimation of serum AAT concentration using standard solution of human plasma AAT.

RESULTS

The following tables show the results of various experiments

Table 1. Determination of Cd, Pb & Ni in blood samples of normal/healthy persons.

SN	Age/sex	Cadmium (Cd)	Lead (Pb)	Nicke (Ni)
1	M/35	0.06	3.3	0.6
2	M/50	0.03	3.6	1.2
3	M/21		2.4	0.9
4	M/27			1.5
5	M/33		3.3	1.3
6	M/38		2.6	
7	M/59		2.9	1.3
8	M/53	0.04	4.1	1.0
9	F/45	0.06	4.4	1.2
10	F/24			0.5
11	F/30		2.4	
12	F/49	0.05	3.2	0.8
13	F/40		2.4	0.6
14	F/29		2.3	
15	F/34		2.1	
16	F/39	0.01	2.4	
Mean				
		0.048	2.95	0.88

Table 2. Determination of Cd, Pb & Ni in blood samples of exposed group (A)

SN	Age/sex	Cadmium	Lead	Nickel
1	M/60	0.18	3.88	4.25
2	M/54	0.25	2.96	7.0
3	M/50	0.09	3.10	5.5
4	M/54	0.06	2.40	4.4
5	M/59	0.10	4.10	4.9
6	M/48	0.07	4.0	3.3
7	M/45	0.04	3.9	3.7
8	M/41	0.11	3.4	3.8
9	F/39	0.08	3.9	3.2
10	F/43		5.6	4.1
11	F/50	0.09	3.6	3.0
12	F/52	0.06	3.4	3.12
13	F/49	0.12	3.36	3.4
14	F/44	0.18	2.9	2.5
15	F/53	0,07	2.8	2.8
16	F/45	0.06	2.6	2.7
Mean		0.104	3.54	3.05

Table 3. Determination of Cd, Pb & Ni in blood samples of exposed group (B).

SN	Age/sex	Cadmium	Lead	Nickel
1	M/62	0.31	4.4	2.4
2	M/59	0.33	4.7	2.9
3	M/60	0.10	4.1	3.0
4	M/57	0.09	3.1	2.6
5	M/55	0.21	3.6	2.7
6	M/48	0.08	3.3	1.6
7	M/51	0.06	3.9	2.0
8	M/50	0.09	3.8	2.1
9	F/52	0.06	3.4	2.2
10	F/49	0.05	2.9	0.09
11	F/48	0.06	2.7	0.86
12	F/52	0.10	3.3	0.70
13	F/40	0.20	3.5	1.3
14	F/44	0.08	3.2	
15	F/42	0.06	2.8	0.5
16	F/39	0.07	2.2	0.5
Mean		0.115	3.4	1.73

Table 4. Group A (Electroplating Industries)

S.No	Parameters	Control	Exposed group (A)
1	Cadmium	0.048 0.01 – 0.06	0.1040 0.04 – 0.25
2	Lead	2.95 2.1 – 4.4	3.54 2.6 – 5.6
3	Nickel	0.88 0.6 – 1.5	3.05 2.5 – 7.0
4	Glutathione Peroxidase U/L blood	18.3 ± 4 11.2 - 28	14.7 ± 3.7 8.4 – 18.1
5	Superoxide dismutase U/L blood	8.0 ±2.56 2.8 – 14.3	6.9 ± 2.2 2.1 – 12.6

Table 5. Group B (Glass industries).

SN	Parameters	Control	Exposed group (B)
1	Cadmium	0.048 0.01 – 0.06	0.115 0.06– 0.33
2	Lead	2.95 2.1 – 4.4	3.54 2.6 – 5.6
3	Nickel	0.88 0.6 – 1.5	3.05 2.5 – 7.0
4	Glutathione Peroxidase U/L blood	18.3 ± 4 11.2 - 28	16.2 ± 4.0 9.7 – 24.4
5	Superoxide dismutase U/L blood	8.0 ±2.56 2.8 – 14.3	7.2 ± 3.0 2.5 – 13.7

Table 6. lpha-1 Antitryptic Activity in control and exposed groups (groups A & B)

	Percent inhibition of Trypsin by 20µl of serum
Control	46.08 ± 4.2
Exposed Group A	59.4 ± 6
Exposed Group B	64.8 ± 3.5

DISCUSSION

Heavy metal toxicity represents an uncommon but clinically significant medical condition, which if unrecognized or inappropriately treated results in significant morbidity and mortality. Many studies have reported toxic and carcinogenic effects induced when humans and animals are exposed to certain metals. These heavy metals are known to cause oxidative deterioration of bio-molecules by initiating free radical mediated chain reaction resulting in lipid per-oxidation, protein oxidation and oxidation of nucleic acid like DNA and RNA (Flora et al., 2013). Present attempt was made in the context to study the level of exposure of human beings to metallic pollutants. The group of workers of electroplating industries (groupA) and those working in the glass industries (group B) were considered. The concentration of heavy metals in this study i: e of cadmium (Cd), Lead (Pb) and nickel (Ni) in parts per million (ppm) were 0.048, 2.95 and 0.88 ppm respectively in control or healthy persons. The level of these toxic metals was 0.104 (Cd), 3.54 (Pb) and 3.05 (Ni) in group A where as in group B, the level of these metals were 0.115 (Cd), 3.4 (Pb) and 1.73 (Ni) ppm respectively. These results indicated that increased ingestion of some these heavy metals over a prolonged period of exposure, cause adverse health effects

Increased production of free radicals in the body demand more and more of antioxidants, leading to reduction in levels of glutathione peroxidase and superoxide dismutase. Mechanism of inorganic metals toxicity include production of reactive oxygen species (ROS) capable of damaging lipids in membrane, proteins or enzymes in tissues, and DNA to induce oxidative stress as balance between generation, and elimination of ROS is essential for maintaining the functional integrity of a cell. Elimination of free radical production takes place via various types of enzymatic and non-enzymatic antioxidants. In this context level of glutathione peroxidase and superoxide dismutase were measured in healthy and diseased group by standardized assay methods. The values show that there is a drastic decline in the antioxidant status of exposed / diseased group of patients. Thus, it may be concluded that occupational exposure to cadmium, lead and other heavy metals disturbs the antioxidant potential of the body leading to various diseases. Alpha-1 antitrypsin AAT has evolved from a simple acute phase reactant protein to a multifunctional anti - inflammatory, immunomodulatory, anti-infective and tissue-repair molecule (Alam et al., 2011; Petrache et al., 2006).

Human AAT, also named α1 proteinase inhibitor (α1-Pi) and SERPINA1 (serine protease inhibitor, group A, member 1), is a water-soluble and tissue-diffusible, medium-sized (6.7 × 3.2 nm) circulating glycoprotein, with a molecular weight of 52-kDa, and a blood half-life of 4–5 days. Over 80% of AAT is synthesized and secreted by hepatocytes. Humans produce ~34 mg/kg/day, resulting in high plasma concentrations of 1-2 g/L (De

Serres and Blanco 2014). In the present study the level of alpha-1 antitrypsin was found to be slightly raised as compared to the healthy subjects. This occurs because the pro-oxidants – antioxidants balance gets disturbed in humans due to chronic heavy metal exposure.

CONCLUSION

Increased ingestion of some heavy metals (as nickel, cadmium and lead), over a prolonged period of exposure, cause serious health effects. This produces imbalance in pro-oxidants – antioxidants balance shifting the equilibrium in the direction of the former in human as compared to the healthy subjects. Increased production of free radicals in the body, demand more and more of antioxidants, leading to reduced level of glutathione peroxidase and superoxide dismutase. Thus heavy metal toxicity is a serious problem to be overcome in order to avoid loss of human life as well as to save our environment.

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