



Heavy Metal Ion and Haptoglobin Association with Career Duration in Pharmaceutical Industries: A Potential Occupational Hazard?

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ABSTRACT

Introduction: Due to the increase growth of industrialization, the problem of environmental pollution has increased worldwide.

Aims & Objectives: A cross-sectional comparative study was designed to find out association between heavy metal ion (lead & nickel), haptoglobin and years of working in Pharmaceutical industries.

Place and duration of study: Pharmaceutical industry located at Quaid-e-Azam Industrial Estate Kot Lakhpat, Lahore, Punjab. Duration of study was January 2019 to June 2019.

Material & Methods: A total of 58 workers of a pharmaceutical industry were included in the study. 35 age matched subjects with no history of any disease were taken as controls. A brief questionnaire was filled by each participant. Heavy metals present in serum sample of patients were digested by concentrated Nitric Acid. The digested samples were analyzed to estimate the level of lead and nickel using atomic absorption spectrophotometry available at the Department of IMBB University of Lahore. Data was entered and analyzed by SPSS 20, $p \leq 0.05$ was taken as significant.

Results: Mean age of workers was 40.64 years with BMI 23.52 kg/m². Their work of experience or duration of work in industries was 1-5 years, 6-10 years and > 10 years. Majority had dry cough followed by running nose, breathless end of day and headache. Levels of lead, nickel and haptoglobin was significantly raised compared to controls. Association of lead and nickel exposure with years of working experience showed that with increasing working experience or duration of workers the levels of lead and haptoglobin was markedly raise (5.9 ± 2.8 mg/l) as compared to the level of nickel (2.58 ± 1.05 mg/l).

Conclusion: Exposure of lead and nickel increases the level of haptoglobin and causes health issue in the workers of Pharmaceutical factors. Levels of these heavy metals increased in circulation of workers with the increasing of their duration of work or work experience.

Keywords: Lead, nickel, haptoglobin, Pharmaceutical workers

INTRODUCTION

Environmental pollutants, present in large amount in any part of environment. The environment has been polluted by number of pollutants like inorganic ions, organ metallic substances, gaseous pollutants etc¹.

The pollutants are usually heavy metals which are toxic to all living creators which may be cause by industrialization and urbanization. Heavy metals are usually present in pharmaceutical material that may be used as catalysts or from raw materials/reagents used in the process of manufacturing. The routes of entering of heavy metals in body are ingestion of polluted food, unhygienic water, inhalation from the air atmosphere and skin

contact from pharmaceutical, agricultural, industrial areas. Heavy metals are metalloids and metallic elements and their toxicity may affect biological activity of living creators and growth or these metals accumulate in different parts or organs of body and may cause serious ailments like lung cancer, cardiovascular problems, hypertension, and other diseases. Some of these metals are essential for body but their deficiency or excess may affect the body system^{2,3}.

It is proposed that metalloids have an ability to form covalent bonds and exhibit toxicological characteristics. These metalloids covalently bind with organic compounds or non-metallic part of macro-molecules, produce lipophilic ions / compounds, and causes toxic effects. Example is seen in lead exposure that binds to SH group of the

amino acid of protein and inhibit the activity of function proteins¹.

Lead and nickel are chemical ingredients with very high specific gravity than water (Five times more) and are deemed as heavy metals⁴. Lead is considered as non-essential heavy metal as it has no important role in mammals. It may accumulate in the body for long period of time and causes serious diseases, even if taken in small quantity. On the contrary, nickel (Ni) is essential element required in very small amount in body^{5,6}.

Toxicity of nickel is not very known as its absorption is poor in body. Though excess exposure of Ni causes unfavorable effect like encephalopathy, dermatitis, allergy, and low count of sperm. Its amount is rises in the environment due to industrial pollutants⁷. The most known effects of over exposure of nickel are lung cancer and fibrosis⁸.

Minute particles of lead can be soaking up via respiratory tract, while large lead particles are transfer to the oropharynx and swallowed. Chronic lead exposure may help to raise blood pressure, impair the neuronal and hormonal systems and in turn may affect heart rate and vascular resistance. Over exposure of lead also affect hematopoietic system and cause anemia and porphyria. Besides lead impair the mechanisms of nervous system, via the impairment of calcium homeostasis⁹.

Haptoglobin (Hp) is acute phase protein secreted form. Its main function is to bind with free haemoglobin and removes it from the bloodstream¹⁰. Metal have ability to reach to the cell membrane and make attachments with plasma proteins¹¹. Exposure to lead impairs the level of haptoglobin and may be related with lead stimulated alteration of body immune system¹². Additionally, lead also has a role in inflammation by increasing the synthesis of pro-inflammatory cytokines by regulating their gene expression. This cause in the variation in the release of various inflammatory cytokines, and some plasma protein involved in inflammatory processes (CRP, haptoglobin, ceruloplasmin)¹¹.

Environmental pollutants are being present in soil, water and into the air atmosphere due to the inappropriate waste disposal of pharmaceutical, metal industries. In our country there is lack of health assessments and screening for acute and chronic diseases of factory workers. It is therefore planned to evaluate the health effects of workers working in pharmaceutical industry. This study may help to take attention of the stake holders towards an appropriate regulatory structure to

offer and monitor the working conditions for the industrial workers.

Study was therefore designed to estimate the level of lead and nickel in workers of pharmaceutical industries. Besides, the association of these metals with the working hours was also noted.

MATERIAL AND METHODS

A cross-sectional comparative study was carried out on 58 workers of a pharmaceutical industry located at Quaid-e-Azam Industrial Estate Kot Lakhpat, Lahore, Punjab. Duration of study was January 2019 to June 2019. 35 age matched subjects with no history of any disease were taken as controls. Factory workers working for more than 2 years and were not taking any precautionary procedures during their work were included in the study. Workers with history of any chronic disease or taking nonsteroidal drugs or tonics were excluded from the study. Chronic smokers were also excluded from the study. Letter of consent and a brief questionnaire was filled by each participant. The study was approved by the ethical Committee of Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.

Blood samples were taken from the participants. EDTA vacutainers were taken for the heavy metal concentration and haptoglobin analysis and 3ml of blood was added into these vacutainers, centrifuge and stored in vials at 20°C until use.

Preparation of sample for heavy metal analysis: Heavy metals were digested by concentrated Nitric Acid. Two ml of serum and 5 ml of concentrated HNO₃ was added in tube and boiled for half an hour. Nitric acid was added until solution become clear. This showed the complete digestion. Mixture was filtered and cool and its volume was making up 100 ml with distilled and stored with cover. These treated samples were analyzed to estimated the level of lead and nickel in atomic absorption spectrophotometry¹³ available in Department of IMBB University of Lahore.

Statistical Analysis:

Data was entered and analyzed by SPSS 20. Variables were expressed as mean \pm SD. Level of metal ions and haptoglobin of workers was compared with controls using student 't' test. $p \leq 0.05$ was taken as significant.

RESULTS

Demographic charactersites of Workers of Pharmaceutical Industries was tabulated as Table-

1. Mean age of workers was 40.64 with BMI 23.52 kg/m². Their work of experience or duration of work in industries was 1-5 years (no of workers 16), 6-10 years (n=18) and > 10 years (n=24). Only 10 workers were smokers and 12 had healthy habits. Majority have dry cough followed by running nose, breathless end of day and headache while 11 showed no symptoms. Exposure of lead and nickel and to workers of pharmaceutical industry in comparison with controls was tabulated (Table-2). Levels of lead and nickel was significantly raised (P<0.001 & 0.05) respectively compared to controls. Level of haptoglobin was also significantly increased in workers compared to controls. Association of lead and nickel exposure with year of working experience or duration of work was tabulated as Table-3. It is observed with increasing working experience or duration of workers the levels of lead and haptoglobin was markedly raise as compared to the level of nickel & in Fig-1 Exposure of metal ions (Pb and Ni) and level of haptoglobin is shown.

Characteristics	Workers (n=58)
Mean age(yrs)	40.64±5.63
BMI (Kg/m ²)	23.5±4.8
Duration of work	
1-5 yrs	16
6-10 yrs	18
>10 yrs	24
Smokers	10
Healthy Habbits (regular walk, exercise)	12
Chronic symptoms	
Dry Cough	21
Frequently running nose	09
Breathlessness end of the day	07
Headache	10
No Symptoms	11

Table-1: Demographic charactersitics of Workers of Pharmaceutical Industries.

Metal Ions	Workers	Control	P-value
Lead (mg/l)	5.9± 2.8**	2.28± 0.99	<0.001
Nickel (mg/l)	2.58± 1.05*	1.07± 0.05	<0.05
Heptoglobin (mg/dl)	255.41± 78.99*	218.69± 58.36	<0.05

Table-2: Exposure of lead and nickel and to workers of Pharmaceutical industry in comparison with controls.

Variables	Level of metals ions in workers	Working expereince Duration of work
*Lead (mg/L) *Nickel (mg/L) Heptoglobin (mg/dl)	4.98±2.12 2.62 ± 1.06 255.41±67.5	1-5 years
Lead (mg/L) Nickel (mg/L) Heptoglobin (mg/dl)	5.55±2.85 2.63±1.11 265.7±66.5	6-10 years
Lead (mg/L) Nickel (mg/L) Heptoglobin (mg/dl)	5.93 ±0.52 2.65±1.01 279.34±68.55	>10year

Table-3: Association of lead and nickel exposure with year of working experience or duration of work.

*Normal levels of Lead 1-5mg/L, Nickel 1.2-2.52mg/L

DISCUSSION

The working in pharmaceutical and other factories for long period is linked with many ailments in the workers due to the exposure of chemical may be a factor of health hazards. Different types of chemicals including solvents and heavy metals increase the risk of intoxications, allergies, cancers, and damage of brain. The exposure routes are mostly inhalation and skin with exposure duration 8 hours/day with long period¹⁴. We agreed with the studies that age is linked with career duration. The occurrence and incident rates of ailments increase really with age. However, it is observed that large number of workers with increasing age physically become weaker but mentally active^{16,17}.

We found that levels of serum lead were significantly increased in industrial workers compared with their controls. We agreed with the studies where research workers found significantly high levels of serum lead in workers in comparison to controls. It is proposed high exposure of lead for longer duration induce toxic effects on haematological, cardiovascular, nervous and reproductive systems^{18,19}. Another study proposed that exposure of lead results oxidative stress and methylation of genes repair DNA damage²⁰. A cross-sectional study was conducted with 114 subjects for estimation the level of lead and cadmium. Study found high levels of these metals and concluded that the level of metals may be related with smoking, days worked per week, duration of work (years), work position, use of mask and gloves, and personal hygiene behavior. The elevated level of toxic metals in the

workers is a sign of metal toxicity associated with duration of work (years) and hygiene practices²¹.

A study was carried on welders working in China. Study reported that welders had significantly high values of the heavy metals like nickel, leads and chromium in their blood circulation compared to controls. It is demonstrated that industrial workers are exposed to heavy metals in mines and industries and may breathe in the metal particles and are prone to develop health issues²². An Indian study reported that among 39 samples of medicine, all sample showed a positive response for lead, cadmium and nickel. However, study reported that all the experimented medicine safe to use and contained lower values of these ions except lead. Study reported that raw material used in preparing these medicines may have high values of lead²³.

An experimental study carried out in rats. The rats were treated with nickel, chromium and cadmium separately. Their gene patterns were determined. Study observed that all these metals are genotoxic. Nickel cause mutation in gene by restores iron in proteins²⁴. Another study carried out in young age group and found that heavy metals (iron, lead, chromium, nickel etc) may affect different systems of body like respiratory system, cardiovascular system, nervous system, reproductive system and make worse pre-existing diseases²⁵. We agreed with a study found that low exposure of metal ion (less duration of work in year) like lead, nickel and others is a hidden threat and may be diagnosed by neuro-psychiatric disorder including anxiety, fatigue and low in low intelligence²⁶. It is reported that low values of exposure of lead showed immunostimulatin effects and high exposure results immunosuppression²⁷. According to our study significantly high levels of haptoglobin was observed in workers as compared to controls. A study was carried out on Indian workers of chemical industry. Study was carried out to find out the level of lead and correlate with hemoglobin/ haptoglobin in the workers based on age and duration of work. Study observed high values of circulating lead in the workers with middle age group that found to be link with anemia and hypertension in workers. It is proposed that Haptoglobin take part in uptake of free form hemoglobin in the circulation¹¹. A study conducted in 192 industrial workers of Poland with age range 22-58 years to find the level of exposed lead and haptoglobin. Study found that mean values of circulating hatoglobin was raised with both increased and less exposure of lead¹². We also agreed with a study that showed an association of aging with markedly increased levels of serum

lead and cadmium. It is proposed that accumulation of metals when exposed for long period of time producing secondary metabolites and their accumulation over time in the living creators can cause serious health issues i.e. impair the activity of various glands and organs such as the liver, kidney, heart, bone etc²⁸. Another study thought that these metals impair cellular function like proliferation, growth, damage repairing process and process of automatic death via generation of reactive oxygen species, weak antioxidant defense and inactivation of enzymes²⁹.

Limitations: The pharmaceutical industrial unit surveyed could be named due to a data confidentiality agreement.

CONCLUSION

It is concluded that exposure of lead and nickel increases the level of haptoglobin and causes health issue in the workers of pharmaceutical factors. Levels of these heavy metals increased in circulation of workers with the increasing of their duration of work or work experience.

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