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NAYAB GUL

ABSTRACT

Mercury (Hg) is a serious environmental pollutant with various recorded nervous system and developmental effects. Despite its hazardous effects, the Hg is continuously used in various products and processes worldwide due to its unique characteristics. Over hundreds of years Hg has widely been used in industry, gold mining, agriculture, cosmetics, dental amalgam and medicine. The recent discoveries on the toxicity of Hg have unfortunately been ignored for long time, the worst effect of which were more pronounced among workers involved in industries. Human exposed to Hg through various pathways among which the selected areas under study was the fluorescent lamp industry, gold mining, dental amalgam and whitening creams. Therefore the study aimed to identify the Hg concentration in the biological samples (blood, urine, hair, nails) of the Hg exposed occupational workers involved in the fluorescent lamp industry and gold mining processes, in addition the Hg was also identified among the blood, urine hair and nails samples of the dental amalgam and whitening cream users other than occupational workers. The first part of the study is concerned with the exposed occupational workers in the fluorescent lamp industry of Khyber Pakhtunkhwa.

This study was conducted to investigate the concentrations of organic (methyl) mercury (Me-Hg), inorganic mercury (I-Hg) and total mercury (T-Hg) in the exposed workers of fluorescent lamp industries. For this purpose biological samples such as red blood cells (RBCs), plasma, urine, hair and nails were collected from Hg exposed workers and non-worker as control group. These samples were analyzed for Hg

concentrations and their correlations with the demographic profile of specimen donating persons were assessed. The mean concentrations of T- Hg (31.9 µg/L), Me-Hg (27.7 µg/L) and I-Hg (5.36 µg/L) in RBCs were significantly (P<0.001) higher in exposed workers (n=40) as compared to the control, whereas T- Hg, Me-Hg and I-Hg concentrations in plasma were observed 15.1, 3.5 and 10.6 µg/L, respectively and significantly higher (P<0.001) than the control group. The mean concentrations of T- Hg (137.5 µg/L), Me-Hg (13.5 µg/L) and I-Hg (137.5 µg/L) in urine were also significantly (P<0.001) higher than the control group which were 2.81, 0.31 and 2.38 µg/L, respectively. The concentrations of Hg species (T- Hg, Me-Hg, and I-Hg) were significantly higher in the samples of hair and nail of the workers as compared to the control group. This study concluded that the workers in fluorescent lamp industries were highly exposed to Hg as indicated by the concentrations of Hg and its species in the biological specimen. Numerous health problems were noticed during questionnaire survey which could be linked with high exposure of Hg; therefore, precautionary measures should be adopted such as protective cloths, masks and minimum exposure time by the workers to minimize the health risks.

The second part of the study is concerned with the individuals with no occupational exposure to Hg. This part of the study is related with the exposure of individuals to Hg through the use of Hg dental filling for their teeth. The study was conducted among the individuals using Hg as dental filling material to investigate the Hg concentration and its distribution with time (days) in the biological samples of Hg dental amalgam users (MDA). Hg concentration was measured in the biological samples (red blood cells (RBCs), plasma, urine, hair, nails) collected from MDA at three different

times (e.g. 1st, 3rd, 12th day) of Hg filling and correlated them with the biological variables (age, weight, restoration, Hg amalgam filling, surface area of filling material, fish consumption). Hg concentrations in the biological samples of MDA were 6-8 times higher than non amalgam users (control). The concentrations of Hg in the RBCs (4.39 µg/L), plasma (3.02 µg/L) and urine (22.5 µg/L) on 1st day of Hg filling was found higher than the concentrations observed on 3rd (2.15, 1.46, 12.3 µg/L for RBCs, plasma, urine, respectively) and 12th (3.05, 2.5, 9.12 µg/L for RBCs, plasma, urine, respectively) day of Hg filling, while in the case of hair and nails, the Hg concentration observed lower on 3rd day (1.53 µg/g for hair) (2.35 µg/g for nails) than 12th day (2.95 µg/g for hair) (3.5 µg/g for nails) of Hg filling. The correlation of Hg concentrations with biological variables indicated that the number of restoration, dental filling, surface area and fish consumption were significantly ($p < 0.05$) correlated with Hg amalgam filling while non significant ($p > 0.05$) correlation was observed for the age and weight of MDA.

Another serious health problem in which the workers exposed to toxic Hg metal is the use of Hg in gold extraction process because they exposed directly through the inhalation and skin contact. Goldsmith involved in the gold extraction and recovery processes are at high risk of health because Hg is a highly toxic metal. The aim of this work was to determine the concentrations of T-Hg, Me-Hg and I-Hg in the biological samples (plasma, RBCs, urine, hair and nails) of the exposed goldsmith workers named nayaragar in local language. Biological samples (RBCs, urine, hair and nails) were collected from goldsmith (n=40) and analyzed for selected Hg using atomic absorption spectrometer (AAS) equipped with mercury hydride system (MHS). The mean T-Hg concentration in RBCs (33 µg/L), plasma (11.8±3.2 µg/L), urine (168.6±29 µg/L), hair

(4.21±0.9 µg/g) and nails (5.91±1.4 µg/g) observed higher than control RBCs (1.64±0.88 µg/L), plasma (0.55±0.27 µg/L), urine (2.72±1.1 µg/L), hair (0.35±0.22 µg/g) and nails (0.51±0.21 µg/g). All the workers participated in this study were suffered from physical and mental diseases. The concentration of Hg was found higher among the workers suffered from mental diseases as compared to those suffered from physical diseases. Among the physical diseases the most serious diseases were included the sexual dysfunction, skin diseases and fatigue because the workers suffered from these diseases have high concentration of Hg than the workers with other diseases. The number of workers suffered from physical diseases (88%) was greater than mental diseases (53%). Among the physical diseases the highest number of workers suffered from chest problem (47%) while 63% from dementia among the mental diseases. The correlation of physical and mental diseases with experience and exposure time showed significant correlation ($p < 0.05$). The burning process of Hg gold amalgamation is a significant source of Hg exposure to goldsmith nayaragar workers and nearby population, therefore awareness and precautionary measures are necessary and the workers should carry out their work outside the residential area.

Another alarming issue found for the common people is the use of Hg in creams and cosmetics. This is the unnecessary exposure of the body to toxic Hg metal. Asians are highly vulnerable to the I-Hg through the use of skin whitening creams. The aim of this work was the quantification of Hg in the skin whitening creams and in the biological samples of whitening cream users (WCU). The Hg concentration observed in the whitening creams was found higher than the permissible limit (1µg/g) set by the US FDA .Similarly when the Hg concentration in the biological samples of these WCU was

analyzed, the T-Hg concentration was found significantly ($p > 0.05$) higher in the plasma (3.61 µg/L), RBCs (9.83 µg/L), urine (21.2 µg/L), nails (1.76 µg/g) and hair (1.23 µg/g) samples of WCU than control plasma (0.57 µg/L), RBCs (1.77 µg/L), urine (3.04 µg/L), nails (0.45 µg/g), hair (0.37 µg/g). Similarly the Me-Hg and I-Hg concentration found in the biological samples was found significantly ($p > 0.05$) higher than control. Among the whitening creams, Stillmans and White Face creams have comparatively the highest Hg concentration of 63.3 µg/g and 57.6 µg/g respectively. The Hg concentration in the biological samples of the Stillmans and White Face users were comparatively higher than other creams users. It was observed from the study that the use of whitening creams containing Hg is a health hazard and the people especially women should encourage not to use these creams due to the high concentration of Hg. The people should enforce the health sector of Pakistan to regulate this sector and increase awareness about the use of Hg in cosmetics and its hazardous effects.

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Mercury

CHAPTER-1

1.1 INRODUCTION

Hg exists in three oxidation states of 0, I and II. They were named as Hg zero, Hg one and Hg two or in other words elemental Hg, mercurous Hg and mercuric Hg. The elemental Hg is a silver color metal which is liquid at room temperature. When the elemental Hg oxidized it form Hg one or Hg two. The Hg attached to a carbon atom form organic Hg i-e methyl Hg and ethyl Hg. The organic Hg is always Hg (II). When not bound to a carbon atom then it is inorganic Hg. The unique characteristic of Hg is that it's liquid at room temperature. The boiling point (357C°) of Hg is quite higher and the solubilities of Hg compounds in water are different which were 69, 60 and 2 g/L for mercuric chloride, meatallic Hg and mercurous chloride respectively (Clarkson and Magos, 2006).

Hg is an environmental pollutant and health hazard for the human however its use still continues in various processes and goods. The Hg released through both natural and anthropogenic activities. The human exposed to the toxic metal the elemental Hg through different ways includes occupational exposure in industries e.g fluorescent lamp industry, through gold mining, electrical switches, thermometers, chlor-alkali production etc. The Hg is also used in the dental amalgam filling, skin whitening creams, paints, pharmaceuticals, in vaccines, in the manufacturing of some batteries and in agriculture pesticides and herbicides. This study is divided into two main parts: one part is concerned with the occupational exposure of the workers to Hg through fluorescent lamp industry

and gold mining while the second part is the exposure through dental amalgam and skin whitening creams.

Hg is present in earth crust and found in ranking on the top of environmental pollutants. It occurs in organic, inorganic, and elemental form in many diverse settings (Karagas et al., 2012). Hg and its compounds have long been recognized as an occupational toxicant. The Hg exerts variety of adverse effects on human kidney, liver, respiratory and nervous systems, skin, reproductive and developmental defects, thyroid gland, immune system, genotoxicities and cardiovascular disorders (Abdel-Rasul et al., 2013; Al-Batanony et al., 2013; Barregård et al., 1994; Carpenter, 2001; Carvalho et al., 2008; Cherian and Clarkson, 1976; De Rosis et al., 1985; El-Demerdash, 2001; Halliwell, 1992; Houston, 2007; Hussain et al., 1999; Kampa and Castanas, 2008; Kishi et al., 1993; Langworth et al., 1992; Rocha et al., 1995; Rodilla et al., 1998; Stohs and Bagchi, 1995; Vallee and Ulmer, 1972; Zahir et al., 2005).

Fluorescent lighting technologies are undergoing rapid market growth as part of a resurgent societal interest in energy efficiency and its need is much higher in developing countries like Pakistan due to energy crises and lesser expansion of electricity projects (Johnson et al., 2008). This fast growth of fluorescent lamps is also associated with great health risks because of emission of Hg that is an essential component in all types of fluorescent lamps due to 75% reduction in energy usage and 10-fold increase in lifetime as compared to incandescent bulbs. Fluorescent lamps contain 0.7-115 mg of Hg per lamp depending on its size, and the subclass of compact fluorescent lamps (CFLs) on average contains 3-5 mg of Hg per lamp (Jang et al., 2005).

Recently, several papers have been reported the effects of Hg exposure in industrial workers of florescent lamp industry including chronic insomnia (Rossini et al., 2000), female reproductive health (De Rosis et al., 1985) and measurement of hand tremor (Fawer et al., 1983; Roels et al., 1989; Verberk et al., 1986). In the past, a few studies have been focused to monitor the creatinine level of Hg in urine samples in both exposed and control groups. (Barregård, 1993; Roels et al., 1991). On the other hand, several papers have been published on the detection of the Hg in biological samples of dental amalgam, used in dentistry (Berglund et al., 2005; Björkman et al., 2007; Nylander et al., 1986; Zolfaghari et al., 2007). Moreover, the Hg concentration in urine of exposed worker in Egypt was reported in florescent lamp industries (Al-Batanony et al., 2013). However, no research work is conducted so far to determine the concentrations of total Hg (T-Hg), organic Hg (Me-Hg)) and inorganic Hg (I-Hg) in blood (plasma and RBCs) nails, hair and urine samples from the exposed workers of florescent lamp industries. This study aimed to investigate the T-Hg, Me-Hg and I-Hg concentrations in biological samples including blood, nails, hair and urine collected from the workers in fluorescent lamp industries and its hazardous effects on human health.

The Hg is the occupational toxicant and exert hazardous effects on human and environment (Agrawal et al., 2014). Hg used in the amalgamation of gold to refine and concentrate this precious metal. Gold mining and subsequent processing of gold ores using amalgamation techniques are known for their potential contamination of environment with Hg and led human health risk (Engstrom et al., 2014; Kristensen et al., 2013). Numerous studies have been conducted on workers exposed to Hg during gold mining in Brazil, Zimbabwe, Columbia, Ecuador, Burkina Faso, Iran and Tanzania

(Charles et al., 2013; Cordy et al., 2011; Harari et al., 2012; Shirkhanloo et al., 2014; Steckling et al., 2014) relatively few papers are published in Africa (Tomicic et al., 2011) while a single paper (D'Ilio et al., 2000) conducted study on hair Hg of those goldsmith workers which were exposed to Hg in lower quantity.

The gold used as ornamental purpose in jewelry throughout the world and also in medicines (Sahin and Erkis, 2013). In Pakistani marriage customs, the gold is the important part of the bride ornamental dress and also the inferiority status symbol of the families (Monger, 2013). Mostly the gold is imported from various other countries but goldsmith makes its different shapes, sizes and polishing of the jewelry. The small invisible gold particles are falling on daily basis due to jewelry production such as polishing, cutting and reshaping processes. The gold from the carpets and dusted-clays of the goldsmith shop usually collected by specialized person called “nayaragar” in local language who process this dusted clays with Hg to concentrate and further subjected it in the furnace for solid mass of the gold which is represented in the diagram below.

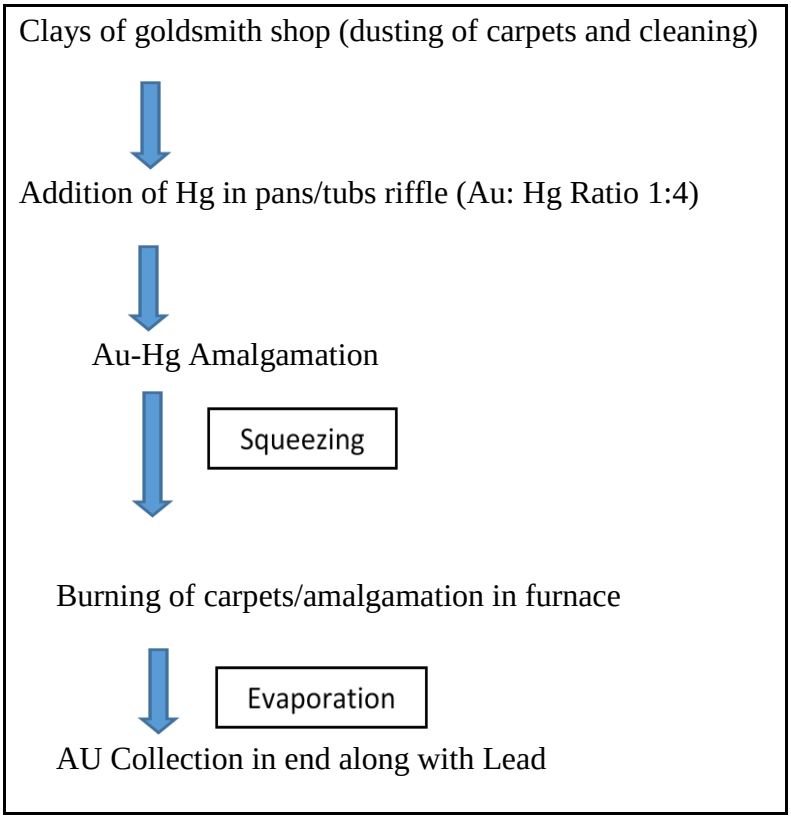


Fig 1.1 Diagrammatic presentation of Hg recovery from the dust/clay

Goldsmith (jewelers) shops are available in every city of the country for the production of jewelry. Besides this, small furnace units are also available in locality of heavy bunch of goldsmith shops for recycling the gold particles using Hg from every goldsmith shop after 3 to 6 months to recover the gold losses during cutting, polishing and reshaping.

Hg is released to environment during amalgamation and roasting processes and the workers (Nayaragar) are exposed directly through skin contact and inhalation (Cordy et al., 2011). This research was designed to investigate the Hg concentrations in the biological samples such as plasma, red blood cells (RBCs), urine, nails and hair collected

from goldsmith workers. The questionnaire concerning the health problems was filled for probing the possible hazardous health effects in the workers. This research work is distinctive in its design due to the determination of the concentrations of Me-Hg, I-Hg and T-Hg in blood (plasma, RBCs) nails, hair and urine specimen collected from the exposed workers involved in Hg gold amalgamation and roasting processes. So far, no single paper reported all these aspects.

Dental amalgam is one of the most commonly used tooth fillings material (Akbal et al., 2014). It is considered a safe, sound, and effective treatment for tooth decay. Amalgam fillings are the chief source of exposure to Hg vapor in the general population. The amalgam consists of approximately 50% Hg combined with other metals such as silver, copper, zinc and tin (Akbal et al., 2014; Homme et al., 2014; Richardson et al., 2011; Shraim et al., 2011; Vamnes et al., 2004). Brain, blood, and urinary concentrations correlate with the number of amalgam surfaces present (Brownawell et al., 2005; Vamnes et al., 2004). It has been estimated that 10 amalgam surfaces would raise urinary concentrations by 1µg of Hg per liter, roughly doubling the background concentrations. It has been estimated that Hg is continuously relieved from Hg-dental fillings in the form of Hg vapor and abraded particles (Al-Saleh, 2011; Clarkson et al., 2003; Woods et al., 2008).

The Hg filling is used from about 150 years as a restorative material because of its stability and easy replacement (Bates, 2006; Brownawell et al., 2005). The ratio of Hg in the biological samples of MDA was 2-12 times higher than without amalgam (Barregård et al., 1999; Mutter, 2011; Mutter et al., 2007). In the intra oral environment the Hg amalgam is subjected to different chemical processes involve mechanical, thermal and

biological. The thermal and mechanical pressure in the mouth due to chewing and brushing of the teeth, use of hot drinks and solution of H_2O_2 (to reduce dental plaque) (Clarkson et al., 2003) corrode the dental filling involve the release of Hg ions to form stable compounds of sulphides, oxides and chlorides (Park and Zheng, 2012; Høl, 2003).

The released Hg from dental filling passed to the gastrointestinal tract and then to the blood (Björkman et al., 2007). The reported papers (Dutton et al., 2013; Geier et al., 2011; Høl; Mutter, 2011; Zeidán-Chuliá et al., 2011) correlated the Hg dental filling with the diseases of the nervous system (e.g. Alzheimer's disease), kidney dysfunction in children, tremor, nausea, weight loss, fatigue, shyness, irritability, headache, anxiety, lack of concentration and impaired memory are all contraindication of Hg amalgam filling**.** Among the toxic metals, Hg is more toxic than Cd, Pd and As due to the formation of covalent bond with thiol groups (cysteins and proteins) causing the accumulation in body tissues specially in brain and kidneys (Mutter, 2011). The autopsy studies found a direct correlation of Hg dental filling with Hg concentration in brain. Hg is lipophilic in nature with high absorption rate and has the ability to pass blood brain barrier and accumulate there which causes neurotoxicity and developmental toxicity and even mortality when exposed to a very higher level (Björkman et al., 2007; Eggleston and Nylander, 1987).

The previous literature indicated that the people using Hg amalgam as their teeth filling material are at a high risk of health. Dutton et al., 2013; Al- Saleh and Elkhatib, 2012; Dunn et al., 2008 and Woods et al., 2008 detected the Hg concentration in urine and kidney biomarkers of children and adults and discussed its toxicity. Furthermore, Lin et al., 2014 and Bjorkman et al., 2012 identified the Hg concentration in serum and whole blood. Similarly, Al Saleh, 2011 and Fakour et al., 2010 detected Hg in the samples of

hair and nails. Some review papers published by Richardson et al., 2011 and Mutter, 2011 focused on the toxicity and health effects of dental amalgam, others like Akbal et al., 2014 and Mutter et al., 2010 studied the neuromuscular effects of dental amalgam. The reported literature observed that recently, several papers were reported that detected Hg concentration in the biological samples blood, urine, hair and nails of MDA, However, no single paper have been reported earlier that detected the Hg concentrations in the biological samples (RBCs, plasma, urine, hair and nails) obtained from MDA at three different times (1^{st}, 3^{rd} and 12^{th} day) from the same individual in a single paper. The importance of the work is that no work has been done in this area before and it is a systematic research following the continuous release of Hg after the dental amalgamation. This research evaluated the Hg in MDA and compared its concentration changes in the biological samples (RBCs, plasma, urine, hair and nails) with time (days) and then correlated them with various parameters (age, weight, surface area, restoration, number of Hg dental filling and fish consumption etc.) that influencing the Hg concentrations in these biological samples.

Hg an important ingredient of beauty because of its use in skin whitening creams, lotion and soap worldwide (Mahaffey, 2005). The Hg was invented by the Greek Philosopher in (371-287 BC) who powdered cinnabar with vinegar in copper pots. The Greek used Hg as cosmetic for skin whitening (Spangler, 2011). Skin whitening is a cause of concern for the women specially Asians because they have dark skin color (Cristaudo et al., 2013; Li et al., 2008a). Dark skin is considered the unattractive aspect of women in Asia which improving the philosophy of west “white is right” (Askari et al., 2013). The darkening of the color is due to the high production of the melanin by the

melanocytes in skin due to environmental factors or inborn (ALqadami et al., 2013). The melanin protects the skin from the sun radiations harmful for skin as well as from the toxic drugs and chemicals (Askari et al., 2013). The high melanin production could be caused by the aging, pregnancy, sun exposure or weakness as well.

In some cultures white skin represents a higher socioeconomic status and success (Benz et al., 2011). Therefore there is worldwide demand for skin whitening creams. In most of the whitening creams in addition to other ingredients Hg is used as a skin whitening agent because the Hg act as a melanin inhibition agent causing whitening of the skin (Peregrino et al., 2011). In cosmetics Hg is also used for the preservation of creams, however Hg is a health hazard causing neurologic, urinary and skin diseases (Benz et al., 2011; Sin and Tsang, 2003). Due to the high toxicity of Hg its use in cosmetics was completely banned and the permissible limit set by the food and drug administration (FDA) for Hg in creams, cosmetics is less than 1ppm (Al-Saleh and Al-Doush, 1997; Hamann et al., 2014).

In spite of its potential toxicity whitening creams containing Hg is still available worldwide (WHO, 2012). The inorganic form of Hg (mercuric chloride, mercurous chloride, ammoniated Hg) is used which induce neurologic and psychiatric symptoms (fatigue, weakness, acrodynia, insomnia) (Benz et al., 2011; Sin and Tsang, 2003). The Hg readily absorbed through the skin depends on skin variability and lipid solubility. The Hg could be ingested through the mouth by the application of cream near the mouth skin and through hands (Chan, 2011). After absorption the Hg is distributed to the body organs and eliminated through urine because major part of inorganic Hg excretion occurs through urine (Benz et al., 2011; Chan, 2011; Sin and Tsang, 2003). The use of whitening

creams containing Hg is a health hazard for developing fetus causing neurologic and intellectual dysfunction (Drescher et al., 2013).

There are many cases raised by the exposure of inorganic Hg in skin whitening creams and lotions. However a study conducted on determination of Hg concentration in creams purchased from different countries including China, USA, Thailand, Japan etc found the highest Hg concentration more than 200 to 1000ppm (Hamann et al., 2014). Another study conducted by Al-Saleh and Al-Doush, 1997 found the highest Hg concentration in creams obtained from Thailand and England which was varied from 1,281-5,650 µg/g whereas the McRill et al., 2000; Peregrino et al., 2011; Sin and Tsang, 2003 and Fakour and Esmaili-Sari, 2014 found the highest Hg concentration in the biological samples (urine, blood, hair and nails) of the individuals used Hg containing whitening creams.

There are many papers related to the detection of Hg concentration in urine, blood, hair and nails but papers related to the detection of Hg concentration in creams and in biological samples of whitening cream users (WCU) are scarce. This study is distinct because of the detection of Hg concentration in whitening creams as well as species of Hg (T-Hg, O-Hg, I-Hg) concentration in the biological samples of WCU in less studied area. The research objective is to study the association between the concentration of Hg in whitening creams and in biological samples of WCU. In Pakistan there are many cosmetics stores where the whitening creams containing Hg are available. The people in this area and even the seller are unaware of the use of Hg in cosmetics and its effects.

1.2 Study Aims and objectives

The present study aims are:

- To analyze the Hg in the blood, hair, nails and urine of goldsmith in Peshawar and its health effects
- To analyze the Hg in the hair, urine, blood and nails of dental amalgam patients
- To investigate the concentration of Hg in the workers of fluorescent lamp manufacturing industries and its health effects
- To identify the Hg concentration in skin whitening creams of different brands and the biological samples of its users.

1.3 Objectives

The proposed study will highlight different aspects of Hg related occupational exposure of the workers and its health effects like gold smith, fluorescent lamp manufacturer, taking into account Hg use in skin whitening creams and dental amalgam in Pakistan and will critically assess its adverse health effect.

1.4 Significance of the Study

Hg is the toxic metal used worldwide in different products and processes. Being its hazardous nature it can affect the human body and cause central nervous system diseases, GIT problems, Infertility in male and female, Skin problems, Kidney damage and respiratory problems. The aim of the study was the simultaneous determination of T-Hg, Me-Hg and I-Hg in biological fluids (Plasma, RBCs, urine, hair and nails) samples of exposed individuals. The present studies help in creating awareness to the general public, exposed workers, dental patients and skin whitening creams user about the hazardous effects of Hg. It can also help the doctors and other health professional about the toxic

effects and symptoms of Hg poisoning. Its significance is highly advantageous for the Environmental protection agency of Pakistan regarding the legislation of industries using Hg. It can also helpful for the future researchers who can study the hazardous effects of Hg in the molecular level specially related to the genotoxicity.

BACKGROUND STUDY

CHAPTER-2

BACKGROUND STUDY

2.1 General Introduction to Hg

The natural occurrence of Hg in environment the three forms, elemental Hg, inorganic compounds (mercuric chloride) and organic compounds (methyl Hg) also called quicksilver. Hg exists in liquid and vapor form at ambient temperature. Coal comprises Hg as a natural component along with other elements in trace amounts (Lyubovskii et al., 1996; Weast et al., 1988).

2.2 Physical Properties

The atomic weight of Hg is 200.59 g/mol having silver-white metal shape (whereas the atomic number of Hg is 80 (1). The density of Hg is 13.6 g.cm-3 at 20°C while the boiling point is 356.6 °C. The Hg exists in liquid state at room temperature and vapor pressure of which is 0.002 mm Hg at 25 °C. The Hg exist in three oxidation states e.g. elemental (Hg), mercurous (Hg^{+}), and mercuric (Hg^{++}). Mercuric chloride $HgCl_2$ (corrosive sublimate - a violent poison), mercuric sulfide (HgS, vermillion, a high-grade paint pigment), mercurous chloride is inorganic Hg compounds whereas Organic Hg compounds comprise mercuric acetate, methylmercuric chloride, dimethyl Hg, and phenyl mercuric acetate (Lyubovskii et al., 1996; Smith et al., 1998; Weast et al., 1988).

2.3 Types of Hg

2.3.1 Elemental Hg

The major source of exposure for Hg-0 is through inhalation in occupational workers, waste incineration, cement manufacturing, coal-fired power plants (CFPP), primary metal production, chemical production and metal mining. The dental amalgamation is another source of exposure to low levels of elemental Hg in the general population released in the mouth after dental filling (Mason et al., 1994; Sanemasa, 1975).

2.3.2 Inorganic Hg

Presently the population is usually not exposed to inorganic Hg ingredients to any important stage these days, as most products containing these ingredients have now been banned. Limited exposure could occur through the container of latex color, which until 1990, could contain Hg ingredients to prevent bacterial and infection growth (Agocs et al., 1990).

2.3.3 Methyl Hg

The most important organic Hg compound, with regards to individual exposure, is methyl Hg. Methyl Hg exposure happens mainly through this diet plan, with fish and fish products as the popular source. Sources of previous get in touch with methyl Hg involve fungicide-treated seeds and various foods from animals fed such grains. Hg has been specific as a pollutant of problem to EPA's Great Waters program due to its persistence in the environment, potential to bio accumulate, and harming to people and the environment (Birke et al., 1972; Hunter et al., 1940; Skerfving et al., 1970).

2.4 Applications

The Hg used in varieties of items due to high density. It is used in barometers, manometers and extensively used in thermometers. Its ease in amalgamating with gold is used in the recovery of gold from its ores (Järup, 2003). Liquid electrode based on Hg metal is used in the manufacture of chlorine and sodium hydroxide by electrolysis of brine (Matson et al., 1965). Hg used in switches, electrical gear and rectifiers, which need to be reliable, and for industrial catalysis (Wong, 2003). It is also used in fluorescent lighting and consumer batteries but it has not been entirely eliminated (Eckelman et al., 2008).

Calomel (mercurous chloride, Hg_2Cl_2) is a Hg compounds used as a standard in electrochemical measurements and in medicine as a purgative (Caldas and Machado, 2004). Mercuric chloride (corrosive sublimate, $HgCl_2$) is used as an insecticide, in rat poison, and as a disinfectant (Fasola et al., 1998). Skin ointments contain Mercuric oxide (Lamar and Bliss, 1966). Mercuric sulphate is used as a catalyst in organic chemistry. Vermilion, a red pigment, is mercuric sulphide; another crystalline form of the sulphide (also used as a pigment) is black. Hg fulminate, $Hg(CNO)_2$, is used as a detonator (Schwartz, 1944).

2.5 Hg Poisoning

Hg is one of toxic metal and exert its toxic effects on nervous system in human's beings. The coastal area in Japan, where sea foods and consumption of fish users was high concentration of Hg was detected and the Minamata disease was spread due to discharge of wastewater from an industrial plant in the sea (Harada, 1995). The US-

EPA's maximum contaminant level of inorganic Hg in drinking water is only $2ugL^{-1}$ (Levin et al., 1991)**.**

The Minamata Disaster after 1956's, methyl Hg disaster happened in Niigata prefecture of Japan in May 1965. The reports declared in survey of the Niigata Minamata 64 – 908 ppb Hg in blood, 56.8 – 570 ppm Hg in hair and 92 – 915 μg/L Hg in urine (Harada, 1995).

During 1970, another case of Minamata disease occurred in Ontario (Canada). Illegal dumping of industrial chemical waste containing Hg into the river by Dryden Pulp and Paper Company was the reason behind the incident and at least seven tonnes of Hg released into the river system. Blood concentration reported more than 200 ppb of Hg accumulated in their blood in 1970s. The hazardous effects were studied in 2010 in the study of Harada et al., 1999 after four decades later; the effects of that Hg are still present. The study revealed the symptoms such as visual disturbances, hearing impairment, difficulty in walking in a straight line, insomnia, headaches, fatigue, exhaustion, etc. were observed in nearly one-third of the people surveyed (Peakall and Lovett, 1972).

Using Hg was effective fungicides in many countries like Mexico and wheat seeds were coated with methyl Hg to protect against the fungus and it was then used as cost effective fungicide. The disastrous was spread in Iraq by using the methyl Hg coated seeds resulted in severe Hg poisoning of Iraq. Nearly 10,000 people died and 1,00,000 were severely and permanently brain damaged (Grant, 1971; Snyder, 1972).

2.6 Hg cycle

The cycle of Hg in the environment undergoes a complex series that is still not completely understood. The Hg is mostly released from the power plants primarily as elemental or ionic Hg. The ionic Hg is non stable than elemental Hg in the atmosphere and deposits near the power plant while elemental Hg transported to the long distance due to fairly stable nature. The Hg becomes oxidized in the air and deposited. The ionic Hg in water attached to the methane group and thus methylated by the bacterial process. This type of Hg conversion and reconversion to different forms is depicted in the Fig 2.1. (Järup, 2003; Mason et al., 1994; Palmer et al., 2009; Svensson et al., 1992).

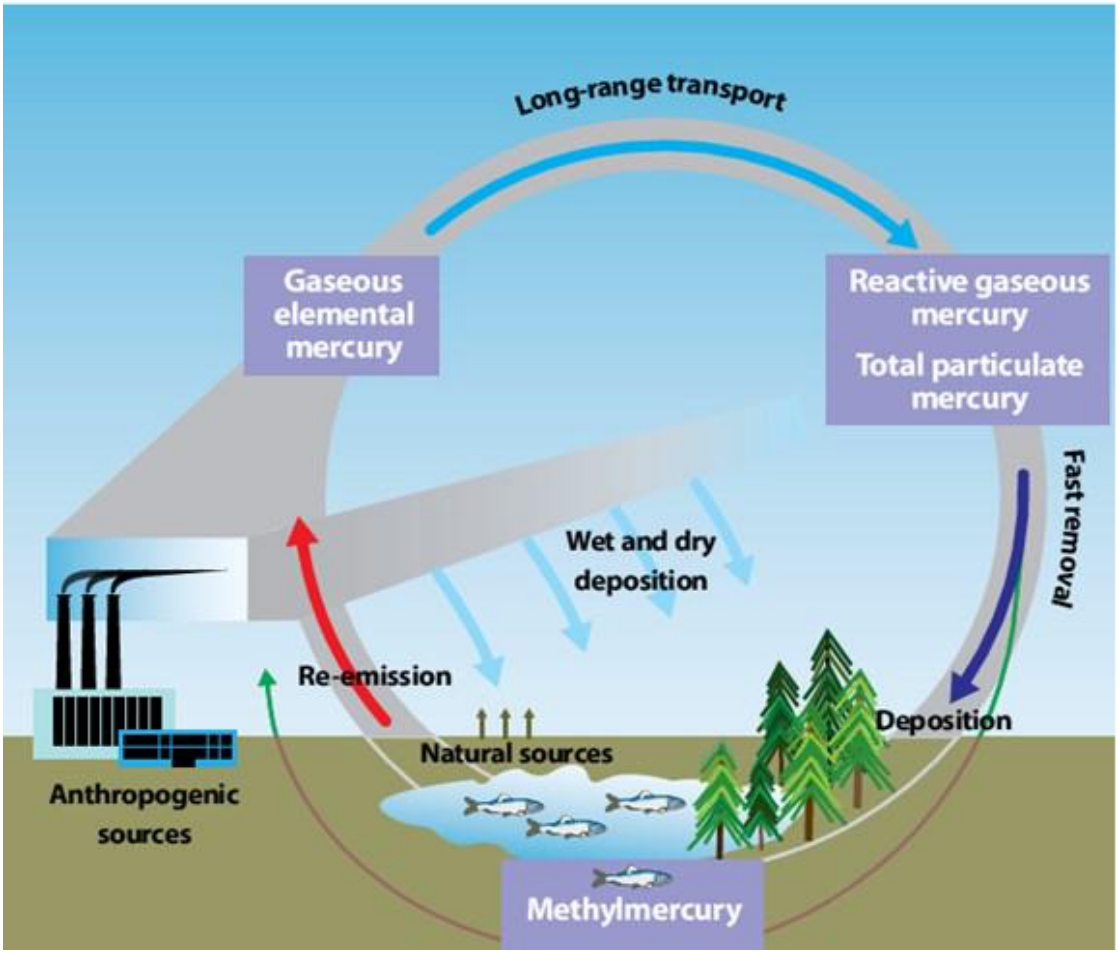


Fig. 2.1 Hg cycle (Crosby, 2013)

2.7 Sources and Exposure

In spite of its well-recognized toxicity, Hg in its various forms is released into the environment at the rate of 11,000 tons annually, supposedly for the "benefit" of mankind. Two important sources of Hg are present i-e natural sources and anthropogenic sources. It is generally assumed that 1/3 of global Hg emissions are natural, 1/3 are anthropogenic and 1/3 are derived from re-emission of previously deposited anthropogenic Hg. The human exposed to the Hg through following sourced (Table 2.1) (Järup, 2003; Palmer et al., 2009).

Table 2.1 Sources of Exposure

Names	Exposure
Cinnabar (mercuric sulphite),	Red pigment in many tattoo salons
Calomel (mercurous chloride)	Treatment for diaper rash
Hg vapor lamps	Lighting
Elemental Hg	Manufacture of plastics, mirrors, thermometers, gold mining
Large fish	As food
Thimerosol	Vaccine
Amalgam	Dental filling material
Cosmetics	skin

2.7.1 Assessing Personal Exposure

The toxicity of Hg are observed in shape of various symptoms in humans when Hg enters the body and is retained at least for some time. The Hg absorption in the body it forms complexes with plasma blood proteins and tissues before targeting important biologically sites. The inhaled Hg half-life (t ½) in blood is 2 – 4 days and 90 % is excreted via urine and faeces whereas methyl Hg has a half-life in human blood of about 50 days (Clarkson, 1972).

Three important pathways for Hg absorption are

(A) Absorption through gastrointestinal tract using foods (B) by Inhalation of Hg vapors in the atmosphere through breathing (C) absorption through the skin but very lesser amount.

2.7.2 Hg exposure through food

Elemental Hg is not soluble in water and hence not absorbed in the gastrointestinal tract (GIT) whereas methyl Hg (Organic Hg) is orally absorbed from the GIT to the extent of 95%. Fishes and other sea food mostly contain methyl Hg and hence readily absorbed. The concentration of methyl Hg in predatory fish is higher due to accumulation and also in food chain in aquatic ecosystems, when consumed by humans, in turns increase the level of Hg concentration and also increased risk of adverse effects in highly exposed or sensitive populations. The mechanism of methyl Hg in human body is the complex formation with free cysteine and with proteins and peptides containing cysteine. This complex formation of methyl mercuric – cysteinyl is accepted by amino acid transporting proteins in the body as methionine, another essential amino acid. This mimicry, it is freely transported in the body using various compartment, cross blood brain barrier (BBB) and placenta and tetrogenic effects of the fetus was observed. It can strongly bind to plasma proteins and not readily eliminated from the body (Clarkson et al., 2007).

2.7.3 Hg exposure through breathing

The burning of coal is used for power generation and also some other industries are one of the major causes for release of Hg in the vapor form in the atmosphere. The populations of such areas are much closed to Hg vapor exposure through inhalation. The inhaled Hg is absorbed through bronchial alveoli by human and is one of the major causes of pollution (Matthes et al., 1958). The National Health and Nutrition Examination survey conducted in 2003 for the assessment of blood Hg level in the United States in 705 children and 1709 women. The geometric mean concentration of total blood

Hg found was 0.34 ppb (95% confidence interval (CI), 0.30 - 0.39 ppb) in children and 1.02 ppb (95% CI, 0.85 - 1.20 ppb) in women (Vupputuri et al., 2005).

The similar studies were conducted in the Canadian population by the Canadian Health Measures Survey (CHMS) evaluating the Hg concentration and other environmental contaminants from 2007 to 2009. The blood concentration of the samples was significantly higher ($P<0.001$) in the subjects as compared to that in controls subjects. The studies revealed that's women (n=100) appearing in the gynaecology clinic have menstrual irregularities, anemia, headache sterility, still births, numbness and tingling of the lower extremities. Hyper-pigmentation, black line over gums, elevated blood pressure and tremors were also seen in some of the cases (Haines and Murray, 2012; Wong and Lye, 2008).

2.8 Toxicity

All types of Hg are quite harmful, and each form exerts different health effects. The Coal combustion evaporates Hg during operating in boiler at temperatures above 11000C. Using coal as fuel in thermal power plant as well as in industrial, commercial and residential burners, The Hg released in considerable amounts into the environment. The utility boilers using coal released Hg in vaporized form and evaporate in a gaseous state (Carpi, 1997; Wang et al., 2000). The report of the United Nations Environmental Program 2008 revealed that approximately 3530 tonnes of Hg were emitted of which 1930 tonnes were attributable to human activity. Hg is a compound and it will re-emit after being deposited on surfaces.

The majority of anthropogenic Hg released into the atmosphere were from 45 % from burning of coal combustion for electric power as the coal is the primary source of

the Hg. Other sources of Hg released to the environment are gold extraction, metal production, cement production, waste incineration, chemical manufacture, and dental amalgams (through cremation), and also contribute significantly to the global Hg releases as shown in Fig. 2.1.

Three countries are the largest Hg emitters (China, the United States, and India) the three largest Hg emitters) are dominated by fossil fuel combustion whereas Indonesia and Brazil are among the top ten Hg emitters. The method used for mining of gold using Hg gold amalgamation have reduced by large mining concerns where as many small scale miners continue the practice in which liquid Hg is mixed with the gold panning slurry, wetting and separating the gold. The heating of this gold-Hg amalgam for gold extraction also create Hg vapor levels up to 16,000 $\mu g/m^3$ which far exceeds the recommended limits for occupational exposure set by the WHO or NIOSH. Frequently burning of these amalgam are almost adjacent to populated areas, effecting nearby buildings, and the escaped Hg can enter local waterways further increasing the risk from consumed fish (Carpi, 1997; Pacyna et al., 2010; Wu et al., 2006).

2.8.1 Hazardous Effects of Hg

- Hg damages the central nervous system (CNS) regardless of how it enters the body (Insomnia, dizziness, tremors, dementia) (Eisler, 2010; Møller-Madsen and Danscher, 1991)
- It has irreparable effects on the kidneys (Langford and Ferner, 1999; Møller-Madsen and Danscher, 1991).
- Hg may harm a developing fetus and decrease fertility in men and women (Koos and Longo, 1976).

- Respiratory diseases (Asthma, bronchitis, wheezing coughing) and effects the lungs
- Fatigue , lethargy, and restlessness (Verity et al., 1975)
- Skin diseases (dermatitis, epidermal eruption, skin allergy and irritation) (Olumide et al., 2008)
- Gastro intestinal problems (Metallic taste Diarrhea, Vomiting, nausea) (Olumide et al., 2008).

2.9 Detoxification

The body deals with toxins in a very ordered fashion:

- Protective barriers and secretions (skin, mucus, tears, saliva)
- Immunologically (inflammation, immunoglobulin response)
- Biotransformation (activation of cytochrome P450 enzyme detoxification systems)
- Raising blood lipids (HDL, LDL and VLDL cholesterol and triglycerides)

By doing a comprehensive blood chemistry, based on the principles of Free Radical Therapy, we can gain a fairly accurate idea of which toxin or combination of toxins we are dealing with, where the toxin is located, how much is there and how it is being transported, and thereby gain some idea of how best to neutralize the toxins and get them out.

The protocol we use to help the patient get rid of Hg is a multi-step process. The first step involves changing the diet to enhance the body's ability to handle contaminant materials. The next step adds specific supplementation and chelation therapy. We then do a comprehensive survey of the mouth to determine the best order for removal of amalgams

and the most compatible type of dental material with which to replace them. Only then do we proceed with the removal of amalgam fillings (Tchounwou et al., 2003).

2.10 Review of the literature

The review of the literature is depicted in the Table. 2.2.

Table 2.2 Literature related with the research area (2000-2014)

S.No	Exposed humans	Sample	Hg concentration	Detection technique	Country	Type of Hg	Ref
1	Chlor alkali industry	Blood, Urine	Blood-22.41± 12.58 µg/l Urine-30.61± 10.86 µg/g Creatinine (Cr)	CVAAS	Iran	T-Hg	(Shirkhanloo et al., 2014)
2	Gold minning	Hg intoxication		Qualitative study	Zimbabwe	T-Hg	(Steckling et al., 2014)
3	Artisanal small scale gold miners	Urine, Blood, Hair	Urine- 7.18 µg/g Cr Blood-4.36 µg/l Hair-1-5 µg/g	Not know	Mongolia	T-Hg	(Steckling et al., 2011)
4	Artisanal small scale gold miners	Health risks associated with Hg		Qualitative study	Tanzania	-	(Charles et al., 2013)
5	Fluorescent lamp factory	Urine	Tremors, emotional lability, memory changes, neuromuscular changes, and performance deficits	Qualitative + Quantitative study	Egypt	T-Hg	(Al-Batanony et al., 2013)
6	Gold-mining	Blood, Urine	Tremor, postural stability	Qualitative + Quantitative study	Ecuador	T-Hg	(Harari et al., 2012)
7	Artisanal Small-Scale Gold Mining	Urine, Hair, Blood	2.4 µg/g Hair	Quantitative study	Asia, Africa	T-Hg	(Baeuml et al., 2011)
8	Small-scale gold mining	Urine	Refining process is the major source of exposure	Qualitative study	Burkina Faso	T-Hg	(Tomicic et al., 2011)
9	Artisanal gold miners	Blood, Urine, Hair	Diseases e.g. ataxia, tremor, dysdiadochokinesia)	Qualitative study	Northern Sulawesi	T-Hg	(Bose-O'Reilly et al., 2010)

10	Smelting workers of artisanal Hg mines	Urine, Hair	Urine-1060 µg/g Cr, Hair T-Hg- 69.3 µg/g Hair Me-Hg 2.32 µg/g	Quantitative study	China	T-Hg, Me-Hg	(Li et al., 2008)
11	Hg mine workers	Hair, Urine	Urine- 258 ng/ml, 226 µg/g Cr Hair-20.0 µg/g	Quantitative study	China	T-Hg	(Sakamoto et al., 2007)
12	Exposed individuals toxicological center	Urine	U-Hg levels Childrens-2.73 µg/g Cr adults 2.55 µg/g Cr shipyard workers-35.47% dentists -23.5%	Quantitative study	Venezuela	T-Hg	(Rojas et al., 2006)
13	Chloralkali Workers		Urine- 5.9 nmol/mmol (Cr)	Quantitative study		T-Hg	(Ellingsen et al., 2001)
14	Goldsmith workers	Hair	Hg level higher than control	flow injection Hg system (FIMS)	Rome	T-Hg	(D'Ilio et al., 2000)
15	Dental amalgam	Urine	0.55 µg/g Cr	Quantitative study	Albertans	T-Hg	(Dutton et al., 2013)
16	Dental amalgam	Review paper	2-12 times more Hg in body tissues of individuals with dental amalgam	---		-	(Mutter, 2011)
17	Dental amalgam	Urine	dental amalgams are a significant chronic contributor to Hg body-burden	Qualitative study	Casa Pia	-	(Geier et al., 2011)
18	Dental amalgam	Questionnaire and interview	Autism is caused by Hg dental filling	Qualitative study	Brazil	-	(Zeidán-Chuliá et al., 2011)
19	Dental amalgam	Brain, Blood, Muscle, Toenails	Occipital cortex-18µg/kg Blood-5µg/l, Abdominal Muscle-5 µg/kg Toenails-280 µg/kg	Cold vapor atomic fluorescence spectrometry, (ICP-SFMS)	Norway	Me-Hg, I-Hg	(Björkman et al., 2007)

20	Dental amalgam	Whole blood, RBC, plasma, Urine, Hair	Whole blood-2.2 µg/l Plasma-0.65 µg/l RBCs-4.1 µg/l Urine-1.9 µg/g Cr Hair-0.76 mg/kg	Multiple injection cold vapour atomic fluorescence spectrophotometry	Sweden	Me-Hg, I-Hg, T-Hg	(Berglund et al., 2005)
21	549 skin-lightening products	Skin creams	6% contain Hg above 1000 ppm 45% - above 10,000ppm	Low-cost portable x-ray fluorescence spectrometer	United States,China, Taiwan, Thailand, Japan, Sri Lanka	T-Hg	(Hamann et al., 2014)
22	Hairdressers	Hair, Nails	Hair- 1.15 µg/g Nails- 1.82 µg/g	Advanced Hg Analyzer accord-ing to ASTM	Iran	T-Hg	(Fakour and Esmaili-Sari, 2014)
23	Whitening creams users	Hair, Urine	Urine-7-135 ug/L Hair-361-5617 ug/g	Quantitative study	Barbados	T-Hg	(Drescher et al., 2013)
24	Misuse of skin products	Questionnaire	Lighter skin is more beautiful. %age use of Fair and lovely cream greater than Garnier and other	Qualitative study	Pakistan		(Askari et al., 2013)
	Whitening creams	Creams	0.207ppm	Flame atomic absorption spectrophotometer	South Africa	T-Hg	(Adepoju-Bello et al., 2012)
25	Skin-lightening creams	Whitening creams	38% creams have Hg- 878 and 36,000 ppm	CV-AAS	Mexico	T-Hg	(Peregrino et al., 2011)
26	Skin-lightening creams users	Urine, blood, Whitening creams	Creams- 660 to 57000 ppm Blood-45.2 µg/L Urine-17.1 µg/L	ICPMS	Hong Kong	T-Hg	(Sin and Tsang, 2003)
27	Whitening creams users	Urine	170 µg/L to 32 µg/L	Quantitative study	Arizona	T-Hg	(McRill et al., 2000)

Industrial Processes in Fluorescent Lamp Industry

CHAPTER-3

QUANTIFICATION OF ORGANIC, INORGANIC AND TOTAL Hg IN THE BIOLOGICAL SAMPLES OF WORKERS IN FLUORESCENT LAMP INDUSTRIES

3.1 Abstract

This study was conducted to investigate the concentrations of Me-Hg, I-Hg and T-Hg in the exposed workers of fluorescent lamp industries. For this purpose biological samples such as red blood cells (RBCs), plasma, urine, hair and nails were collected from Hg exposed workers and non-worker as control group. These samples were analyzed for Hg concentrations and their correlations with the demographic profile of specimen donating persons were assessed. The mean concentrations of T- Hg (31.9 µg/L), Me-Hg (27.7 µg/L) and I-Hg (5.36 µg/L) in RBCs were significantly ($P<0.001$) higher in exposed workers (n=40) as compared to the control, whereas T- Hg, Me-Hg and I-Hg concentrations in plasma were observed 15.1, 3.5 and 10.6 µg/L, respectively and significantly higher ($P < 0.001$) than the control group. The mean concentrations of T-Hg (137.5 µg/L), Me-Hg (13.5 µg/L) and I-Hg (137.5 µg/L) in urine were also significantly ($P<0.001$) higher than the control group which were 2.81, 0.31 and 2.38 µg/L, respectively. The concentrations of Hg species (T- Hg, Me-Hg, and I-Hg) were significantly higher in the samples of hair and nail of the workers as compared to the control group. This study concluded that the workers in fluorescent lamp industries were highly exposed to Hg as indicated by the concentrations of Hg and its species in the biological specimen. Numerous health problems were noticed during questionnaire

survey which could be linked with high exposure of Hg; therefore, precautionary measures should be adopted such as protective cloths, masks and minimum exposure time by the workers to minimize the health risks.

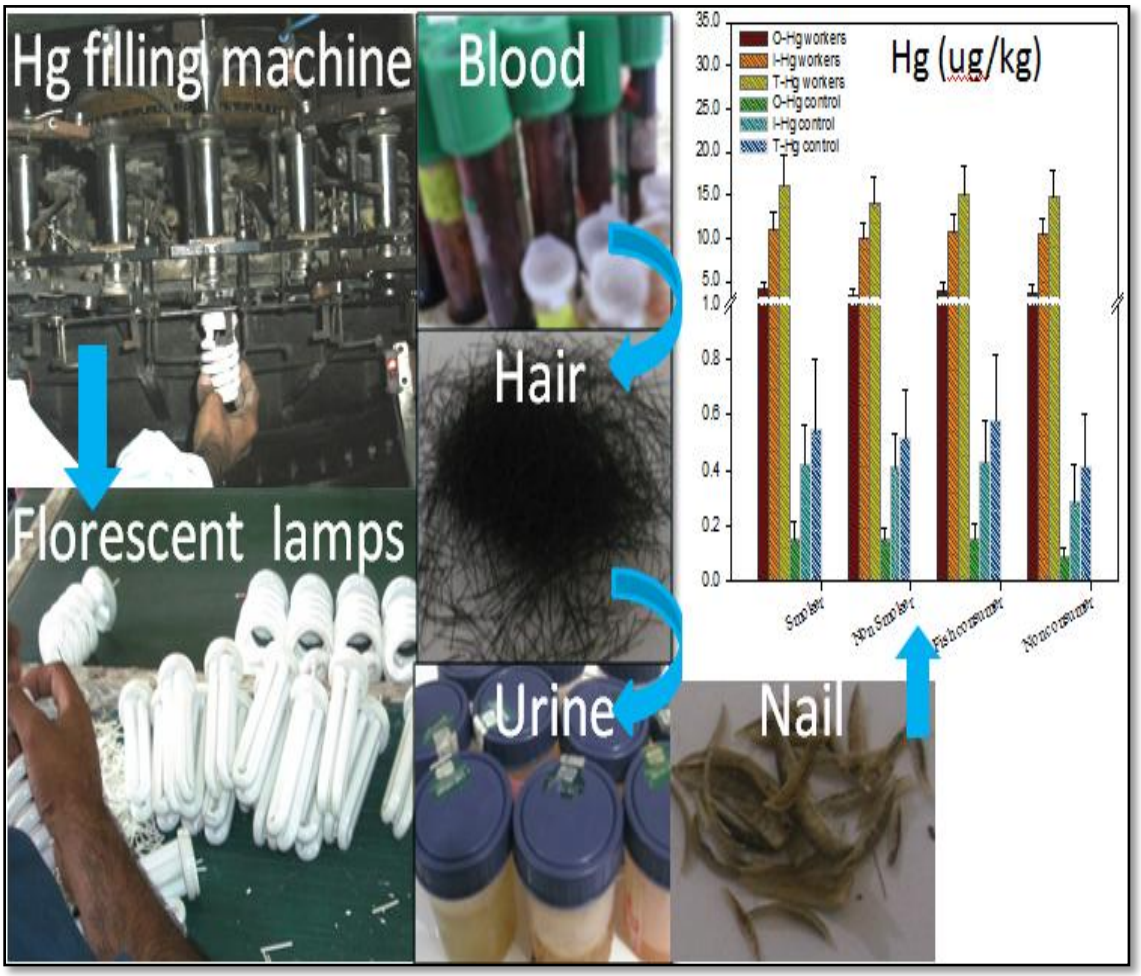


Fig. 3.1 Graphical abstract of fluorescent lamp industry chapter

3.2 Introduction

Hg (Hg) is present in earth crust and found in ranking on the top of environmental pollutants. It occurs in organic, inorganic, and elemental form in many diverse settings (Karagas et al., 2012). Hg is used widely in agriculture, medicine and industries and circulates in the environment (Park and Zheng, 2012). Hg and its compounds have long been recognized as an occupational toxicant. The Hg exerts variety of adverse effects on human kidney, liver, respiratory and nervous systems, skin, reproductive and developmental defects, thyroid gland, immune system, geno toxicities and cardiovascular disorders (Abdel-Rasul et al., 2013; Al-Batanony et al., 2013; Barregård et al., 1994; Carpenter, 2001; Carvalho et al., 2008; Cherian and Clarkson, 1976; De Rosis et al., 1985; El-Demerdash, 2001; Halliwell, 1992; Houston, 2007; Hussain et al., 1999; Kampa and Castanas, 2008; Kishi et al., 1993; Langworth et al., 1992; Rocha et al., 1995; Rodilla et al., 1998; Stohs and Bagchi, 1995; Vallee and Ulmer, 1972; Zahir et al., 2005).

Fluorescent lighting technologies are undergoing rapid market growth as part of a resurgent societal interest in energy efficiency and its need is much higher in developing countries like Pakistan due to energy crises and lesser expansion of electricity projects (Johnson et al., 2008). This fast growth of fluorescent lamps is also associated with great health risks because of emission of Hg that is an essential component in all types of fluorescent lamps due to 75% reduction in energy usage and 10-fold increase in lifetime as compared to incandescent bulbs. Fluorescent lamps contain 0.7-115 mg of Hg per lamp depending on its size, and the subclass of compact fluorescent lamps (CFLs) on average contains 3-5 mg of Hg per lamp (Jang et al., 2005).

Recently, several papers have been reported the effects of Hg exposure in industrial workers of florescent lamp industry including chronic insomnia (Rossini et al., 2000), female reproductive health (De Rosis et al., 1985) and measurement of hand tremor (Fawer et al., 1983; Roels et al., 1989; Verberk et al., 1986). In the past, a few studies have been focused to monitor the creatinine level of Hg in urine samples in both exposed and control groups. (Barregård, 1993; Roels et al., 1991). On the other hand, several papers have been published on the detection of the Hg in biological samples of dental amalgam, used in dentistry (Berglund et al., 2005; Björkman et al., 2007; Nylander et al., 1986; Zolfaghari et al., 2007). Moreover, the Hg concentration in urine of exposed worker in Egypt was reported in florescent lamp industries (Al-Batanony et al., 2013). However, no research work is conducted so far to determine the concentrations of total Hg (T-Hg), organic Hg (Me-Hg)) and inorganic Hg (I-Hg) in blood (plasma and RBCs) nails, hair and urine specimens from the exposed workers of florescent lamp industries. This study aimed to investigate the T-Hg, Me-Hg and I-Hg concentrations in biological samples including blood, nails, hair and urine collected from the workers in fluorescent lamp industries and its hazardous effects on human health.

3.3 Materials and Methods

3.3.1. Reagents

Hg standard solution (1 mg/L in 10% HNO_3) Perkin Elmer manufactured under ISO 9001 quality assurance system, HNO_3 (68%), HCl (37%), H_2SO_4 (98%), $HCLO_4$ (70 %) were purchased from Sigma–Aldrich® (via Analytical Measuring Systems, Karachi, Pakistan). Analytical grade ethanol (CH_3CH_2OH), acetic acid (CH_3COOH) analytical-grade sodium boro hydride ($NaBH_4$), tin chloride ($SnCl_2$), cadmium chloride ($CdCl_2$), sodium

hydroxide (NaOH), cysteine ($HO_2CCH(NH_2)CH_2SH$) and potassium hydroxide (KOH) were purchased from either Sigma–Aldrich®, Merck chemicals (via Science Centre, Rawalpindi, Pakistan), or Scharlau (via Musaji Adam & Sons, Karachi, Pakistan). Ultra-pure water was prepared by a Millipore ultra-pure water system (Milford, USA). All these chemicals and reagents were used without further purification.

3.3.2. Study design

The proposed hypothesis was evaluated by designing a prospective observational case-control clinical study as per the principles of the "world medical association (WMA) declaration of Helsinki−Ethical principles for medical research involving human subjects" and its amendments. The protocol of this study was approved by the Ethical Committee of the University of Peshawar, Pakistan.

3.3.3 Subjects

Exposed workers (n=40) selected from different production sections of the fluorescents lamp industries, including those working in the vicinity of exhaust, basing and sealing machine and healthy non exposed age and gender matched subjects (n=40) were enrolled based screening type of study. Signed written "*Informed consent*" was also obtained from all the participants at the start of the study.

A brief questionnaire was also filled from the individuals in which the detailed medical profile and duration of the work in the florescent lamp industries were recorded.

3.3.4. Inclusion criteria

The study includes the workers involved in working near the exhaust, sealing and basing machines in the fluorescent lamp industry.

3.3.5 Exclusion criteria

The workers involved working other than the exhaust, sealing and basing machines in the industry were excluded from the exposed workers study.

3.3.6 Collection of Samples

3.3.6.1 Blood

Venous blood samples were collected from subjects in heparinized vacutainer tubes. These samples were then divided in plasma and erythrocytes aliquots by centrifugation at 3000 x *g* for 10 min. These samples were then stored in eppendorf tubes at -20 °C until analyses.

3.3.6.2 Urine

Urine samples were collected in clean polyethylene bottles. To avoid volatilization of Hg, 1 ml of HNO_3 (10% aqueous solution) was added to each urine sample.

3.3.6.3. Nails

Nail clipping were obtained by cutting all 10 fingernails and toenails. Nails were coded and stored in plastic bags at room temperature for further processing. Before extraction, nails samples were thoroughly washed three times with acetone, deionized water, 0.5% Triton X-100 aqueous solution and again with deionized water. Finally, samples were dried at 35°C for 24 h.

3.3.6.4 Hair

A lock of scalp hair from the occipital region about three inches near the root was obtained from each participant and stored in plastic bags at room temperature for further processing. Before extraction, nails were also washed twice with acetone and deionized

water. Finally, samples were dried in an oven at 60°C for 24 h. These samples were also cut into smaller pieces with scissors.

3.3.7 Sample preparation

The concentrations of T-Hg, I-Hg and Me-Hg were determined in the prepared biological samples (plasma, RBCs, urine, nails and hair) as given below:

3.3.7.1. Quantification of T-Hg

The concentrations of T-Hg from plasma, RBCs and urine samples were extracted using the method adopted by Sakamoto et al., 2010 with slight modification. Briefly, plasma (0.5 ml) and urine (1 ml) samples were thawed to room temperature and then transferred to a vial containing a 3 ml mixture of HNO_3 (70%), $HCLO_4$ (70%) and H_2SO_4 (98%) (in 5:2:1 ratio, respectively). This digestion mixture was heated to 40 $^{\circ}$C for 1 h and then at 90 $^{\circ}$C for another 1 h with frequent mixing until the brown fumes of oxides of nitrogen dissipated and the remaining liquid is golden yellow in color. After cooling the samples, 30 ml of deionized water was added and ready for analysis.

The concentrations of T-Hg in nails and hair samples were extracted using the method adopted by Anim et al. (2011) with slight modification. Briefly, the finely cut hair (0.2 g) and grounded nails (50 mg) samples were digested with 1 ml deionized water, 2 ml of a mixture of concentrated HNO_3 and $HClO_4$ in 1:1 ratio and 5 ml H_2SO4 at 220-240 $^{\circ}$C for 20 min. After cooling, homogenized the samples by shaking and the volume was made 30 ml with deionized water. The concentrations of T-Hg in the extracts of RBCs, plasma, urine, nails and hair were determined using atomic absorption spectrometry (ASS 700, Perkin Elmer) equipped with Hg hydride system (MHS-15) using 1.5% HNO_3, 3% sodium borohydride ($NaBH_4$) as reducing agent and 2-3 drops of 5% $KMNO_4$.The

samples internal laboratory standards and controlled were carried through the digestion procedure. The precision and accuracy of this method have been repeatedly verified by inter-laboratory calibration exercises.

3.3.7.2 Quantification of Me-Hg

The concentration of Me-Hg was determined in biological samples RBCs, plasma and urine using the method adopted by Vahter et al. (2000) with slight changes. The plasma (0.5 ml), RBCs (1 ml) and urine (1 ml) samples were treated with a mixture of 1 ml of L-cysteine solution (0.0124 M), 1.5 ml of KOH (11 M) and 0.5 ml deionized water and stored overnight at ambient temperature, while $NaBH_4$ (3%) was used as reducing agent. The extracts were diluted to 30 ml with deionized water before analysis for Me-Hg.

The Me-Hg was extracted from hair and nails samples by using the method Harada et al., 1999). Briefly The finely cut hair (0.2 g) and grounded nails (50 mg) samples were digested with 1 ml deionized water, 2 drops of ethanol and 5 ml of HCl (2.0 N). The mixture was heated up to 100 $^{\circ}$C for 15 min. After cooling, 3 ml acetate buffer solution (4.5 pH) was added. Deionized water (30 ml) was added into each sample. Like blood and urine samples, the $NaBH_4$ was used as reducing agent during time of analysis. The concentrations of Hg were determined using ASS 700.

3.3.7.3 Quantification of I-Hg

The I-Hg was extracted from biological samples using the method adopted by Littlejohn et al. (1976) with slight modification.

RBCs (0.5 ml), plasma (0.5 ml) and urine (1 ml) samples were taken in reaction flask (50 ml) along with 0.5 ml of stannous chloride (100 g/L of HCl) and 3 ml of NaOH (300 g/L)

with continuous stirring. Stannous chloride was used as a reducing agent before analysis and the concentrations of Hg were determined using ASS 700.

3.3.8 Statistical Analyses

The mean and standard deviation of replicates (n=3) for T-Hg, Me-Hg and I-Hg concentrations in plasma, RBCs, urine, hair and nails were calculated. Calibration standards of 10, 25 and 50 µg/L for the instrument were prepared freshly by the dilution of Hg standard stock solution 1 mg/L in 10% HNO_3. The analytical performance was evaluated in terms of limit of detection were 0.34 µg/L, 0.109 µg/L, 0.91 µg/L for RBCs, plasma and urine respectively and 0.093 µg/g, 0.16 µg/g for hair and nails respectively. The method was validated by analyzing three different certified reference materials namely blood material level I, MR4206 (Nycomed company, Oslo, Norway) and urine control level II (Sigma chemical company) and human hair NIES No.13 (National Institute of Environmental Studies, Japan). The observed average (n=3) and reference values for blood were 2.2±0.4 µg/L comparable with reference value 2.4 µg/L while 72±14 µg/L and 98 µg/L for urine and 4.27±0.25 µg/g comparable with reference value 4.42 µg/g for hair. The Statistical analysis was performed used sigma plot version 10, Minitab® and Xcel® software. The difference between exposed workers and the control group was evaluated using unpaired t-test at 95% confidence interval. The correlation between Hg concentration in biological samples and demographic characteristics were examined by linear regression analysis.

3.4 Results

Table 3.1 summarizes the demographic profile of the individual volunteer participated in this study. The mean values of age, weight, gender, working experience,

smoking (cigarette), exposure time and fish consumption of the workers and control subjects were calculated. In fluorescent lamps industries, the workers were only male with a mean age of 26.7 y; therefore no female was included from the control group. The height (5.28±0.20 feet), weight (56.9±10.5 kg) and experience (5.35±4.81 y) of the exposed workers and control group were recorded. The working time was 8 h per day with one holiday in a week. The precautionary measures such as masks and protective cloths were not adopted by most of the workers during work, while 70% of the workers were used gloves. Among selected workers, only 40% workers were consumed fish. Two workers were used dental amalgam of Hg based in tooth filling one year before giving the biological samples for this research. All the data collected during questionnaire survey were correlated with Hg concentrations in biological samples collected from the workers.

Table 3.1 The Demographic profile and the concentrations of Hg in the RBCs, plasma, urine, hair and nails in exposed workers of Fluorescent lamps industries and control subjects.

Parameters	**Exposed workers**	**Control group**	**Parameters**		**Exposed occupational workers**	**Control group**	
					Mean+S.D	**Mean+S.D**	**P value**
Age (y)	26.7 ± 6.72	29.9 ± 5.27	**RBCs**	Me-Hg	27.7±5.36	1.67±0.86	0.001
Gender (male*)	40	40	µg/L	I-Hg	3.32±0.75	0.14±0.074	0.001
Height (ft)	5.28 ± 0.20	5.31 ± 0.16		T-Hg	31.87±5.96	1.85±0.94	0.001
Weight (kg)	56.9 ± 10.5	55.1 ± 8.92	**Plasma**	Me-Hg	3.5±0.68	0.16±0.084	0.001
Experience (y)	5.35 ± 4.81	NA*	µg/L	I-Hg	10.6±1.95	0.41±0.21	0.001
Working (h)	8	NA*		T-Hg	15.1±2.77	0.59±0.301	0.001
Smoker (%)	60	55	**Urine**	Me-Hg	13.5±6.89	0.31±0.23	0.001
Masks user (%)	NR*	NA*	µg/L	I-Hg	137.5±24.2	2.38±1.14	0.001
Gloves user (%)	70	NA*		T-Hg	154.6±28	2.81±1.3	0.001
Goggles user (%)	NR*	NA*	**Hair**	Me-Hg	3.21±0.73	0.3±0.18	0.001
Fish consumer (%)	40	70	µg/g	I-Hg	0.31±0.17	0.03±0.016	0.001
Protective cloths	NR*	NA*		T-Hg	3.64±0.84	0.33±0.2	0.001
Hg based tooth filling (%)	10	NR*	**Nails**	Me-Hg	5.52±0.92	0.37±0.2	0.001
			µg/g	I-Hg	0.6±0.26	0.034±0.021	0.001
				T-Hg	6.3±1.62	0.41±0.22	0.001

*No female worker was present in these industries; therefore in control group no female was included.
*Each value of mean ± SD represents standard deviation (n=40)
NA*. Not applicable
NR*. Not reported

The concentrations of Hg (T-Hg, Me-Hg and I-Hg) in the RBCs, plasma, urine, hair and nails samples collected from the exposed workers and control individuals are given in Table 3.1. Hg concentrations were significantly higher in the exposed workers as

compared to the control subjects. Fig. (3.1-3.5) show the concentrations of Me-Hg, I-Hg and T-Hg in RBCs, plasma, urine, hair and nails with the demographic characteristics (age, weight, experience, smoking, fish consumption) of exposed workers and control. The age of the exposed workers were divided in to four groups ranging from 18-23, 24-29, 30-34 and 35-40 y, whereas the weights were also categorized in to four groups ranging from 40-49, 50-59, 59-68 and 69-74 kg.

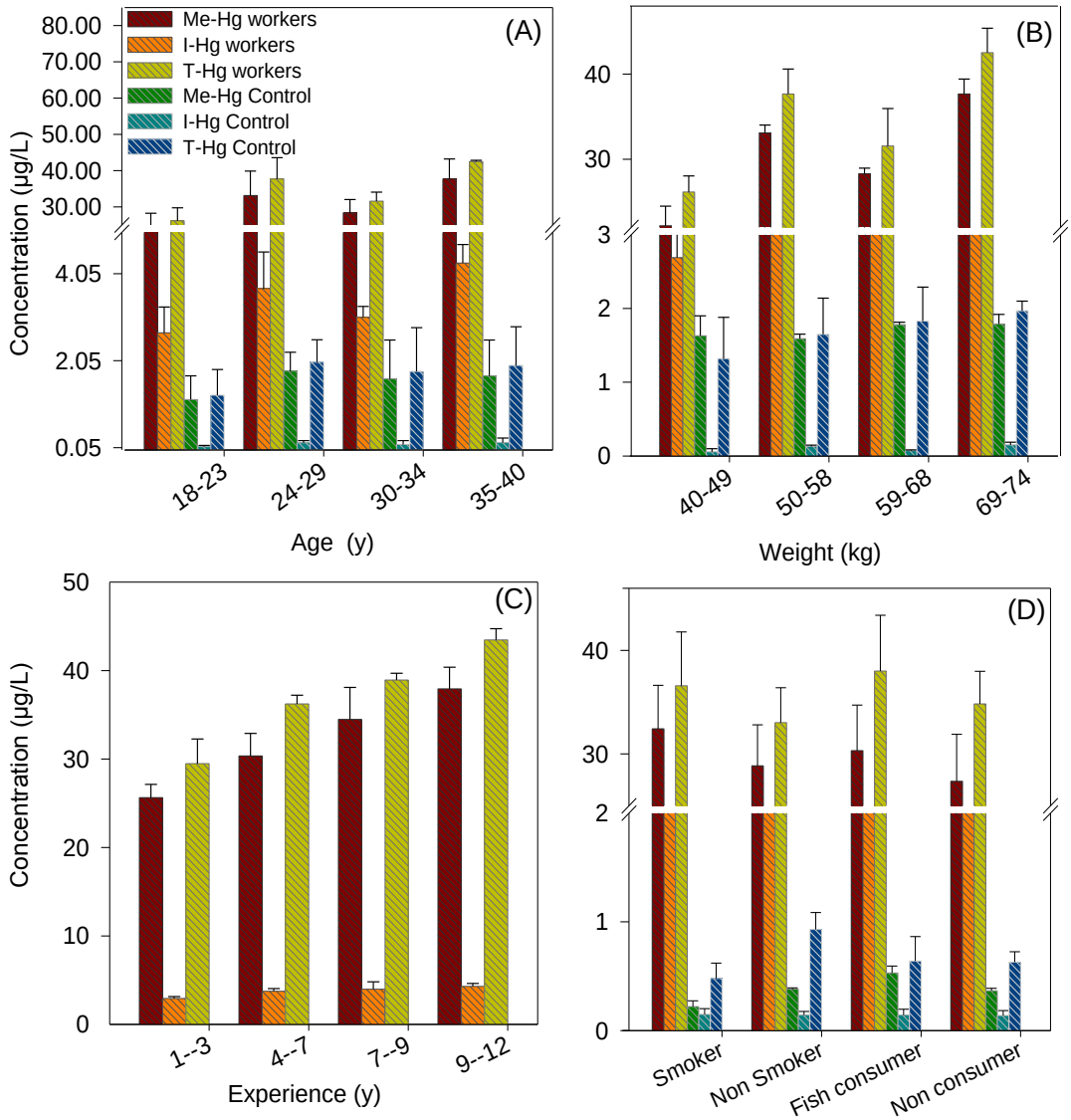


Fig. 3.2 The concentrations of O-Hg, I-Hg and T-Hg in RBCs with A) age, B) weight, C) experience, D) smoking and fish consumption of the exposed workers and control subjects.

The mean concentrations of T-Hg (31.9 µg/L), Me-Hg (27.7 µg/L) and I-Hg (3.3 µg/L) in RBCs were significantly ($P<0.001$) higher in exposed workers (n=40) as compared to the control subjects (n=40) T-Hg (1.85 µg/L), Me-Hg (1.67 µg/L) and I-Hg (0.14 µg/L) concentrations are given in Table 3.1. The concentrations of Hg with age and weight were comparatively higher in exposed workers than the control but the linear regression model between Hg concentrations and age/weight did not seemed significant effect (Fig. 3.1A, 3.1B). The workers with age ranged from 24-29 y and weight from 50-59 kg showed an elevated level of T-Hg as compared to the third group (age, weight) which might be linked with the fish consumption, smoking, experience length or their working locations in the industry where they were exposed to high concentration of Hg. The regression model showed a stronger correlation between the Me-Hg in RBCs and the age of worker (R^2=0.61 for T-Hg, R^2=0.65 for Me-Hg, R^2=0.56 for I-Hg), while weak correlation (R^2=0.24 for T-Hg, R^2=0.34 for Me-Hg, R^2=0.32 for I-Hg) was observed for weight of the exposed workers and Hg concentrations in RBCs. The experience of workers is categorized in to 4 groups (1-3, 4-7, 7-9 and 9-12 y). The long-term working exposure to Hg is directly related with the elevated concentrations of the Hg species as shown in Fig. 3.1C. The linear regression model was used to assess the correlation between the experience length and Hg concentrations in RBCs, which indicated a strong corelationships (R^2=0.932 for T-Hg, R^2=0.994 for Me-Hg and R^2=0.978 for I-Hg) for the concentration of Hg in workers RBCs having longer experience time. The concentration of T-Hg, Me-Hg and I-Hg in the RBCs of smokers were 36.6, 32.4 and 3.48 µg/L, respectively, whereas as in non-smokers group of the exposed workers were 33.0, 28.9 and 3.30 µg/L, respectively (Fig. 3.1D). These results showed a significant (P=0.001)

difference in Hg concentrations of RBCs collected from the exposed smokers and non smokers. Furthermore, the Hg concentrations in smokers and fish consumers were also significantly higher than the nonsmokers and non-fish user in both workers and control groups (Fig. 3.1D).

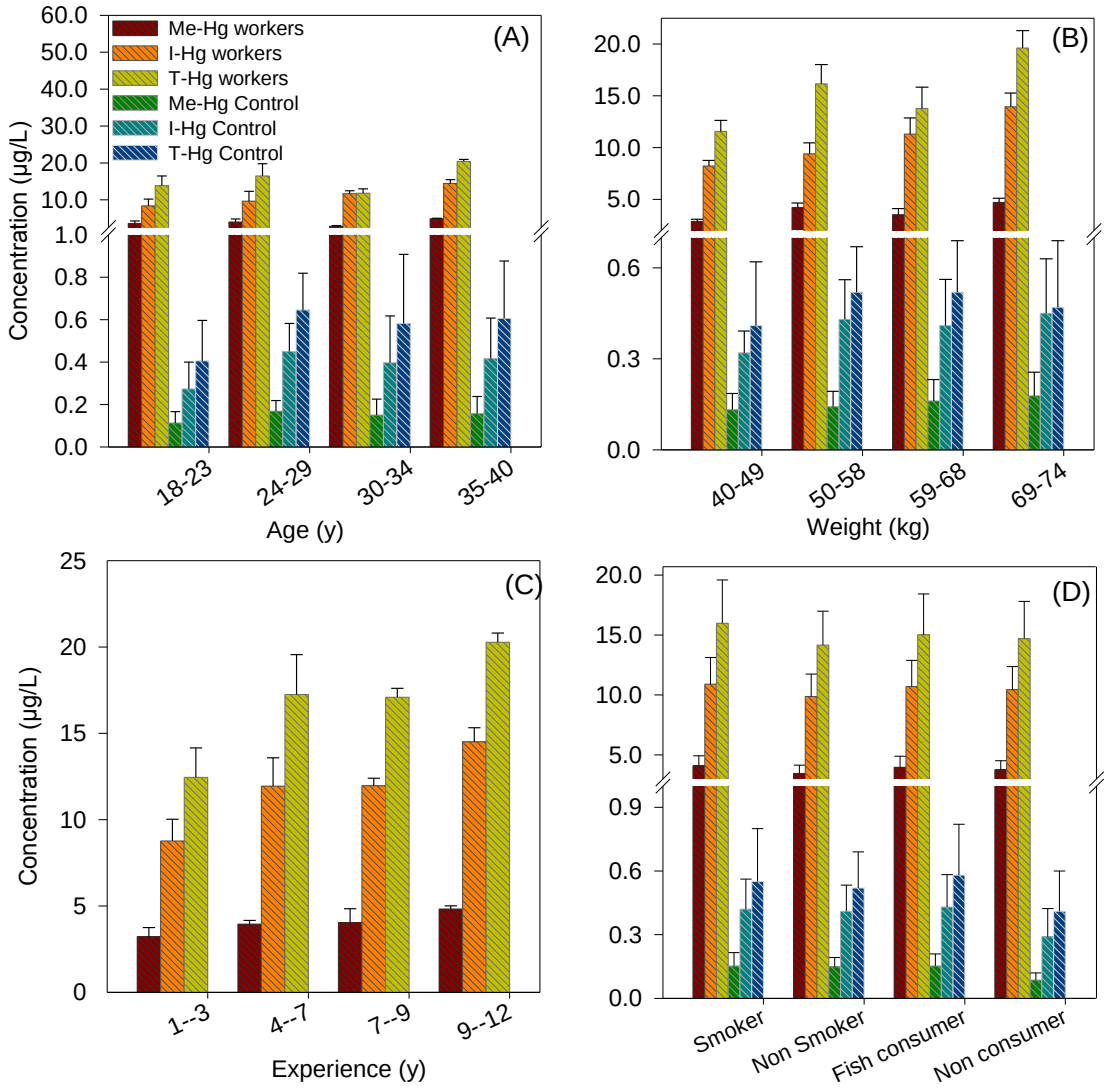


Fig. 3.3 The concentrations of Me-Hg, I-Hg and T-Hg in plasma with A) age, B) weight, C) experience, D) smoking and fish consumption of the exposed workers and control subjects.

The mean concentrations of T-Hg, Me-Hg and I-Hg in plasma was found 15.1, 3.5 and 10.6 µg/L as compared to control 0.59, 0.16 and 0.41 µg/L, respectively which indicated that workers had significantly ($p< 0.001$) higher concentrations of Hg than the control (Table 3.1). The comparison of Hg concentrations in the plasma and the demographic characteristic of workers were studied. The Hg in the blood plasma was varied greatly with the changes in the demographic properties (Fig. 3.2A-3.2B). The concentrations of T-Hg, Me-Hg and I-Hg in the smokers plasma were 15.97, 4.08 and 10.9 µg/L, respectively, whereas as non-smokers group of the exposed workers were 14.15, 3.46, 9.86 µg/L, respectively (Fig. 3.2D). Like RBCs, a significant ($p< 0.001$) increase was observed in plasma Hg concentrations with experience and fish consumption but age, weight and smoking showed less effects on Hg concentrations in plasma. The linear regression model of Hg concentration in plasma with experience represented a strong correlationship of (R^2= 0.869 for T-Hg, R^2= 0.923 for Me-Hg R^2= 0.897 for I-Hg). The correlationship of Hg species with age and weight of the workers showed a weak correlation with T-Hg in the plasma in both age (R^2=0.27) and weight (R^2=0.665). The above data clear that statistically no significant differences were found in the Hg concentration of blood (RBCs, plasma) with regard to age and weight.

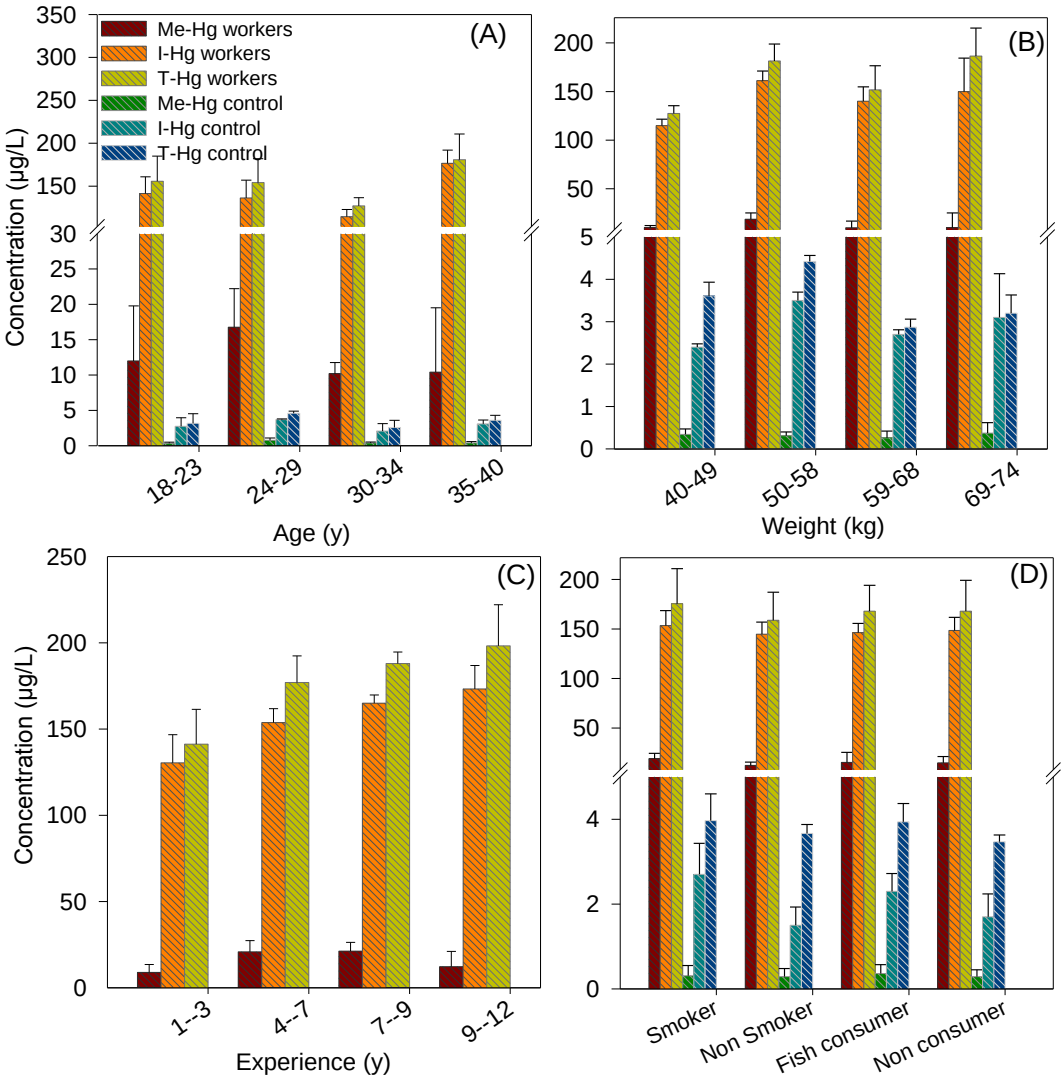


Fig. 3.4 The concentrations of Me-Hg, I-Hg and T-Hg in urine with A) age, B) weight, C) experience, D) smoking and fish consumption of the exposed workers and control subjects.

The mean concentrations of T-Hg (154 µg/L), Me-Hg (13.5 µg/L) and I-Hg (138 µg/L) in urine were significantly ($p < 0.001$) higher than control subjects (Table. 3.1). The concentrations of Hg species T-Hg, Me-Hg and I-Hg in the urine of smoker personnel's versus non-smokers were 175.5, 19.11 and 153.5 µg/L, respectively whereas for nonsmokers the Hg level was monitored as 158.9, 12.33 and 144.7 µg/L, respectively (Fig. 3.3D). The experience of the workers ranged from 9-12 y showed higher

concentration of T-Hg, Me-Hg and I-Hg among the four groups and were 198.2 12.16, 173.3 µg/L and are depicted in Fig. 3.3C. The linear regression model between the experience length of the workers with Hg concentration in urine was significant (p < 0.001) and indicated a strong correlation for the T-Hg (R^2= 0.896) and I-Hg (R^2=0.942) concentrations. No correlation (0.046) was found between the Me-Hg concentration in urine and experience. The correlation data of age (R^2 = 0.08) and weight (R^2 = 0.47) of the workers indicated that these parameters were weakly correlated with Hg concentrations in urine.

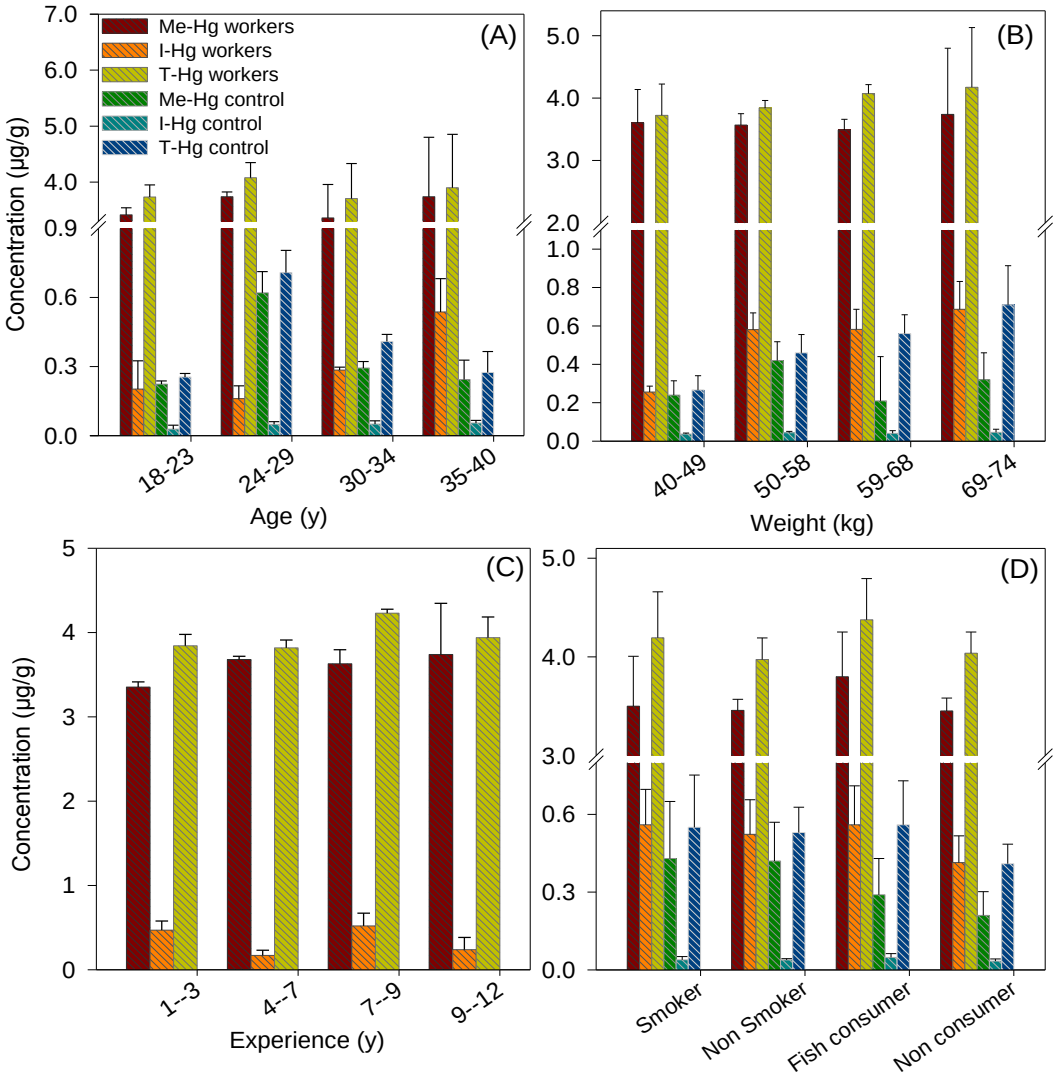


Fig. 3.5 The concentrations of Me-Hg, I-Hg and T-Hg in hair with A) age, B) weight, C) experience, D) smoking and fish consumption of the exposed workers and control subjects.

The Hg (T-Hg, Me-Hg, I-Hg) concentrations were studied in the hair of industrial workers and control subjects. The mean Hg concentrations in hair was determined significantly high ($p < 0.0001$) and was found 3.5, 3.2 and 0.3 µg/g, respectively in workers whereas 0.33, 0.3 and 0.03 µg/g, in control subjects respectively (Table 3.1). The Hg species in the hair of industrial workers were also compared with the demographic variables. The T-Hg, Me-Hg, and I-Hg level in the smokers hair were 4.19, 3.5 and 0.56

µg/g, respectively whereas these concentrations in the hair of the nonsmokers were determined as 3.97, 3.45 and 0.53 µg/g (Fig. 3.4D). The highest experience length among the 4 groups of the workers ranged from (9-12 y) found a high concentration of T-Hg (3.94 µg/g), Me-Hg (3.74 µg/g) and I-Hg (0.24 µg/g) in the hair (Fig. 3.4C). The correlation regression of the hair Hg with experience length assessed that the Me-Hg (R^2= 0.702) were strongly and T-Hg (R^2= 0.232, R^2= 0.167) were weakly correlated. Furthermore no correlation was found between hair I-Hg concentration with age, weight and experience of the workers with the exception of the T-Hg and weight of the workers (R^2= 0.978 for T-Hg) which were strongly correlated.

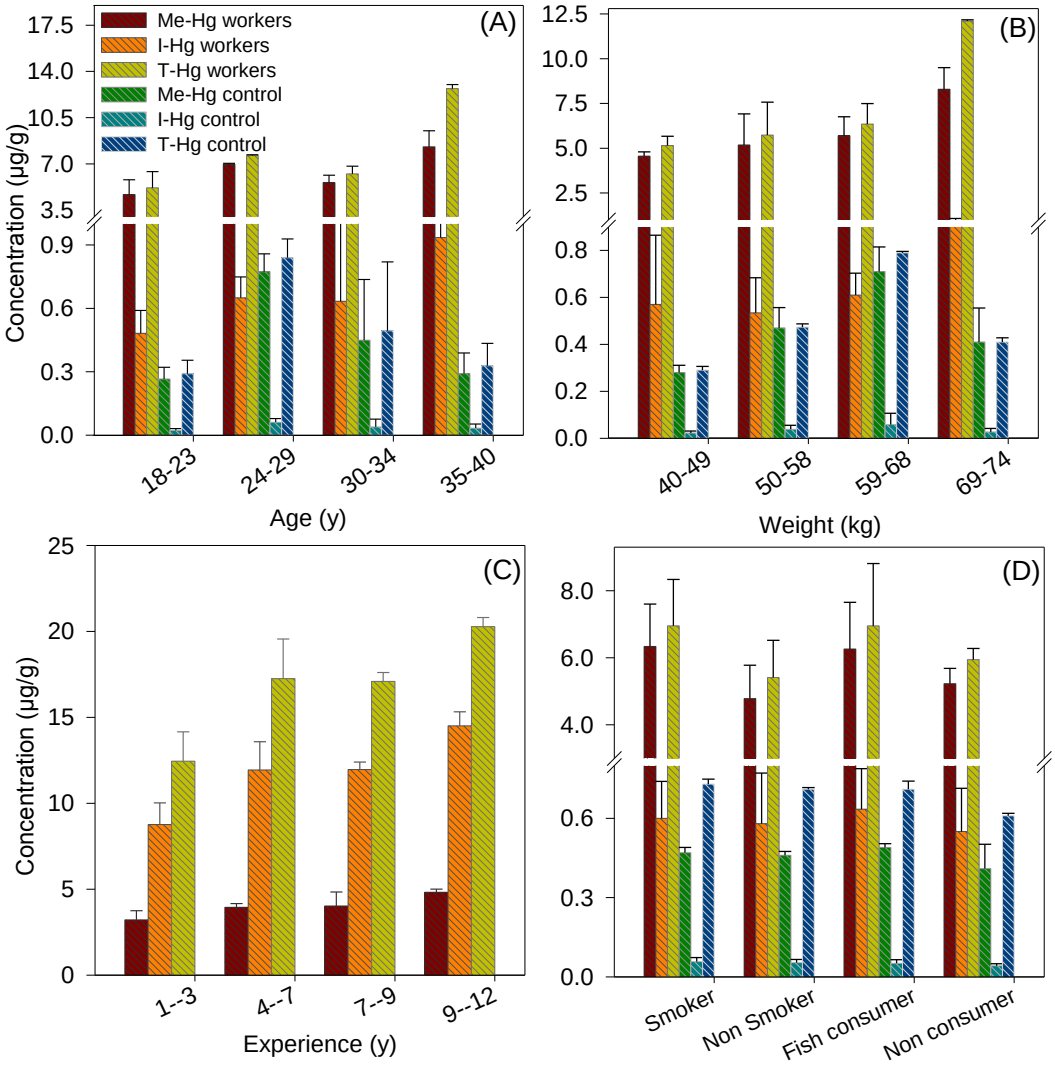


Fig. 3.6 The concentrations of Me-Hg, I-Hg and T-Hg in nails with A) age, B) weight, C) experience, D) smoking and fish consumption of the exposed workers and control subjects.

The mean concentrations of T-Hg (6.3 µg/g), Me-Hg (5.52 µg/g) and I-Hg (0.6 µg/g) were examined in workers nails which were significantly ($p< 0.001$) high as compared to the control, T-Hg (0.41 µg/g), Me-Hg (0.37 µg/g), I-Hg (0.034 µg/g) respectively (Table 3.1). The nails T-Hg, Me-Hg and I-Hg concentrations were compared with the age, weight, experience, smoking and fish consumption to ascertain whether these variables could be related to differences in the mean Hg concentrations in workers

nails (Fig 3.5A-3.5D). The nails T- Hg in age group ranged from (35-40 y) and weight group ranged from (69-74 Kg) was observed the highest Hg level of 12.69 µg/g in nails samples (Fig. 3.5A-3.5B). The T-Hg concentration in the nails of smoker's workers was 6.95 µg/g whereas in non-smokers the concentration was determined 5.41 µg/g which was indicated in Fig. 3.5D. The exposure of workers having maximum experience time (9-12 years) had elevated concentrations of T-Hg 12.71 µg/g (Fig. 3.5C) whereas the fish consumer group of workers had 6.95 µg/g and non-fish consumer the level was determined 5.94 µg/g (Fig. 3.5D). The linear regression was used for the correlation studies between nails Hg concentration and variables (age, weight, experience etc.). The T-Hg concentration in nails was significantly ($p< 0.001$) high and strongly correlated with age ($R^2= 0.674$), weight ($R^2= 0.743$) and experience length ($R^2=0.869$) of the exposed workers.

3.5 Discussion

The present study investigated that industrial exposed workers exhibited significantly (P = 0.001) higher concentrations of Hg in their blood (RBCs, plasma), urine, hair and nails samples than the control. The T-Hg concentrations were exceeded the guide line value (5.0 µg/L) set for the whole blood in the adults (Sahu et al., 2014). However, more recent data in non-institutionalized US population suggested that 95% for whole blood Hg was 2.30 µg/L for young children and 7.10 µg/L for adults (Aranda et al., 2009; Barbosa Jr et al., 2004). The concentration of T- Hg in the blood was found 6 times higher than its respective permissible limit. Hg concentration in RBCs was consisted on 80-90% of Me-Hg might be the reason of conversion of ionic Hg into Me-Hg by the anaerobic bacteria present in the RBCs (Table 3.1). These findings are agreed

with those reported by (Zolfaghari et al., 2007) and (Sakamoto et al., 2012). However, demethylation of Me-Hg occurs in RBCs which leads to increase the I-Hg concentrations counting for 10-20%. The findings of this study showed that the ratio between Me-Hg and I-Hg in RBCs was very high (9:1). Thus the blood can be used as a good indicator for Me-Hg (Berglund et al., 2005). The comparison and correlation studies between the Hg concentration in RBCs and demographic variables assessed that age and weight were not found significant factors in influencing the Hg changes in the RBCs. It was observed in this study that older workers with increased weight have no effect on Hg changes in RBCs suggesting that major part of Hg derived from exposure have short half life (Björkman et al., 2007) while Me-Hg correlated with age suggesting that Me-Hg excreted slowly. On the other hand long time experience of workers had greatly affected the Hg changes in RBCs. The study observed that experienced workers have high concentration of Hg in their RBCs which was an indication that Hg accumulated in the RBCs with the passage of time. The assessment of smoking and fish consumption effects on Hg in workers RBCs unveiled that fish consumers and smokers had higher level of Hg than non fish consumers and nonsmokers but the difference was not significant. It was noted in the previous papers (Berglund et al., 2005; Levy et al., 2004) that fish consumption was the important parameter for Me-Hg concentration in biological samples but here the difference was not significant because all workers were used fish in little quantity (once in a month). The concentration of Me-Hg in RBCs was attributed by the conversion of elemental Hg into Me-Hg though industrial exposure and not by the fish consumption. The data of this research and previously reported findings in published papers (Mortada

et al., 2002) indicated that smoking was not proved to be important parameter for assessing Hg concentration in RBCs.

The difference between Me-Hg and I-Hg in plasma samples of the workers (n=40) calculated from the data and observed as 30-40% of Me-Hg and 60-70 % of I-Hg level which are agreed with those reported by (Berglund et al., 2005). The ratio calculated for Me-Hg and I-Hg in plasma was 1:2, therefore blood plasma is a good indicator for both organic and inorganic Hg analysis. Like RBCs, the same results were found when the plasma Hg concentration was compared with the demographic characteristics. The age, weight and smoking of the workers have no effect on plasma Hg concentration, while a significant affect was found with experience. Thus the age, weight and smoking were observed as independent variables for the assessment of Hg concentration in blood (RBCs and plasma) in this study. Me-Hg and I-Hg distributions in the whole blood (RBCs and plasma) was calculated 75:25% whereas the half-life (t ½) of Hg in the blood was about 90-120 d and Me-Hg was about 50 d in the whole body indicating that Me-Hg leaves the body slowly (Berglund et al., 2005; Jung et al., 2013; Montuori et al., 2006).

The data showed that the concentration of urine Hg was higher in the workers with longer experience range of (9-12 y) among the 4 groups. The distribution of Me-Hg and I-Hg was calculated for urine and found that 90% of the urine samples had I-Hg whereas a small amount of organic Hg may be found due to the reason of rupturing of RBCs after spending life span of 120 d in body which contains bounded organic Hg as reported earlier (Berglund et al., 2005; Park and Zheng, 2012). The ratio calculated from the data for Me-Hg and I-Hg in urine was 1:10 which indicated all of the Hg in urine was I-Hg. Therefore urine is a good indicator for inorganic Hg determination (Li et al., 2011).

The permissible limit set by WHO for urinary T-Hg is 4 µg/L (Sakamoto et al., 2007). T-Hg concentration in urine was 39 times higher than the permissible limit set by WHO in exposed workers. This study showed that workers exposed near exhaust machine had a high level of Hg concentration in their urine. The exhaust machine released all of the processed Hg in the industry as a smoke; therefore, the workers working near exhaust machine were greatly vulnerable to high concentration of Hg. The half-life (t ½) of Hg in the urine was about 25-41 d lower than the half life (90-120) of Hg in the blood indicated that I-Hg rapidly leaves the body than Me-Hg (Nuttall, 2004). The correlation regression of urine Hg with demographic characteristics assessed that urinary Hg was positively correlated with experience while no significant correlation was found with age and weight of the workers. All of the Hg present in urine was I-Hg with a shorter half life. Therefore I-Hg was negatively correlated with age and weight. In the case of fish consumption the correlation was weak because the consumption of fish among the workers was very low. Smoking was found to be an independent parameter for Hg assessment in urine as reported earlier (Levy et al., 2004).

Regarding Hg in hair, 80-90% of Hg in workers was Me-Hg (Li et al., 2011) and its ratio was 10:1 with I-Hg. The half-life (t ½) of the hair Hg is 65 d with the range of 35-100 d and the small amount of I-Hg detected may be linked with the conversion of Me-Hg in the hair. Me-Hg is lipophilic in nature and strongly attached to the sulfhydrill groups of the protein which were abundantly present in the hair (Park and Zheng, 2012) (Río-Segade and Bendicho, 1999). The concentrations of Me-Hg in the hair samples were two folds higher than the permissible limit (less than 1 µg/g) set for hair Hg (Harada et al., 1999). Statistically significant correlation was found between Me-Hg in the hair

and experience of the workers which is an indication that all of the Hg in hair was Me-Hg and accumulated with the passage of time. According to regression model, no significant correlation was found between hair Hg and fish consumption because the workers consume fish very rare (one time per month) thus hair Me-Hg derived by the exposure to elemental Hg and not by fish consumption. The age, weight and smoking were not significantly affected hair Hg concentrations indicating that these parameters were found independent variables which agreed the findings reported in previous paper (Barbosa et al., 2001).

The significant differences in the nails Hg concentration was observed in exposed workers than control which was found 15 times higher than control. The permissible limit set for nail Hg is less than 5 µg/L. The 85-95% of Me-Hg was observed in nails samples which is a good indicator for Me-Hg detection reported earlier (Björkman et al., 2007). The mean data results of Me-Hg and I-Hg in the nails of exposed and control individuals showed a ratio of 10:1 respectively. The comparison of nails Hg with demographic variables showed that the changes in Hg level with age, weight, smoking, fish consumption and experience was quite evident. Therefore age, weight, long term exposure near exhaust machine, fish consumption and smoking were found good parameters in assessing nails Hg concentration. The linear regression model was used for nails Hg and demographic variables showed that significant correlation was found with weight, experience and fish consumption while no correlation was found with age and smoking. It was indicated that nails Hg was influenced by long term exposure and fish consumption even in minor quantity as it is accumulated in nails with the passage of time.

Therefore nails can be used for the past exposure of the Hg as well (Björkman et al., 2007; Zolfaghari et al., 2007).

The total Hg concentration can be calculated from the formula Me-Hg + I-Hg = T-Hg by identifying the concentration of other two species (Montuori et al., 2006). All of the three species of Hg were identified and obtained the % age difference between the calculated and identified total Hg. The % age difference obtained for the Plasma, RBCs, urine, hair and nails samples with a mean of 2.8 ± 2.75 µg/L (range 0.2-9.2) for RBCs, 6.7 ± 4.2 µg/L (range 0.4 - 14.99) for plasma, 2.3±2.29 µg/L (range 0.4 - 13.9) for urine, 3.3 ± 4.34 µg/g (range 0.063-15.38) for hair, 2.8 ± 3.73 µg/g (range 0.03-16.86) for nails respectively.

The highest Hg concentration was found in the biological samples of four (4) workers aged 26, 29, 35 and 40 y. The two workers were working on the exhaust machine whereas the other two had experience of 12 and 14 y. This data showed that high concentration of Hg was detected in the biological samples of workers worked near the vicinity of exhaust machine as compared to the basing and sealing machine. This study also unveiled that the age and weight were found independent variables for the assessment of Hg concentration while exposure time and vulnerability greatly influence the Hg concentration in the biological samples.

The diseases noted among the workers from the questionnaire data showed 20 % of the workers complaining the symptoms of insomnia and loss of appetite whereas the 30 % reported memory disturbances. Among them 35 % of the workers complaining the headache and body ach with fatigue. The urinary problems regarding burning maturation, some often blood seen in urine was reported by exposed workers near exhaust machine.

The results of this study unveiled that a high concentration of Hg was found in all biological samples (RBCs, plasma, urine, hair and nails) which indicated that Hg was distributed in the whole body after got entry into the body but the species of Hg (Me-Hg, I-Hg, T-Hg) showed different pattern of distribution among the body organs. The Me-Hg was found dominant in the RBCs, hair and nails samples while I-Hg was dominant in the urine and plasma samples.

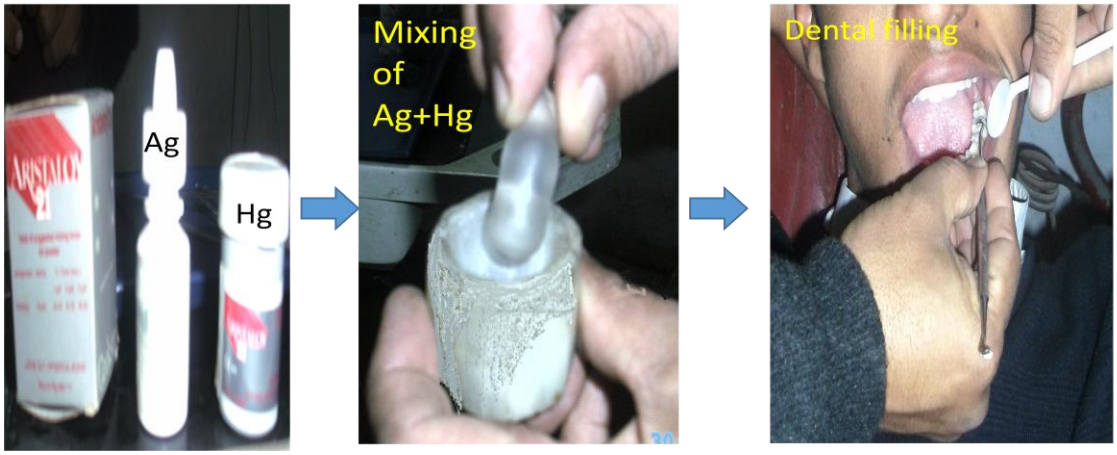
The relationship of biological samples with demographic properties observed that a positive and strong correlation was found between the RBCs, plasma and hair with experience, while a weak correlation with age and weight. Furthermore a strong correlation was found between urine (I-Hg, T-Hg), hair (Me-Hg, T-Hg) and nails (Me-Hg, T-Hg) while no correlation was found between Me-Hg in urine and I-Hg in hair and nails.

3.6 Conclusion

The concentrations of Hg in biological samples of workers exceed the permissible limits recommended by WHO and USEPA. Significant differences were observed in the Hg concentrations of biological samples collected from exposed workers and control individuals. Strong and weak correlations were observed between the demographic variables (age, weight, experience, smoking, fish consumption) of the workers with the Hg concentration in the biological samples. RBCs, plasma, urine, Hair and nails Hg concentration were found positive correlation with experience of workers while weak correlation with age, weight, smoking and fish consumption. RBCs, hair and nails were found good indicators for Me-Hg concentration while urine and plasma were observed good indicator for I-Hg concentration. The study recommended that workers must use the gloves, mask and other protective clothing and the manufacturer should regularly monitor

the health of the individuals and precaution measurement should be taken to avoid any serious problems. The Environmental Protection Agency (EPA) of the Pakistan has to strictly implement the law and regulations to regularly monitor the industries to ensure safe and protected environment for workers and general public living near surroundings.

Dental Amalgam Filling



CHAPTER-4

THE EFFECT OF Hg DENTAL FILLING ON Hg CONCENTRATION IN BIOLOGICAL SAMPLES AND ITS CORRELATION WITH BIOLOGICAL VARIABLES

4.1 Abstract

The study was conducted among the individuals using Hg as dental filling material to investigate the Hg concentration and its distribution with time (days) in the biological samples of Hg dental amalgam users (MDA). Hg concentration was measured in the biological samples (red blood cells (RBCs), plasma, urine, hair, nails) collected from MDA at three different times (i.e 1^{st}, 3^{rd}, 12^{th} day) of Hg filling and correlated with the biological variables (age, weight, restoration, Hg amalgam filling, surface area of filling material, fish consumption). Hg concentrations in the biological samples of MDA were 6-8 times higher than non amalgam users (control). The concentrations of Hg in the RBCs (4.39 µg/L), plasma (3.02 µg/L) and urine (22.5 µg/L) on 1^{st} day of Hg filling were higher than the concentration observed on 3^{rd} (2.15, 1.46, 12.3 µg/L for RBCs, plasma, urine, respectively) and 12^{th} (3.05, 2.5, 9.12 µg/L for RBCs, plasma, urine, respectively) day of Hg filling, while in the case of hair and nails the Hg concentration observed lower on 3^{rd} day (1.53 µg/L for hair) (2.35 µg/L for nails) than 12^{th} day (2.95 µg/L for hair) (3.5 µg/L for nails) of Hg filling. The correlation of Hg concentrations with biological variables indicated that number of restoration, dental filling, surface area and fish

consumption were significantly ($p < 0.05$) correlated with Hg amalgam filling while non significant ($p > 0.05$) correlation was found with age and weight of MDA.

4.2 Introduction

Dental amalgam is one of the most commonly used tooth fillings material (Akbal et al., 2014). Its considered a safe, sound, and effective treatment for tooth decay. Amalgam fillings are the chief source of exposure to Hg vapor in the general population. The amalgam consists of approximately 50% Hg combined with other metals such as silver, copper, zinc and tin (Akbal et al., 2014; Homme et al., 2014; Richardson et al., 2011; Shraim et al., 2011; Vamnes et al., 2004). Brain, blood, and urinary concentrations correlate with the number of amalgam surfaces present (Brownawell et al., 2005; Vamnes et al., 2004). It has been estimated that 10 amalgam surfaces would raise urinary concentrations by 1µg of Hg per liter, roughly doubling the background concentrations. It has been estimated that Hg is continuously relieved from Hg-dental fillings in the form of Hg vapor and abraded particles (Al-Saleh, 2011; Clarkson et al., 2003; Woods et al., 2008).

The Hg filling is used from about 150 years as a restorative material because of its stability and easy replacement (Bates, 2006; Brownawell et al., 2005). The ratio of Hg in the biological samples of MDA was 2-12 times higher than without amalgam (Barregård et al., 1999; Mutter, 2011; Mutter et al., 2007). In the intra oral environment the Hg amalgam is subjected to different chemical processes involve mechanical, thermal and biological. The thermal and mechanical pressure in the mouth due to chewing and brushing of the teeth, use of hot drinks and solution of H_2O_2 (to reduce dental plaque)

(Clarkson et al., 2003) corrode the dental filling involve the release of Hg ions to form stable compounds of sulphides, oxides and chlorides (Park and Zheng, 2012; Høl, 2003).

The released Hg from dental filling passed to the gastrointestinal tract and then to the blood (Björkman et al., 2007). The reported papers (Dutton et al., 2013; Geier et al., 2011; Høl; Mutter, 2011; Zeidán-Chuliá et al., 2011) correlated the Hg dental filling with the diseases of the nervous system (e.g. Alzheimer's disease), kidney dysfunction in children, tremor, nausea, weight loss, fatigue, shyness, irritability, headache, anxiety, lack of concentration and impaired memory are all contraindication of Hg amalgam filling**.** Among the toxic metals, Hg is more toxic than Cd, Pd and As due to the formation of covalent bond with thiol groups (cysteins and proteins) causing the accumulation in body tissues specially in brain and kidneys (Mutter, 2011). The autopsy studies found a direct correlation of Hg dental filling with Hg concentration in brain. Hg is lipophilic in nature with high absorption rate and has the ability to pass blood brain barrier and accumulate there which causes neurotoxicity and developmental toxicity and even mortality when exposed to a very higher level (Björkman et al., 2007; Eggleston and Nylander, 1987).

The previous literature indicated that the people using Hg amalgam as their teeth filling material are at a high risk of health. Dutton et al. (2013), Al- Saleh and Elkhatib (2012) Dunn et al. (2008) and Woods et al. (2008) detected the Hg concentration in urine and kidney biomarkers of children and adults and discussed its toxicity. Furthermore, Lin et al. (2014) and Bjorkman et al. (2012) identified the Hg concentration in serum and whole blood. Similarly, Al Saleh (2011) and Fakour et al. (2010) detected Hg in the samples of hair and nails. Some review papers published by Richardson et al. (2011) and Mutter (2011) focused on the toxicity and health effects of dental amalgam, others like

Akbal et al. (2014) and Mutter et al. (2010) studied the neuromuscular effects of dental amalgam. The reported literature observed that recently, several papers were reported that detected Hg concentration in the biological samples blood, urine, hair and nails of MDA, However, no single paper have been reported earlier that detected the Hg concentrations in the biological samples (RBCs, plasma, urine, hair and nails) obtained from MDA at three different times (1^{st}, 3^{rd} and 12^{th} day) from the same individual in a single paper. The importance of the work is that no work has been done in this area before and it is a systematic research following the continuous release of Hg after the dental amalgamation. This research evaluated the Hg in MDA and compared its concentration changes in the biological samples (RBCs, plasma, urine, hair and nails) with time (days) and then correlated them with various parameters (age, weight, surface area, restoration, number of Hg dental filling and fish consumption etc.) that influencing the Hg concentrations in these biological samples.

4.3 Material and Methods

4.3.1 Reagents

Hydrochloric acid (HCl 37% purity), perchloric acid ($HClO_4$), nitric acid (HNO_3, 68% purity), sulfuric acid (H_2SO_4, 98% purity) were obtained from sigma-Aldrich® (via Analytical Measuring Systems, Karachi, Pakistan). Sodium borohydride ($NaBH_4$), sodium hydroxide (NaOH), potassium permanganate ($KMNO_4$) were obtained from Scharlau (via Musaji Adam & Sons, Karachi, Pakistan) and Sigma–Aldrich®, Merck Chemicals (via Science Centre, Rawalpindi, Pakistan). Ultra-pure water was prepared by a Millipore ultra-pure water system (Milford, USA).

4.3.2 Instrumentation

The Perkin Elmer atomic absorption spectrometry (AAS Perkin Elmer 700, Waltham, USA) with Hg hydride system (MHS-15) using electrodless discharge lamp (EDL) with wavelength-253.7λ, energy 62J and current 185MP was used for Hg determination in all biological samples.

4.3.3 Study design

The anticipated research study was designed to assess the future observation case control clinical study. This study approved the principles of world medical association declaration (WMA) of Helsinki −Ethical principles for medical research involving human subjects" and its amendment. The protocol of the study was approved by the University of Peshawar, Pakistan.

4.3.4 Sampling

Hg dental amalgam patients (n=30) were recruited for samples collection with one or many dental restoration. Blood, urine, hair and nails samples were collected on 1^{st}, 3^{rd} and 12^{th} day of Hg amalgam used. The research project was explained and dental patients volunteered to participate in the sampling. Healthy non exposed volunteers (n=30) with age and gender matched subjects have no dental filling and occupational exposure to Hg selected as control. The participants were asked to fill a questionnaire to get information about height, weight, eating habit, gender, restoration, amalgam filling, teeth brushing frequency, fish consumption, smoking and use of paste/gel etc.

The samples of blood, urine, hair and nail from the same person were collected on 3^{rd} and 12^{th} day of Hg dental amalgam. On average 10 biological samples were collected from each subject under study.

4.3.5 Inclusion criteria

The study included the subjects used fresh Hg amalgam as the filling material for teeth cavities.

4.3.6 Exclusion criteria

The dental patients used filling material (composite, resin etc.) other than Hg or the individuals exposed to Hg occupationally, environmentally or subjects with old Hg dental filling were excluded from the study.

4.3.7 Samples collection and preparation

4.3.7.1 Blood. Blood samples were collected on 1st day, after 6 h of amalgam used, on 3rd day and 12th day from the same person. RBCs and Plasma were separated by means of centrifugation of blood at 3000 rpm. RBCs and plasma were stored in heparinized tubes and eppendorf tubes at -20ºC in the freezer.

4.3.7.2 Urine. Urine samples were collected on 1st day after 6 h, on 3rd and 12th day of dental filling. Urine samples were stored at 4ºC in clean polyethylene bottles. Then 1 ml nitric acid (10%) was added in each urine sample to protect Hg from volatilization.

4.3.7.3 Nails. Nail samples were collected on 3rd and 12th day of Hg dental filling by cutting all 10 fingers and toenails. Each nail sample was washed separately with 0.5% Triton X 100, once with acetone, once with distilled water and again rinsed with acetone. The volume of washing in each step was 4ml. The nail samples were dried in an oven at 40ºC overnight, stored and coded in plastic bags for further analysis.

4.3.7.4 Hair. Hair **s**amples from the head were obtained near the occipital region from each participant on 3rd and 12th day of Hg dental amalgam. The samples were cut into

smaller pieces of about 1 cm in length with scissors. Then the samples were washed with distilled water and acetone dried in oven at 50ºC overnight and stored in plastic bags.

All glassware and containers were washed with distilled water, soaked in 30% nitric acid for 24 h, once more with distilled water and then air dried.

4.3.8 Extraction

The concentrations of T-Hg were determined in the prepared biological samples (plasma, RBCs, urine, nails and hair) as given below:

4.3.8.1 Detection of T-Hg concentration

The concentration of T-Hg was detected according to method adopted by Sakamoto et al. (2010) with some minor changes. Briefly, a vial containing 3 ml mixture of H_2SO_4 (98%), $HCLO_4$ (70%) and HNO_3 (70%) (in ratio 1:2:5, respectively) added into RBCs (0.5 ml), plasma (0.5 ml) and urine (1 ml) and then heated at 40ºC for 60 min and then raised temperature to 90Cº for another 60 min. Continued stirring on a regular basis until the light brown fumes of Nitrogen disappear and the liquid color becomes golden yellow. Cooled down the samples to room temperature and diluted to 30 ml with deionized water.

The T-Hg concentration in the hair and nails samples were detected by adopted the method of Anim et al. (2011) with slight modification. Briefly, the grounded nails (50 mg) and hair (0.2 g) samples were digested with a mixture of 2 ml of $HClO_4$ and HNO_3 (1:1 ratio), 1 ml of deionized water and 5 ml of H_2SO_4. Heated the mixture in digestion block at 210-230ºC for 25 min. Homogenized the samples by shaking, cooled at room temperature and made volume up to 30 ml with deionized water.

4.3.8.2 Analysis

The Hg concentration in the biological samples (plasma, RBCs, urine, nails and hair) were detected using ASS 700. Calibration standards of 10, 25 and 50 µg/L were prepared from Hg standard (1g/kg) stock solution before analysis. At the time of analysis $NaBH_4$ was used as reducing agent in the reduction flask containing HNO_3 (1.5%) and HCl (1.5%) with potassium permanganate (5%). The laboratory control, standards and samples were passed through the digestion process.

4.3.9 Quality control

The replicates (n=3) and standard deviation of the blank and T-Hg concentration was analyzed. The limit of detection for plasma, RBCs, urine, nails and hair was calculated as 0.02 µg/L, 0.015 µg/L, 0.21 µg/L, 0.08 µg/g and 0.12 µg/g, respectively. The accuracy of the method was checked by analyzing three certified reference materials such as urine control level II (Sigma chemical company), human hair NIES No.13 (National institute of Environmental studies, Japan) and blood material level I, MR4206 (Nycomed company, Oslo, Norway). The recommended value was 4.42 µg/g equivalents to the detected value in hair 4.28±0.24 µg/g and the recommended value for blood was 2.4 µg/L equivalents to the detected value 2.15±0.5 µg/L, while for urine the value was 98 µg/L similar to the observed value 72±14 µg/L.

4.3.10 Statistical analysis

The correlation analysis for the biological samples and variables were achieved by using Pearson correlation analysis. The statistical software including sigma plot version 10, Graph pad version 5 and Xcel® were used. The difference in Hg concentration of MDA and control group was assessed by unpaired t-test (Two tailed) at 95% CI.

4.4 Results and Discussion

Table 4.1 Summarizes the mean profile of MDA including age, gender, weight, amalgam restoration, number of amalgam, smoking, surface area of filling, fish consumption, brushing frequency and gum chewing habit etc.

The T-Hg concentration was analyzed and evaluated in the selected Hg dental amalgam users (MDA) (n=30) and control (n=30) for their biological samples (RBCs, plasma, urine, hair and nails). The MDA included both male (70%) and female (30%), while in control included male (62.5%) and female (37.5%). In MDA group the male number was greater than control. The average age of the selected MDA were 41.14± 8.88 y out of which 39.2 ± 10.1 y were male and 42.3±6.4 y were female age. The control individuals were mostly young having average age of 36.2±7.856 y. The average weight and height of the MDA were 68.28±9.46 kg and 5.66±0.15 ft respectively, among which the male have 71.2±7.5 kg, 5.7±0.12 ft and female have 61±11.7kg, 5.5±0.21 ft, respectively. The average weight and height of the MDA and control was almost the same. The surface area of the dental filling material among the MDA was 3.07±1.54 mm whereas the male have 3.5±1.66 mm and female have 1.8±0.29 mm. Among the MDA 90% were brushing their teeth regularly in which 60% of the individual filled their teeth more than one time. The data was obtained about their habits in which 15% were gum chewers and smokers and 40-60% of the amalgam users were consuming fish.

The mean T-Hg concentrations in the biological samples (RBCs, plasma, urine, hair and nails) of the MDA and control individuals with time (days) is given Table 4.1.

Table 4.1 The Demographic profile and the concentrations of Hg in RBCs, plasma, urine, hair and nails of MDA and control subjects**.**

Parameters	**MDA**			**Control group**			**Parameters**		**MDA**	**Control group**
									Mean+S.D	**Mean+S.D**
	Mean+S.D	**Male**	**Female**	**Mean+S.D**	**Male**	**Female**	**RBCs** µg/L	1^{st} day	4.39±1.4	1.91±0.9
Gender	70/30	70	30	62.5/37.5	62.5	37.5		3^{rd} day	2.15±0.7	1.87±0.7
Age (y)	41.14±8.9	39.2±10	42.3±6.4	36.2±7.9	37.5±7.6	40.3±8		12^{th} day	3.05±1.04	1.61±0.9
Height (ft)	5.7±0.2	5.7±0.12	5.5±0.2	5.7±0.2	5.72±0.1	5.57±0.2	**Plasma** µg/L	1^{st} day	3.02±0.9	0.61±0.3
								3^{rd} day	1.46±0.6	0.66±0.3
Weight (kg)	68±9.5	71.2±7.5	61±11	68.3±9.5	73.3±7	61.7 ± 8.5		12^{th} day	2.5±0.7	0.58±0.3
Surface area (mm)	3.07±1.5	3.5±1.7	1.8±0.3	0	0	0	**Urine** µg/L	1^{st} day	22.5±6.5	2.79±1.3
Smoking (%)	15	15	0	20	20	0		3^{rd} day	12.3±3.6	2.4±1.01
Restoration more than one	60	20	40	0	0	0		12^{th} day	9.12±2.8	2.91±1.2
Teeth filled more than one	60	20	40	0	0	0	**Hair** µg/g	3^{rd} day	1.53±0.4	0.35±0.2
Teeth brush Once/day	90	60	30	80	50	50		12^{th} day	2.95±0.6	0.39±0.2
Fish consumer (%)	1/week	40	30	10	20	10 10		3^{rd} day	2.35±1.2	0.43±0.2
	1/month	60	45	15	70	40 30	**Nails** µg/g			
Gum chewing	15	15	0	30	10	20		12^{th} day	3.5±1.41	0.42±0.25
Tooth paste use	80	50	30	0	45	65	**P value**	0.001	0.001	0.001
Oral breathing	60	45	15	40	20	20				

*Each value of mean ± SD represents standard deviation (n=30)

Significant ($P<0.001$) differences was observed between Hg concentration in RBCs, plasma and urine of MDA and control individuals on 1st day of dental filling that the MDA have comparatively higher concentration of Hg in RBCs (4.39 µg/L), plasma (3.02 µg/L), urine (22.5 µg/L) than control RBCs (1.45 µg/L), plasma (0.36 µg/L), urine (1.64 µg/L) (Table 4.1) as reported earlier that MDA have higher concentration of Hg in the biological samples than control (Barregård et al., 1999; Mutter, 2011; Vamnes et al., 2004) and higher than the permissible limit (5 µg/l for blood and urine, 1 µg/g for hair) set by the WHO 1991 and USEPA 2005 except nails which was observed lower than the permissible limit set for nails Hg (5 µg/g) (Al-Saleh, 2011; Mutter, 2011). The Hg concentrations in RBCs, plasma, urine, hair and nails on 3rd day and 12th day of Hg filling were comparatively higher in MDA than control as shown in the Table 4.1. The concentration of Hg found in RBCs, plasma, urine, hair and nails observed on 12th day in MDA was 3.05 µg/L, 2.5 µg/L, 9.12 µg/L, 2.95 µg/g, 3.5 µg/g, respectively which were higher than control 1.16 µg/L, 0.33 µg/L, 1.76 µg/L, 0.39 µg/g and 0.42 µg/g, respectively (Table 4.1). These findings are in close proximity with those reported in literature (Vamnes et al., 2004; Zolfaghari et al., 2007; Aranda et al., 2009; Stube et al., 2011; Jung et al., 2013; Mortada et al., 2002).

The changes in Hg concentration of RBCs were observed with time (days) and used to identify the distribution and excretion of Hg from the body. The concentration of Hg (4.39 µg/L) found in the RBCs on 1st day of dental filling was comparatively higher than those observed on 3rd and 12th day of Hg dental filling. The Hg concentration in RBCs on 12th day of dental filling was comparatively higher than found on the 3rd day (Fig 4.1A). The similar results were observed in the case of plasma as well which is

depicted in Table 4.1. The Hg concentration in plasma observed on 1st, 3rd and 12th day of dental filling was 3.02, 1.46 and 2.5 µg/L, respectively (Fig 4.1B). The pattern of Hg concentration changes observed in the urine of Hg amalgam with time was quite different than those observed in RBCs and plasma (Fig. 4.1C). The Hg concentration observed in the urine on 1st, 3rd and 12th day of dental filling was 22.5, 12.3 and 9.12 µg/L, respectively as shown in the Table 4.1, which was higher than control (Akbal et al., 2014; Levy et al., 2004). The samples of hair and nails were collected on 3rd and 12th day of dental filling because Hg accumulation take some days to reached to the hair and nails. The Hg concentration found on 12th day (2.95 µg/g for hair) (3.5 µg/g for nails) of Hg dental filling in hair and nails was found higher than on 3rd day (Fig 4.1D-4.1E) which indicated that Hg was slowly accumulated in the hair and nails after distribution in the body. The hair and nails therefore used as indicators for past exposure of Hg (Al-Saleh, 2011).

The comparison of Hg concentrations in the biological samples (RBCs, plasma, urine, hair and nails) collected on different days (1st, 3rd, 12th) of dental filling from each individual showed a continuous decrease in case of urine, continuous increase in case of hair and nails and a random pattern of increase and decrease in case of RBCs and plasma (Fig 4.3A-4.3D). The results indicated that Hg after exposure in the body soon distributed to the blood and urine as reported earlier that Hg from dental filling after exposure rapidly localized in the liver and kidneys (Hahn et al., 1989) therefore, we found a high concentration on 1st day. With the passage of time the Hg was excreted through the urine because 90% of the Hg removed from the body through the urine slowly with longer half life of 40-60 days (WHO, 2008). The accumulated Hg from the plasma and RBCs on the

3rd day of dental filling transferred through the blood to other organs and to the brain pass the blood brain barrier (BBB) where it is converted into inorganic form through oxidation and not be able to recross the BBB and accumulate there (Björkman et al., 2007; Levy et al., 2004). This could be the reason of lower concentration of Hg in blood on 3rd day. On 12th day again an increase was observed in blood (RBCs, plasma) was due to the continuous released of Hg from dental filling due to chewing and brushing of teeth and use of hot drinks etc. (Al-Saleh, 2011; Levy et al., 2004; Sällsten et al., 1996). Thus Hg dental filling is also a source of Hg accumulation in the brain through blood (Björkman et al., 2007). The results of this study indicated that Hg reached to the hair and nails very slowly and accumulated with the passage of time, (Al-Saleh, 2011) therefore Hg concentration found in the hair and nails on 3rd day was found lower as compared to the 12th day. However, the total Hg concentrations on different days in the hair and nails were lower indicating that Hg released from Hg dental filling is not enough to increase the Hg concentration in the hair and nails (Mortada et al., 2002). These observations support the findings of previous studies conducted by Levy et al. (2004), Al Saleh (2011) and Woods et al. (2008).

In the intraoral environment, the Hg of dental filling absorbed through three different ways. Lungs, gastrointestinal tract and absorption through jaw cells (Hahn et al., 1989). The most rapid and high percentage absorption takes place through lungs (80%) (Mutter, 2011). The Hg concentration in the RBCs was compared and correlated with different variables (age, weight, surface area of Hg dental filling, number and restoration of Hg dental filling), as shown in Fig. 4.1-4.4. The age was categorized into 4 groups ranged from 24-31, 32-39, 40-46, 47-52 y. Similarly, the weight of the MDA was grouped

into 4 groups ranged from 52-59, 60-66, 67-74, and 75-82 kg (Fig. 4.1). Restoration was categorized into 4 groups on the basis of number of removal of previous filling by the new filling as 1, 2, 3, and 4 (Fig. 4.2). The tooth filling involved a specific surface area depend on the size of the cavity in the tooth of the persons have. The MDA on the basis of surface area were categorized as 1.5, 2, 4 and 5 mm (Fig. 4.3). Similarly, the person filled their tooth previously or newly one, two, three or four times were grouped into 1, 2, 3 and 4 (Fig. 4.4). The comparison of Hg concentration was studied with fish consumption frequency to know its effect on Hg concentration in the biological samples. The fish consumption was very less in the selected people, therefore its frequency was categorized as persons consume fish once per week and once per month (Fig.4.5).

The comparison of age with Hg concentration in the biological samples indicated that lower age group 24-31 y have higher concentration of Hg in all biological samples than the age group of 47-52 y. The correlation of the age with Hg concentration in RBCs, plasma, urine on 1^{st} day filling showed non significant and negative correlation ($r= -0.38$, $P=0.4$ for both RBCs and plasma) ($r= -0.08$, $p=0.9$ for urine). The same negative and non significant correlation was observed between age and Hg concentration in plasma ($r= -0.66$, $p=0.11$), urine ($r= -0.75$, $p=0.06$), hair ($r= -0.63$, $p= 0.13$) on 3^{rd} day of Hg filling except for RBCs ($r= -0.76$, $p <0.05$) and nails ($r= -0.85$, $p= 0.02$) which were significantly correlated. On 12^{th} day, the correlation of age with Hg concentration in RBCs, plasma, urine, hair, and nails showed significant but negative correlation. The correlation study observed that there was random pattern of changes in Hg concentration and negative correlation exist which indicate that Hg concentration in the biological samples decreases with age as previously reported (Björkman et al., 2007).

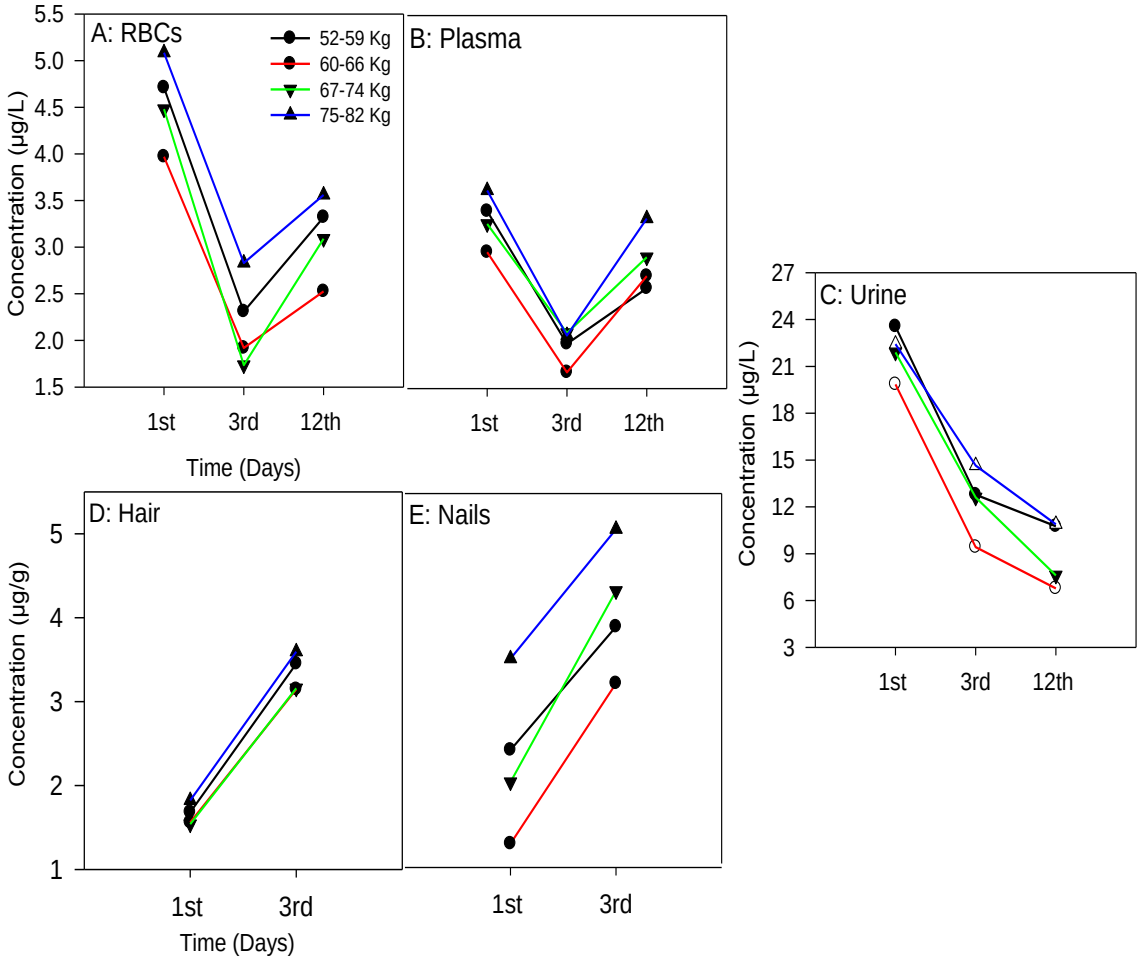


Fig. 4.1 The concentrations (ug/L) of Hg in the RBCs (A), plasma (B), urine (C), hair (D) and nails (E) on 1st, 3rd and 12th day of Hg dental filling with weight of MDA

The weight of DMA was compared with the Hg concentration in the biological samples showed that increased weight group ranged from 75-82 kg have higher concentration of Hg than the lower weight group range from 52-59 kg but these changes was not uniform as the lower group 52-59 had higher concentration of Hg than higher weight groups 60-66 kg and 67-74 (Fig 4.1A-4.1E). The changes may be due to increased number of restoration, number of filling, chewing or continues brushing etc. The correlation between weight and Hg concentration in RBCs and plasma (r=0.29, p=0.52), urine (r=0.05, p=0.92) on 1st day of Hg amalgam observed non significant correlation

which is also reported earlier (Levy et al., 2004). The same results were observed when the weight was correlated with the 3rd and 12th day Hg concentration in RBCs, plasma, urine, hair and nails. Thus it was observed from the study that obesity or lower weight had no effect on Hg concentration in the biological samples of MDA (Zolfaghari et al., 2007).

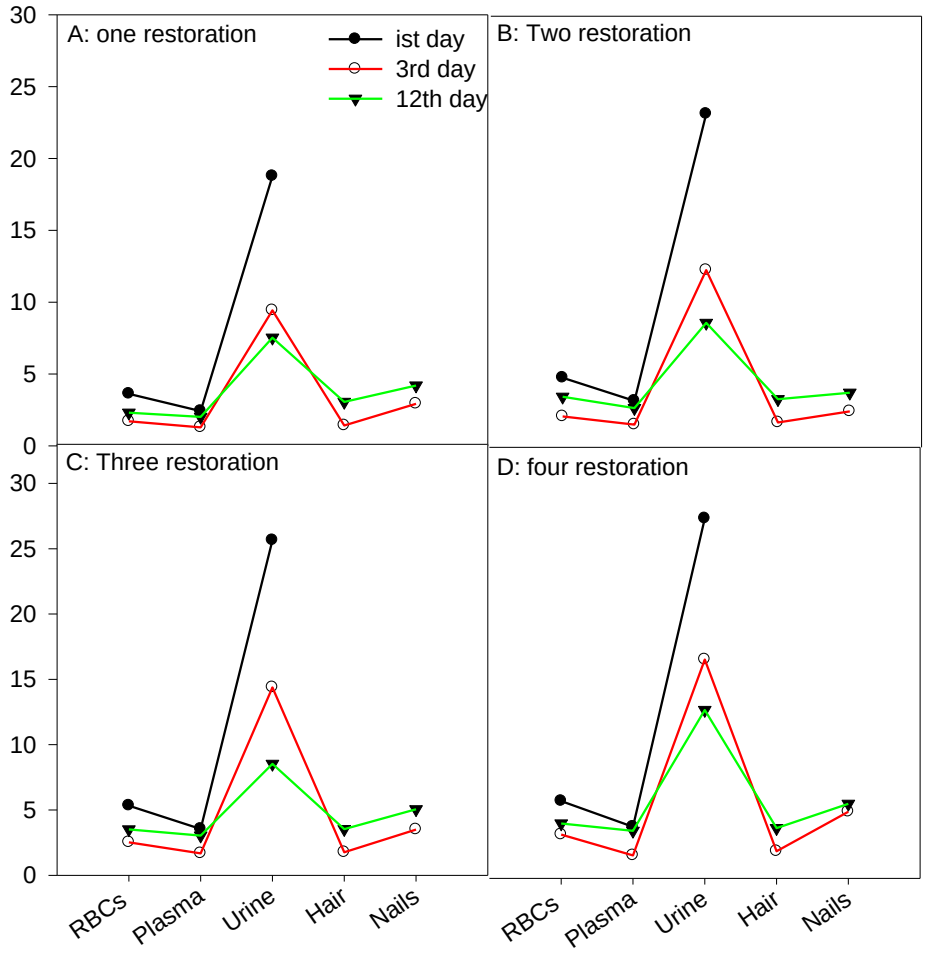


Fig. 4.2 The concentrations of Hg in the RBCs (A), plasma (B), urine (C), hair (D) and nails (E) on 1st, 3rd and 12th day of Hg dental filling with dental restoration of MDA

The restoration of Hg filling is the replacement of corroded and abraded previous Hg filling with the fresh filling which plays an important role in the concentration of Hg in biological samples. The MDA were divided into four groups on the basis of restoration

(1, 2, 3 and 4 restorations) and found that restoration had significant effect on Hg concentrations in the RBCs, plasma, urine, hair and nails (Fig 4.2A-4.2D). MDA with 4 restorations had higher concentration of Hg in all biological samples than the MDA with 1, 2 and 3 restorations (Fig 4.2C-4.2D). Similarly the MDA with 2 restorations have higher concentration of Hg than 1 restoration (Fig 4.2A-4.2B). The correlation coefficient and *p* value obtained between restoration and Hg concentration in the biological samples (RBCs, plasma, urine (r= 0.98, p= 0.02) on 1st day of Hg filling observed that significant and positive correlations exist. The correlation of the restoration with the Hg concentration in RBCs (r= 0.98, p= 0.02), urine (r= 0.99, p= 0.002), hair (r= 0.97, p= 0.03) on 3rd day filling observed significant and positive correlation except for plasma (r=0.95, p= 0.06) and nails (r= 0.85, p= 0.15) had non significant correlation. The correlation between the number of restoration and 12th day Hg concentration observed non significant correlation with RBCs, plasma (r= 0.93, p= 0.07), urine and nails (r= 0.87, p= 0.13) except for hair (r= 0.99, p= 0.004) which were strongly correlated. It indicated that the concentrations of Hg released from restoration drop down on 12th day of Hg filling due to the distribution and excretion through the biological samples (Björkman et al., 2007), while the Hg from amalgam filling was not enough to increase the nails Hg concentration. The strong correlation found for hair Hg on 12th day observed that Hg accumulated in the hair therefore hair can be used for the previous Hg exposure of about 5-6 months as reported earlier (Al-Saleh, 2011; Zolfaghari et al., 2007). It indicated that restoration released high amount of Hg in the biological samples because the removal of previous filling and replacement of fresh one both increased the Hg concentration in the biological samples (Brownawell et al., 2005; Homme et al., 2014).

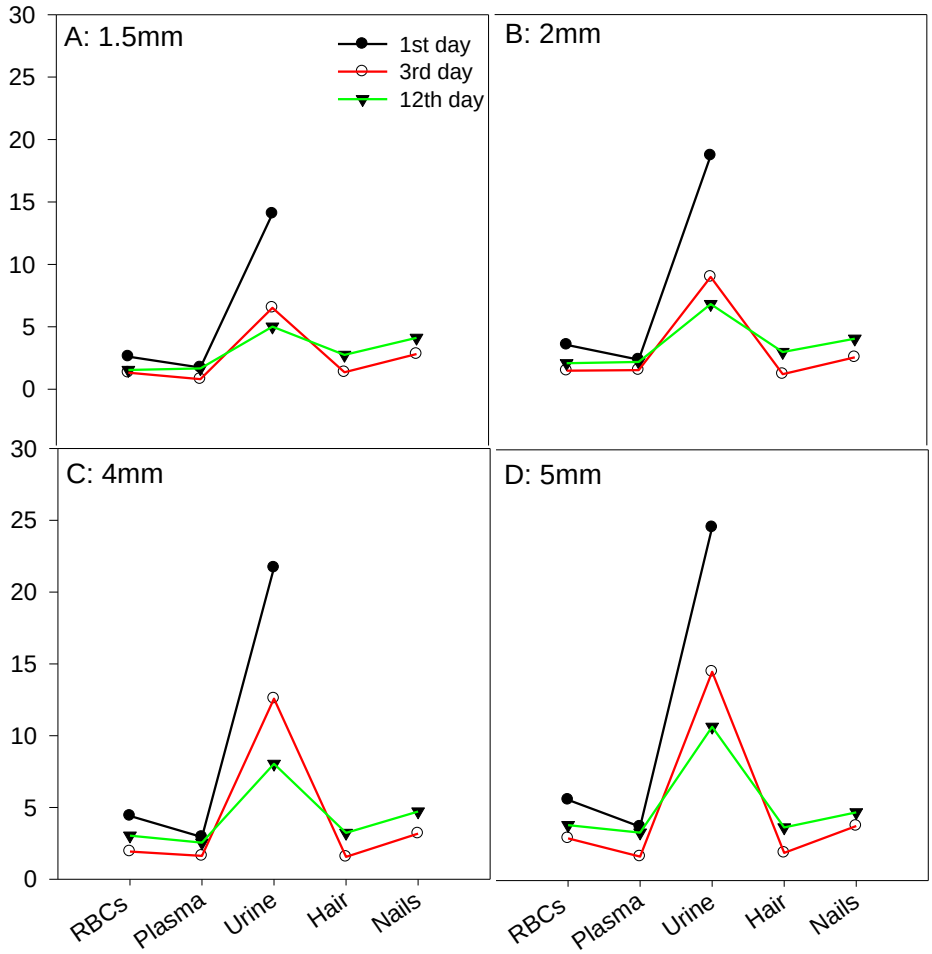


Fig. 4.3 The concentrations of Hg in the RBCs (A), plasma (B), urine (C), hair (D) and nails (E) on 1st, 3rd and 12th day of Hg dental filling with dental surface area of MDA

The surface area of Hg dental filling was grouped in 1.5, 2, 4 and 5 mm. The filling with surface area 5 mm had higher concentration of Hg in the RBCs, plasma, urine, hair and nails than the groups with surface area of 2 and 4 mm. As shown in the Fig. (4.3A - 4.3D), the Hg concentration increased with increasing the surface area but the effect was not too much pronounced between the groups with little difference in surface area. The changes in the Hg concentration in RBCs, plasma, urine, hair and nails was more evident when compared group of 2 mm surface area with 5 mm surface area rather than 1.5 mm surface area (Fig. 4.3A, 4.3B, 4.3D).

The surface area of Hg filling was correlated with the Hg concentration in RBCs, plasma, urine, hair and nails observed a significant and positive correlation on 1st day (r=0.97, p= 0.03 for both plasma and RBCs) (0.95, p>0.05 for urine), respectively and 12th day (r=0.99, p= 0.008 for RBCs) (r=0.95, p>0.05 for both plasma and urine) (r=0.96, p= 0.04 for hair) (r=0.96, p>0.05 for nails), respectively. The correlation coefficient and p values observed in RBCs, plasma, hair and nails on 3rd day of Hg filling was (r=0.94, p= 0.06) (r=0.76, p= 0.24) (r=0.91, p= 0.09) (r=0.92, p= 0.09), respectively observed non significant correlation except for urine (r=0.99, p= 0.013) which were significantly correlated. It was observed from the comparison and correlation that increased surface area would increased the Hg concentration in the biological samples (Brownawell et al., 2005). The fresh filling with increased surface area release high amount Hg, therefore we found a high Hg concentration on 1st day filling. On the 3rd day the released Hg distributed to different organs through the blood. Furthermore, the studies also indicated that surface area corroded with brushing and chewing, therefore increased Hg concentration in the biological samples on 12th day (Al-Saleh, 2011; Mutter, 2011).

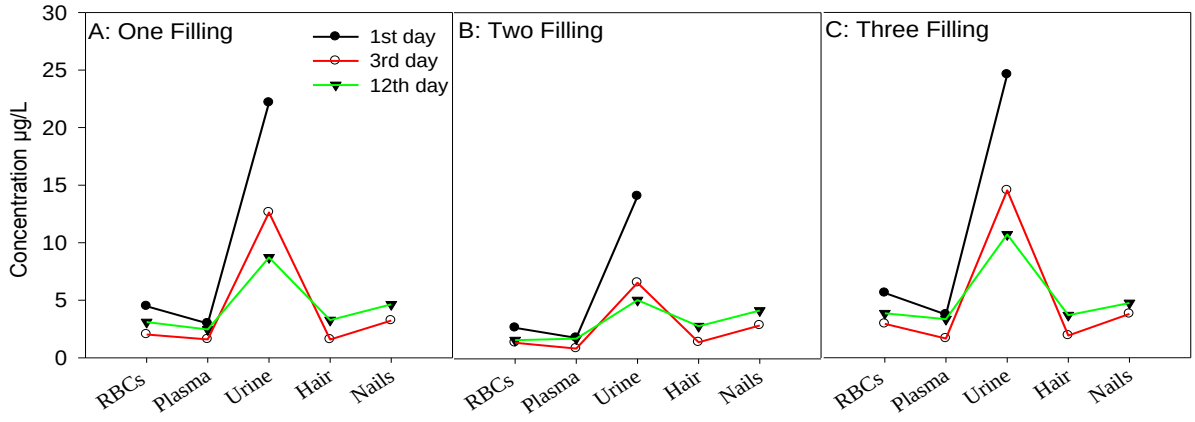


Fig. 4.4 The concentrations of Hg in the RBCs (A), plasma (B), urine (C), hair (D) and nails (E) on 1st, 3rd and 12th day of Hg dental filling with number of teeth filled by the MDA

Fig. 4.5A-4.5C shows the comparison of Hg concentration in the biological samples with dental filled teeth. The persons with five filled teeth have higher concentration of Hg in RBCs, plasma, urine, hair and nails than the persons with lower number of teeth filled (Oskarsson et al., 1996) but the persons with 3 teeth filled had lower Hg concentration than the persons with 1 tooth filled which showed little effect on Hg concentration in biological samples (Berglund et al., 2005). The correlation of number of teeth filled with Hg concentration in the biological samples observed significant but weak correlation which indicated that number of teeth filled had not much effect on the Hg concentration in the RBCs, plasma, urine, hair and nails. It may possible that MDA with lower number of teeth had older Hg amalgam filled teeth that older teeth due to corrosion add more Hg than the new dental filling (Dutton et al., 2013) or the MDA involve in continuous brushing and chewing (Al-Saleh, 2011).

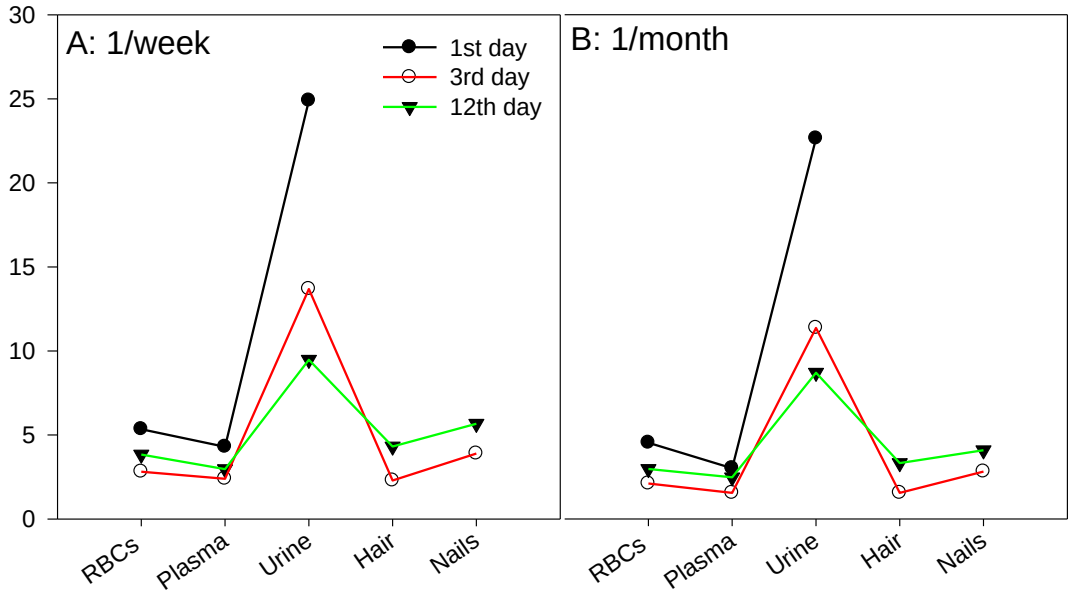
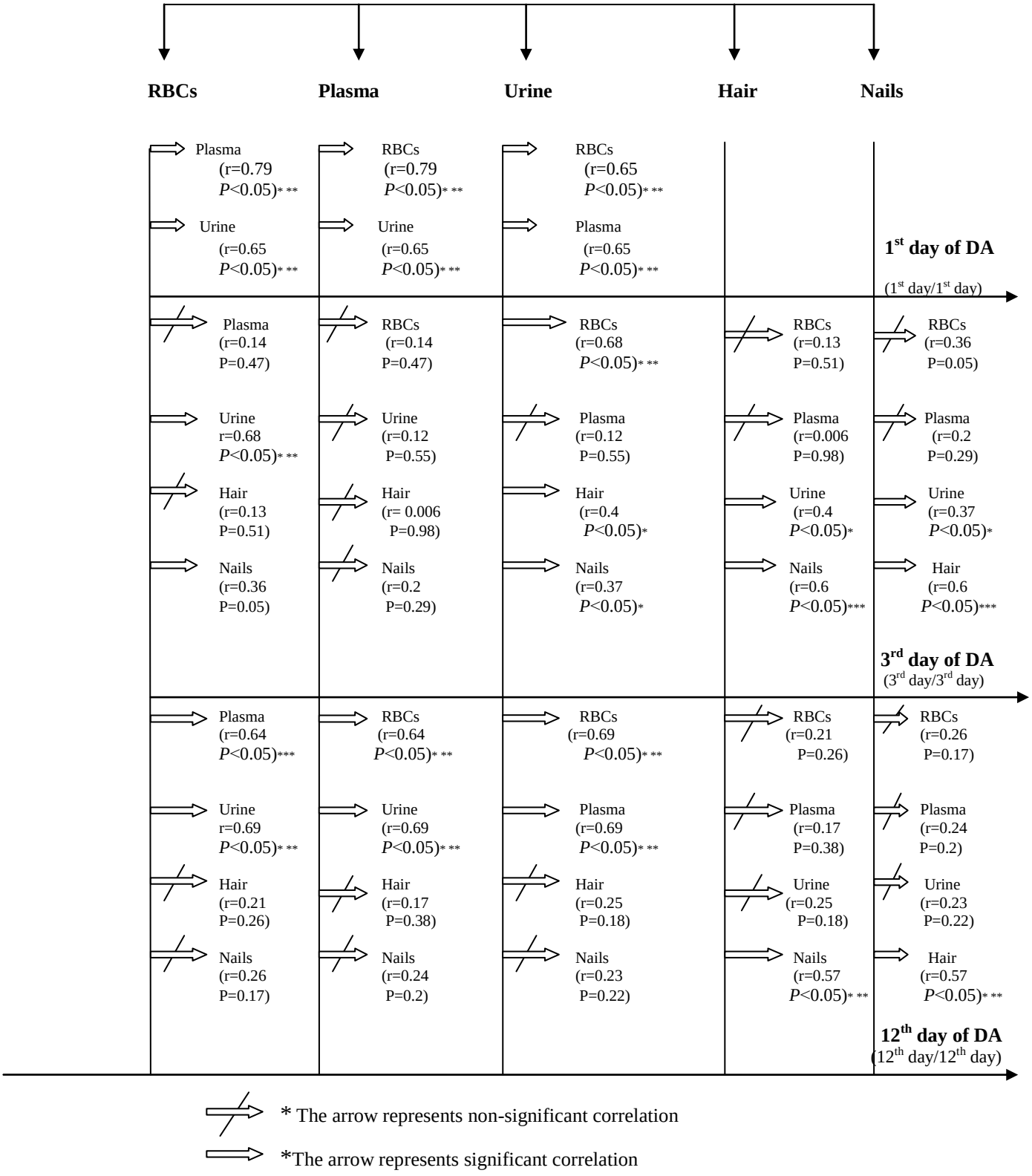


Fig. 4.5 The concentrations of Hg in the RBCs (A), plasma (B), urine (C), hair (D) and nails (E) on 1st, 3rd and 12th day of Hg dental filling with fish consumption of MDA

The consumption of fish affects the Hg concentration in biological samples (Levy et al., 2004). The Hg concentration in different samples and its relations with fish consumption is shown in Fig. 4.5. The fish consumption significantly increased the Hg concentration in the biological samples which were more pronounced in persons used fish one time a week than those consuming fish twice in a month (Levy et al., 2004).

The correlation of Hg concentration between the biological samples (plasma, RBCs, urine) observed that a strong and positive correlation was found between RBCs and plasma ($r=0.79$, $p=0.0001$) and between RBCs and urine ($r=0.65$, $p=0.0001$) Hg concentration on 1^{st} day of MDA as reported earlier (Lin et al., 2014). Similarly, strong and positive correlation was found by the correlation of Hg concentration of 3^{rd} and 12^{th} day RBCs with urine and 12^{th} day RBCs with plasma except 3^{rd} day Hg concentration of RBCs with plasma found non significant correlation ($r=0.14$, $p=0.47$). Furthermore, non significant correlation was found between RBCs with hair and nails on 3^{rd} and 12 day of dental amalgam except RBCs and nails Hg concentration on 3^{rd} day. Similarly, non significant correlation was found by the correlation of 1^{st}, 3^{rd} and 12^{th} day Hg concentration of plasma, urine with hair and nails Hg concentration except 3^{rd} day urine Hg concentration with hair ($r=0.4$, $p=0.03$) and nails ($r=0.37$, $p=0.04$) (Table 4.2). It was assumed from the study that the Hg concentration in the biological samples was correlated with the Hg in dental amalgam (Björkman et al., 2007).

Table 4.2. Correlation between the Hg concentrations in the biological samples of MDA



The correlation study indicated that dental amalgam Hg after release from dental filling distributed to the blood and urine of MDA on 1st day (Berglund et al., 2005). The blood is the main route for Hg distribution in the body tissues (Lin et al., 2014). The Hg released from dental filling was not in that much quantity to reach to the hair and nails because no significant correlation was found between plasma, urine with hair and nails Hg concentration on 3rd and 12th except the 3rd day RBCs Hg concentration was weakly correlated with nails ($r=0.35$, $p=0.05$). It was assumed from the study that it was the RBCs in the blood that transfer Hg to the nails as reported earlier that nails Hg has close relationship with blood Hg (Björkman et al., 2007) and that the presence of Hg in the hair was due to the adsorption of Hg on hair. The diseases were not noticed among the MDA except minor oral thrush, metallic taste and mouth ulcers.

4.5 Conclusion

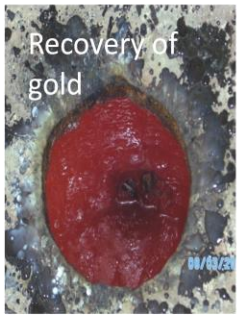
The Hg concentration in the biological samples of MDA was found higher than non amalgam users and the permissible limit for the biological samples by USEPA except nails which was found lower indicated that dental filling Hg reached to the hair and nails in little quantity. The correlation between the Hg concentration with biological variables and with time (days) showed an increase in Hg concentration with passage of time (days) in the case of hair, a decrease in the case of urine and random pattern of increase and decrease in the case of plasma and RBCs and that some parameters like chewing, continuous brushing and fish consumption, number of dental filling and restoration etc. enhanced the Hg concentration in these biological samples. The concentration of Hg decreased with time due to the excretion through urine. It was observed that the use of Hg dental amalgam for dental restoration is the unnecessary exposure to toxic Hg vapors.

Gold Recovery Process

Falling of gold particles on carpets in shops

Nayaragar buy the carpets and dust at high price

Collection of visible Gold particles by using Hg



CHAPTER-5

Hg EXPOSURE AND HEALTH EFFECTS AMONG THE GOLDSMITH WORKERS EXTRACTING GOLD FROM CARPETS AND DUSTED-CLAYS THROUGH AMALGAMATION AND ROASTING PROCESSES

5.1 Abstract

Mercury (Hg) is a highly toxic metal which can cause serious health effects. The aim of this research was to determine the concentrations of total Hg (T-Hg), methyl Hg (Me-Hg) and inorganic Hg (I-Hg) in the biological samples (plasma, RBCs, urine, hair and nails) of the exposed goldsmith workers. This is the first study that determines the detailed Hg concentrations in the biological samples (plasma, RBCs, urine, hair and nails) of the exposed goldsmith workers and correlates them with the diseases noted among the workers in a single paper. Biological samples were collected from goldsmith workers (n=40) and analyzed for T-Hg, Me-Hg and I-Hg using atomic absorption spectrometer equipped with mercury hydride system. The mean T-Hg concentration in RBCs (33 µg/L), plasma (11.8 µg/L), urine (167 µg/L), hair (4.21 µg/g) and nails (5.91 µg/g) were higher than the control RBCs (1.64 µg/L), plasma (0.55 µg/L), urine (2.72 µg/L), hair (0.35 µg/g) and nails (0.51 µg/g). All workers participated in this study were suffering from physical and mental diseases. The concentration of Hg was found higher among the workers suffering from mental diseases as compared to those suffering from physical diseases. Among the physical diseases the most serious diseases were sexual dysfunction, skin diseases and fatigue because the workers suffering from these diseases had higher concentration of Hg than the workers with other diseases. The occurrence of

physical diseases (88%) was greater than the mental diseases (53%) among the workers. The correlations of physical and mental diseases with experience (years of work) and exposure time were significant ($p < 0.05$), while non significant ($p > 0.05$) correlation was observed between demographic parameters and Hg concentrations in the biological samples of the workers. The burning process of amalgamated gold is a significant source of Hg exposure to goldsmith workers, therefore awareness precautionary measures are needed to provide protection to them.

Key words: Mercury, physical diseases, mental diseases, gold extraction, worker

5.2 Introduction

The Hg is the occupational toxicant and exert hazardous effects on human and environment (Agrawal et al., 2014). Hg used in the amalgamation of gold to refine and concentrate this precious metal. Gold mining and subsequent processing of gold ores using amalgamation techniques are known for their potential contamination of environment with Hg and led human health risk (Engstrom et al., 2014; Kristensen et al., 2013). Numerous studies have been conducted on workers exposed to Hg during gold mining in Brazil, Zimbabwe, Columbia, Ecuador, Burkina Faso, Iran and Tanzania (Charles et al., 2013; Cordy et al., 2011; Harari et al., 2012; Shirkhanloo et al., 2014; Steckling et al., 2014) relatively few papers are published in Africa (Tomicic et al., 2011) while a single paper (D'Ilio et al., 2000) conducted study on hair Hg of those goldsmith workers which were exposed to Hg in lower quantity.

The gold used as ornamental purpose in jewelry throughout the world and also in medicines (Sahin and Erkis, 2013). In Pakistani marriage customs, the gold is the important part of the bride ornamental dress and also the inferiority status symbol of the families (Monger, 2013). Mostly the gold is imported from various other countries but goldsmith makes its different shapes, sizes and polishing of the jewelry. The small invisible gold particles are falling on daily basis due to jewelry production such as polishing, cutting and reshaping processes. The gold from the carpets and dusted-clays of the goldsmith shop usually collected by specialized person called “nayaragar” in local language who process this dusted clays with Hg to concentrate and further subjected it in the furnace for solid mass of the gold which is represented in the diagram below.

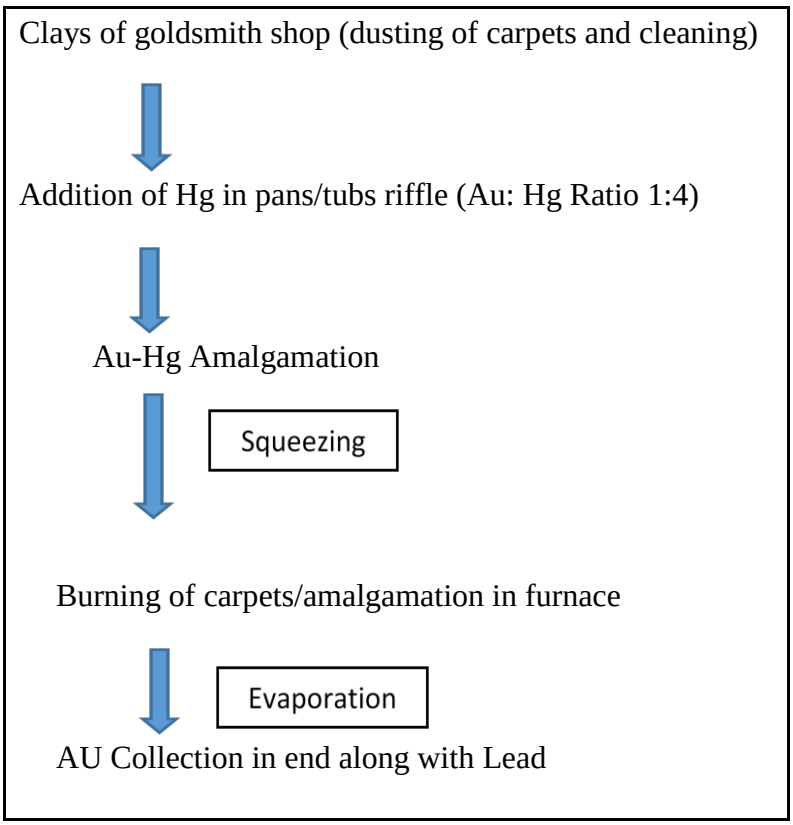


Fig 5.1 Diagrammatic presentation of Hg recovery from the dust/clay

Goldsmith (jewelers) shops are available in every city of the country for the production of jewelry. Besides this, small furnace units are also available in locality of heavy bunch of goldsmith shops for reprocessing the gold particles using Hg from every goldsmith shops after 3 to 6 months to recover the gold losses during cutting, polishing and reshaping.

Hg is released to environment during amalgamation and roasting processes and the workers (Nayaragar) are exposed directly through skin and inhalation (Cordy et al., 2011). This research was designed to investigate the Hg concentrations in the biological samples such as plasma, red blood cells (RBCs), urine, nails and hair collected from goldsmith workers. The questionnaire concerning the health problems was filled for

probing the possible hazardous health effects in the workers. This research work is distinctive in its design due to the determination of the concentrations of Me-Hg, I-Hg and T-Hg in blood (plasma, RBCs) nails, hair and urine specimen collected from the exposed workers involved in Hg gold amalgamation and roasting processes. So far, no single paper reported all these aspects.

5.3 Materials and Methods

5.3.1 Reagents

Ultra-pure water was prepared by a Millipore ultra-pure water system (Milford, USA), Hg standard solution (1 mg/L in 10% HNO_3), H_2SO_4 (98%), $HClO_4$ (70 %), HCl (37%), HNO_3 (68%) obtained from Sigma–Aldrich® (via Analytical Measuring Systems, Karachi, Pakistan), Analytical grade acetic acid (CH_3COOH) analytical-grade sodium boro hydride ($NaBH_4$), ethanol (CH_3CH_2OH), tin chloride ($SnCl_2$), sodium hydroxide (NaOH), cysteine ($HO_2CCH(NH_2)CH_2SH$), cadmium chloride ($CdCl_2$), potassium hydroxide (KOH) were obtained from both (via Musaji Adam & Sons, Karachi, Pakistan) and Sigma–Aldrich®, Merck chemicals (via Science Centre, Rawalpindi, Pakistan). These chemicals were used without any further purification.

5.3.2 Instrumentation

The Perkin Elmer atomic absorption spectrometry (AAS Perkin Elmer 700, Waltham, USA) with Hg hydride system (MHS-15) connected with the electrodless discharge lamp (EDL) used for Hg detection. The instrument setting involve with current 185MP, energy 62J and wavelength-253.7λ, which was used for the detection of Hg in all biological samples.

5.3.3 Study design

The study was approved by the world medical association declaration (WMA) of Helsinki (WMA 2013). The expected research was designed to evaluate the future declaration cross sectional clinical study. The method of the research was approved by the University of Peshawar, Pakistan.

5.3.4 Sampling

Goldsmith workers exposed to the Hg through burning from different shops and unexposed age and gender matched subjects other than goldsmith shops were selected as control. Before beginning the study a written "*Informed consent*" was obtained from the participants. A questionnaire was attained from the workers and control included detailed information about demographic profile, exposure time of Hg, years of work and health problems

The samples of blood, urine, hair and nails from the recruited goldsmith workers (n=40) and control (n=40) were collected with a sampling frequency range from 60 seconds/sample to 120 seconds/sample. The research study was open label and the workers and control volunteered to join the sampling.

5.3.5 Inclusion criteria

The workers occupationally exposed to Hg in the goldsmith shop through burning named nayaragar in local language were included in the study.

5.3.6 Exclusion criteria

The workers exposed to Hg through other ways (industrial exposure, dentist etc) except goldsmith nayaragar were excluded from the study.

5.3.7 Samples collection and preparation

5.3.7.1 Urine. Clean polyethylene bottles were used for the collection of urine samples (50ml) from each individual and stored at 4C^{o} after collection. One ml of HNO3 (15%) was added in each sample for protection against Hg vaporization.

5.3.7.2 Blood. Sample of 5 ml blood from each individual was collected in heparinized tubes and centrifuged at 3000 rpm to separate RBCs and plasma. The RBCs were stored in the heparinized and plasma in eppendorf tubes at -20C^{o} in the deep freezer..

5.3.7.3 Nails. Nails (0.25 g) clippings were obtained from each individual by cutting all finger nails and stored in plastic bags for further use in analysis. Before analysis nails samples were washed with 0.5% Triton X 100, then with acetone and once with distilled water. All samples were dried in an oven at 40C^{o}.

5.3.7.4 Hair. The hair samples were obtained from the occipital region by cutting (1g) hair near the scalp and stored in plastic bags. Before analysis hair samples were washed with distilled water and acetone and dried at 45 ^{o}C in oven for 24 h. The hair samples were cut with the help of stainless steel scissor in smaller length of about 1cm.

All glassware used in the preparation and analysis of the samples were soaked in 40% HNO_3 overnight, washed with distilled water and then dried in oven at 70^{o}C.

5.3.8 Extraction

The Hg concentration (T-Hg, Me-Hg, I-Hg) was determined from biological samples (urine, RBC, plasma, hair and nails) by using the reported methods as follows:

5.3.8.1 Determination of T-Hg concentration

The concentration of T-Hg was detected according to the method adopted by (Sakamoto et al. 2010) with some minor changes. Briefly, 3 ml mixture of H_2SO_4 (98%), $HClO_4$ (70%) and HNO_3 (70%) (in ratio 1:2:5, respectively) was added into a vial containing RBC (0.5ml), plasma (0.5 ml), urine (1 ml), hair (0.2g) and nails (0.05 g). Then the mixture was warmed up to 40ºC for 1hour and then the temperature raised up to 90 ºC for another 1 hour for RBCs, plasma and urine extracts while the hair and nails extracts were heated upto 200 ºC for 20 minutes Regularly continued samples stirring till Nitrogen fumes disappear. Cooled the mixture at room temperature and diluted up to 30 ml with deionized water.

5.3.8.2 Determination of Me-Hg

The concentration of Me-Hg was determined in the RBCs, plasma and urine samples by using the method adopted by (Vahter et al. 2000) with slight changes. The plasma (0.5 ml), RBCs (0.5 ml) and urine (1 ml) samples were treated with a mixture of 1 ml of L-cysteine solution (0.0124 M), 1.5 ml of KOH (11 M) and 0.5 ml deionized water in a vail and stored overnight at ambient temperature. The extracts were diluted uto 30 ml with deionized water before analysis for Me-Hg. $NaBH_4$ (3%) was used as reducing agent during analysis.

The Me-Hg was extracted from hair and nails samples by using the method (Harada et al. 1999).The finely cut hair (0.2 g) and grounded nails (0.05g) samples were digested with 1 ml deionized water, 2 drops of ethanol and 5 ml of HCl (2.0 N). The mixture was heated up to 100 $^{\circ}$C for 15 min. After cooling, 3 ml acetate buffer solution (4.5 pH) was added. The samples were diluted upto 30 ml. Like blood and urine samples,

the $NaBH_4$ was used as reducing agent during the time of analysis by AAS 700, Perkin Elmer.

5.3.8.3 Determination of I-Hg

The I-Hg was extracted from biological samples (plasma, RBCs, urine, hair and nails) using the method adopted by (Ehrenberg et al. 1991) with slight modification. RBCs (0.5 ml), plasma (0.5 ml), urine (1 ml), hair (0.2g) and nails (0.05 g) samples were taken in the reaction flask (50 ml) along with 0.5 ml of stannous chloride ($1 X 10^5$ µg/L of HCl) and 3 ml of NaOH ($3 X 10^5$ µg/L) with continuous stirring. The reaction mixture was analyzed by AAS 700, Perkin Elmer.

5.3.9 Analysis

Biological samples (plasma, RBCs, urine, nails and hair) were analyzed for Hg concentration using ASS 700, Perkin Elmer connected with MHS-15. Hg standard stock solution (1g/kg) was used for the preparation of calibration standards of 10, 25 and 50 µg/L freshly at the time of analysis. Moreover $NaBH_4$ used as a reluctant for T-Hg and Me-Hg detection while SnCl2 for I-Hg detection. 2-3 drops of potassium permanganate (5%) with 10ml HCl (1.5%) for each sample was added before analysis through ASS 700. The laboratory control and standards were passed through the same process.

5.3.10 Quality control

The precision and accuracy of the methods were verified by analysis of certified reference materials for blood (blood material level I, MR4206, Nycomed company, Oslo, Norway), urine (urine control level II, Sigma chemical company) and hair (NIES No.13, National institute of Environmental studies, Japan). The deviations between observed (2.17±0.6 µg/L, 75±15 µg/L, 4.26±0.25 µg/g) and certified (2.4 µg/L, 98 µg/L, 4.42

µg/g) values for blood, urine and hair, respectively, were acceptable. The accuracy was calculated in terms of limit of detection for plasma, RBCs, urine, nails and hair 0.11µg/L, 0.33µg/L, 0.92µg/L, 0.15µg/g and 0.094µg/g, respectively. The recovery values from spiked samples ranged from 78% to 96%. The reference materials (n=3) and reagent blanks were included in each batch.

5.3.11 Statistical analysis

The statistical analysis was performed by using statistical software sigma plot version 10, Graph pad version 5 and Xcel®. The correlation of Hg concentration in the biological samples with years of work, exposure time and diseases reported among the workers was evaluated by using Spearman correlation analysis. The unpaired (Two tailed) non parametric t-test at 95% CI was used to assess the difference between Hg concentration in workers and control. A p value less than 0.05 is considered significant.

5.4 Results and Discussion

The profile data and mean Hg concentration in the biological samples (RBCs, plasma, urine, hair and nails) of goldsmith workers and the control (unexposed individuals) participated in this study are given Table 5.1. The mean values of age, weight, years of work, exposure time and percentage values for gender, smoking and fish consumption of the workers and control subjects were calculated. The data distribution of the exposed workers is mentioned in the Table 5.2. In goldsmith furnace, all the workers were male with a mean age of 31.5±9.8 y, therefore no female was enrolled in the control group. The mean height (5.5±0.19 feet), weight (68.6±18.8Kg) and years of work (15.2±9.5 y) of the goldsmith workers and control were recorded; the working time was 8±2.3 per day with one holiday in a week. The workers involved in the process of

recovery of gold using Hg amalgamation and evaporation of residues in furnace were directly exposed to smoke and fumes. They were not adopted any precautionary measures such as masks, protective cloths and gloves. Among selected workers, only 40% were fish consumer on monthly basis and 30% used fish weekly. Five workers (0.46 ± 0.83) used dental amalgam of Hg based tooth filling two years ago before giving the biological samples for this research (Table 5.1). All the data collected during questionnaire survey was correlated with the Hg concentrations in the biological samples collected from the workers and also with the diseases reported by them.

Table 5.1 Goldsmith workers profile and Hg concentrations in their biological samples

Workers Profile			**Hg Concentration**				
Parameters	**Goldsmith**	**Control**	**Parameters**		**Goldsmith**	**Control**	**P value**
Gender (male)	n=50	n=50					
Age (y)	31.5±9.8	29.9 ± 5.27			**Mean+S.D**	**Mean+S.D**	
Weight (kg)	68.6±18.8	55.1 ± 8.92	**RBCs µg/L**	T-Hg	33±8.8	1.64±0.88	0.0001
Height (ft)	5.5±0.19	5.31 ± 0.16		Me-Hg	29±7.8	1.57±0.76	0.0001
Experience (y)	15.2±9.5	NA*		I-Hg	2.7±1.1	0.13±0.08	0.0001
Working h/day	8±2.3	NA*		T-Hg	11.8±3.2	0.55±0.27	0.0001
Working days/week	6	NA*	**Plasma µg/L**	Me-Hg	2.91±1.3	0.15±0.04	0.0001
Contact with Hg (h)	4.75±2.8	NA*		I-Hg	8.14±2.1	0.34±0.11	0.0001
Age of work start (y)	17.3±9.5	NA*		T-Hg	168.6±29	2.72±1.1	0.0001
Teeth amalgam filling (n)	0.46±0.83	NR*	**Urine µg/L**	Me-Hg			
Smoking (%)	42	40			13.3±3.4	0.28±0.22	0.0001
Use of mask, gloves (%)	0	NR*		I-Hg	152±27	2.35±1.3	0.0001
Oral breathing (%)	46	NA*		T-Hg	4.21±0.9	0.35±0.22	0.0001
Fish consumption (%)	42	70	**Hair µg/g**	Me-Hg	3.82±0.9	0.29±0.17	0.0001
Once/week	30	30					
Once/month	40	50		I-Hg	0.31±0.2	0.04±0.01	0.0001
Mental/physical problem after work (%)	52	NR*	**Nails µg/g**	T-Hg	5.91±1.4	0.51±0.21	0.0001
Mental problem	53	NR*		Me-Hg			
Physical problem	88	NR*			5.24±1.3	0.39±0.22	0.0001
Urinary problem (%)	25	NR*		I-Hg	0.49±0.2	0.033±0.01	0.0001

Each value of mean ± S.D represents standard deviation (n=40)
NA*. Not applicable
NR*. Not reported

The concentrations of T-Hg in the RBCs, plasma, urine, hair and nail samples were significantly higher (*P<0.001)* in workers as compared to the control subjects. The mean concentrations of T-Hg in RBCs, plasma, urine, hair and nails were 33 µg/L, 11.8

µg/L, 167 µg/L, 4.21 µg/g and 5.91 µg/g, respectively, while in control group the T-Hg concentrations were 1.64 µg/L, 0.55 µg/L, 2.72 µg/L, 0.35 µg/g and 0.51 µg/g, respectively. The highly significant values were recorded ($P < 0.001$) using 95% confidence interval for T-Hg concentration.

Me-Hg concentrations in the biological samples (RBCs (29 µg/L), plasma (2.91 µg/L), urine (13.3 µg/L), hair (3.82 µg/g) and nails (5.24 µg/g) were significantly ($P < 0.0001$) higher in workers as compared to control subjects (Table 5.1). The concentrations of I-Hg in biological samples of workers were significantly ($p > 0.05$) higher than the control group. The I-Hg concentrations in RBCs, plasma, urine, hair and nails of workers were 2.7 µg/L, 8.14 µg/L, 152 µg/L, 0.31 µg/g and 0.49 µg/g, respectively as compared to the control 0.13 µg/L, 0.34 µg/L, 2.35 µg/L, 0.04 µg/g and 0.03 µg/g (Table 5.1). The data showed that Hg exposed workers have a high concentration of Hg in their biological samples and associated potential health risks (Castilhos et al. 2015). During questionnaire survey mental and physical diseases were noted which showed that these workers were vulnerable to high concentration of this toxic metal (Ritchie et al. 2002).

Table 5.2. Data distribution of the Hg concentration in biological samples of workers

Parameters	**RBCs**			**Plasma**			**Urine**			**Hair**			**Nails**		
	Me-Hg	I-Hg	T-Hg	Me-Hg	I-Hg	T-Hg	Me-Hg	I-Hg	T-Hg	Me-Hg	I-Hg	T-Hg	Me-Hg	I-Hg	T-Hg
Total number of values	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Number of excluded values	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of binned values	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Minimum	16.7	0.73	19.1	0.59	3.95	5.73	5.27	100	111	2.22	0.08	2.91	3.23	0.18	3.49
25% Percentile	20.3	1.69	23.4	1.91	6.72	9.43	11.1	129	143	3.01	0.17	3.33	4.13	0.32	4.46
Median	31.8	2.98	36.5	2.93	8.17	11.9	13	161	179	3.915	0.27	4.26	5.24	0.53	6.05
75% Percentile	36.1	3.7	41	3.92	9.3	13.8	15	175	192	4.28	0.35	4.7	6.11	0.57	6.93
Maximum	39	4.34	44	6	12.5	18	22	190	213	5.89	0.89	6.32	7.99	1.19	8.69
Mean	29	2.7	33	2.91	8.14	11.8	13.3	152	169	3.82	0.31	4.21	5.24	0.49	5.91
Std. Deviation	7.83	1.08	8.8	1.33	2.08	3.15	3.36	27.1	29.8	0.85	0.21	0.86	1.25	0.23	1.44
Std. Error	1.24	0.17	1.39	0.21	0.33	0.5	0.53	4.28	4.71	0.14	0.03	0.14	0.2	0.04	0.23
Lower 95% CI of mean	26.5	2.36	30.2	2.5	7.47	10.8	12.2	144	159	3.55	0.25	3.93	4.84	0.42	5.46
Upper 95% CI of mean	31.5	3.05	35.8	3.34	8.8	12.9	14.4	161	178	4.1	0.38	4.48	5.64	0.57	6.37

*Number of workers (n=40)

5.4.1 Mental Diseases

Among the workers, the most common mental diseases noted were dementia, dizziness and stress (Fig. 5.4). Dementia is a type of brain disease involving decline in memory and other thinking skills i.e. memory loss. Among the mental diseases the highest percentage of workers (63%) complained of dementia which is caused by long term exposure to Hg (Bełdowska et al. 2015; Björkman et al. 2012; Mutter et al. 2005). Dizziness is a type of mental disorder in which a person losses balance. In this study, 38% of workers were suffering from dizziness, while 25% from stress (Fig. 5.4). As a whole 53% of the workers were suffered from mental diseases. The T-Hg concentrations observed in the RBCs, plasma, urine, hair and nails of workers suffered from dementia were 37.2 µg/L, 13.6 µg/L, 184 µg/L, 4.52 µg/g and 6.57 µg/g, respectively. T-Hg concentrations in RBCs (37.2 µg/L), plasma (13.8 µg/L), urine (184 µg/L), hair (4.66 µg/g) and nails (6.43 µg/g) in stress suffered workers were higher than the RBCs (30 µg/L), plasma (11.2 µg/L), urine (161 µg/L), hairs (3.84 µg/g) and nails (5.58 µg/g) of dizziness suffered workers (Fig. 5.1C). Similarly, the concentrations of Me-Hg and I-Hg were also found higher in the workers with dementia than other mental diseases as shown in Fig. 5.1A-5.1C. The data showed that the workers suffered from dementia showed higher concentration of Hg in their biological samples than those suffered with stress and dizziness indicating that dementia was found to be the worst disease than stress and dizziness caused by Hg. The high accumulation of Hg in biological samples (brain) has resulted in neurotoxicity (Anim et al. 2011; Bełdowska et al. 2015; Harada et al. 1999; WHO 2008).

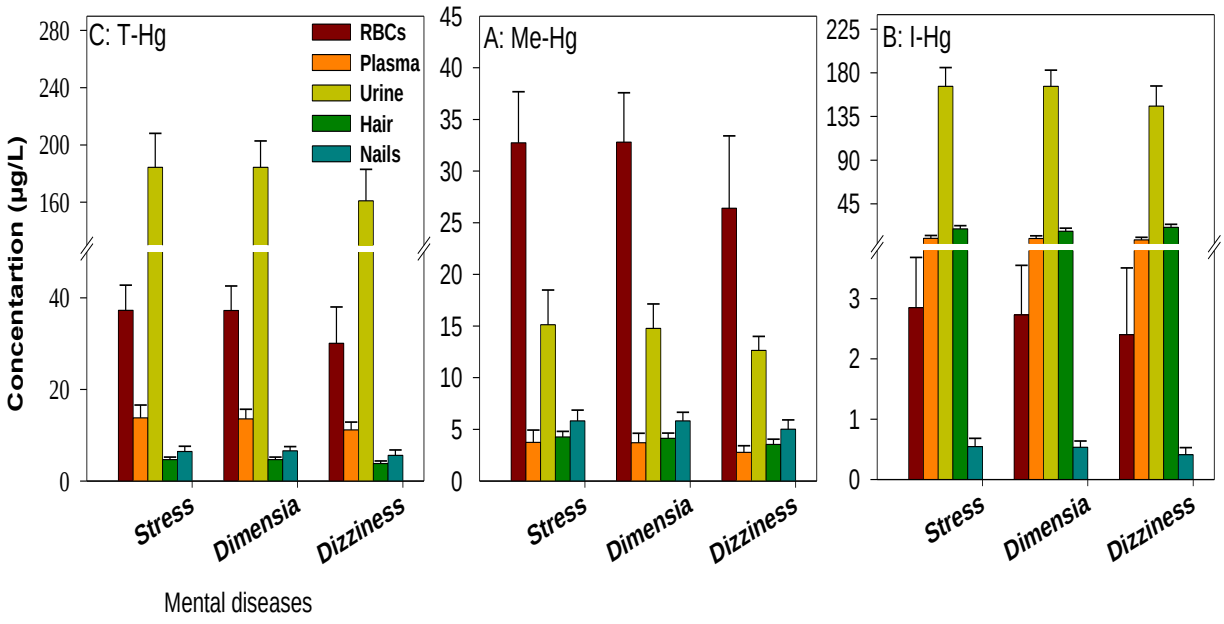


Fig 5.2 The concentrations of A) T-Hg, B) Me-Hg and C) I-Hg in the RBCs, plasma, urine, hair and nails of workers suffered from mental diseases.

The mental diseases were correlated with the years of work and Hg exposure time of the workers. It was observed that the workers with more years of work had higher concentration of Hg in their biological samples than observed in the workers with less years of work and lower exposure time. Among the workers, 52% suffered from both mental and physical diseases, while 35% were found to have physical diseases only. However, no worker was found to have mental diseases alone. The mean concentrations of T-Hg, Me-Hg and I-Hg found in the RBCs, plasma, urine, hair and nails of workers suffered from both mental and physical diseases were comparatively higher than the workers suffered from physical diseases only. Thus it was observed that mental diseases were the severe form of Hg toxicity and appeared after long term exposure and accumulation of Hg, therefore, mental diseases raised after physical diseases (Ngim and Ngim 2013; Sahu et al. 2014).

5.4.2 Physical diseases

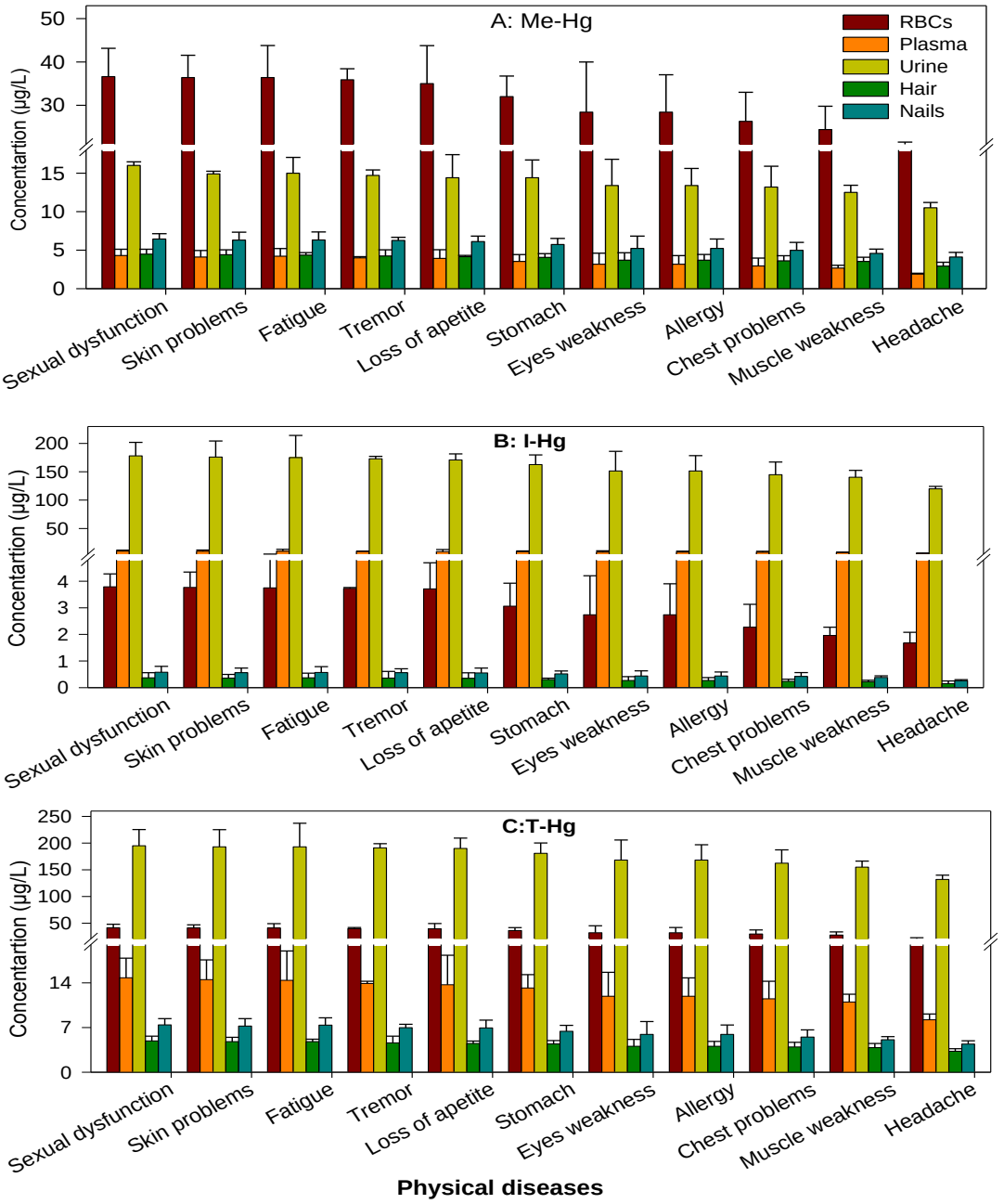


Fig 5.3 The concentrations of A) T-Hg, B) Me-Hg and C) I-Hg in the RBCs, plasma, urine, hair and nails of workers suffered from physical diseases.

The physical diseases noted during field survey were stomach disorders, chest problems (coughing, congestion, asthma etc.), allergy, muscle weakness, eyes weakness,

skin problems (rashes, dermatitis, skin irritation, cutaneous eruption), continuous headache, fatigue, sexual dysfunction, tremor and loss of appetite (Abdel-Rasul et al. 2013; Harada et al. 1999; Leiva G and Morales 2013; Sahu et al. 2014). Among the studied workers, 88% were suffering from physical diseases. Hg concentration was found higher in the biological samples of those workers suffered from such diseases. The highest T-Hg concentration was found higher in the biological samples of workers suffered from sexual dysfunction as compared to those suffered with skin problems. The concentrations of T-Hg were 41.5 µg/L, 14.8 µg/L, 195 µg/L, 4.9 µg/g and 7.41 µg/g, in the RBCs, plasma, urine, hair and nail samples, respectively collected from the workers suffered with sexual dysfunction, while 41.4 µg/L, 14.5 µg/L, 193 µg/L, 4.8 µg/g and 7.22 µg/g, respectively in the workers suffered with skin diseases. Similarly, those workers suffered with fatigue, tremor and stomach problems had higher concentration of Hg than the control but lower than those found in workers suffered with sexual dysfunction and skin problems (Abdel-Rasul et al. 2013). It was observed that the worst physical diseases noted after sexual dysfunction were skin problems > fatigue > tremor > loss of appetite > stomach problem > eyes weakness > allergy > chest problems > muscle weakness > headache.

In this study, the diseases appeared due to high Hg concentration were found in few workers, indicating that the worst diseases may appear after long term exposure. The correlation of worst diseases with the percentage of distribution of diseases showed a significant and negative correlation ($r= -0.95$, $p= 0.0001$).

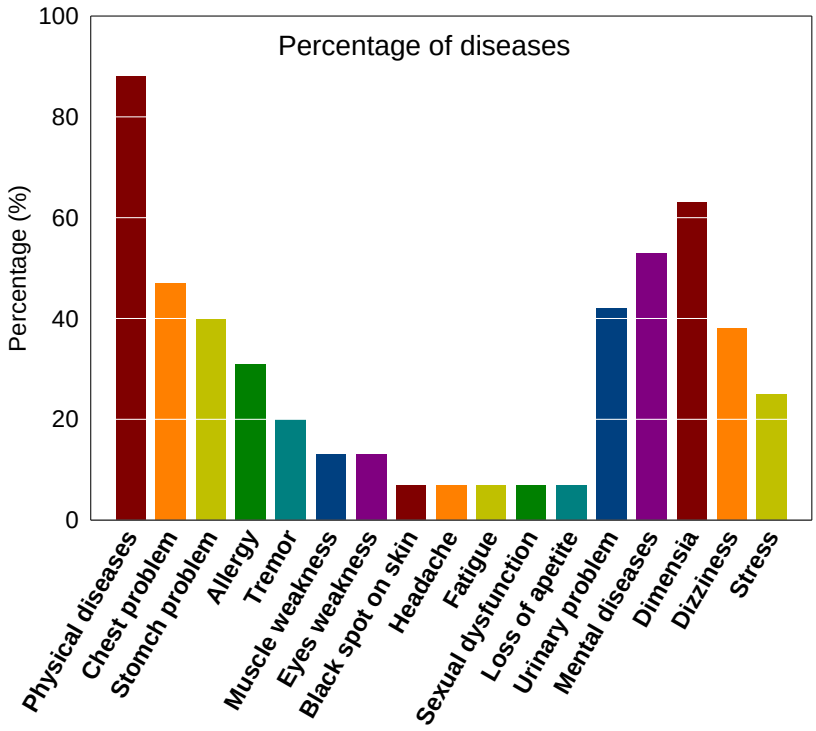


Fig 5.4 Physical, Mental, Urinary diseases (%) found among the workers participated in the study.

The chest problems were noted in 47% of workers because of Hg fume evolved during burning process (Abdel-Rasul et al. 2013; Harada et al. 1999). The absorption of Hg is highest through lungs counting for 80% (Sahu et al. 2014) leading to respiratory diseases (Li and Tse 2015). Similarly, 40% workers complained from stomach problems, 31% allergy, and 13% muscle weakness, while only 7% fatigue, tremor, loss of appetite, sexual dysfunction, continuous headache and skin problems (Fig. 5.2). The previous studies have also been reported these kinds of diseases in the Hg exposed persons (Fawer et al. 1983; Harada et al. 1999; Park and Zheng 2012).

5.4.2.1 Percentage distribution of diseases noted:

Chest problems > Stomach problem > Allergy > Muscle weakness > Eyes weakness
(47%) (40%) (31%) (13%) (13%)

Fatigue > Sexual dysfunction > Headache > Loss of appetite > Skin problems
(7%) (7%) (7%) (7%) (7%)

5.4.3 Urinary problem

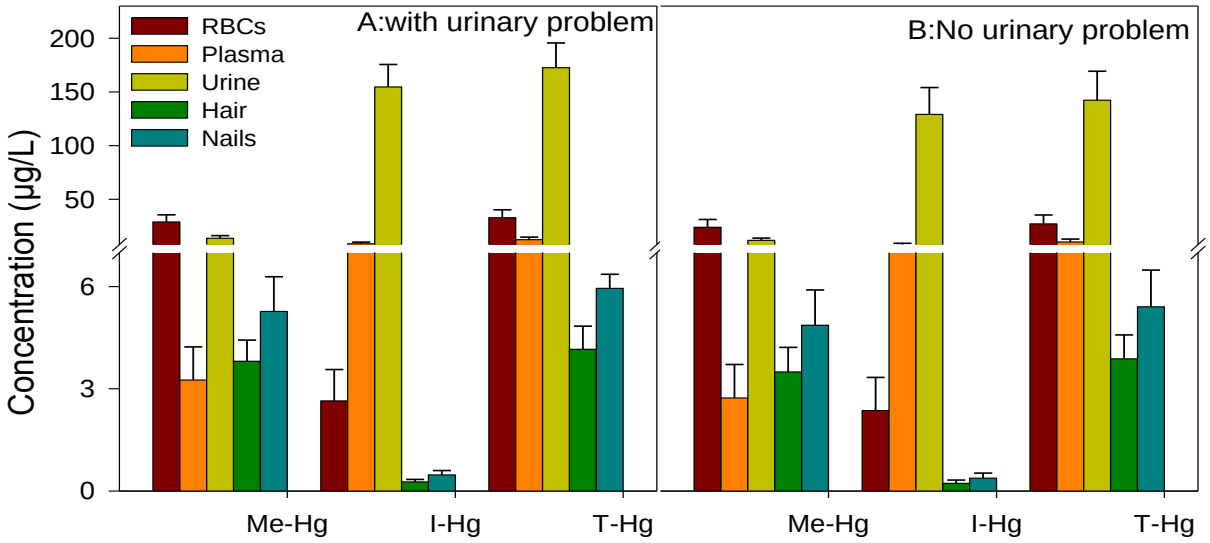


Fig 5.5 The differences between the T-Hg, Me-Hg and I-Hg concentration in the RBCs, plasma, urine, hair and nails of workers suffered from A) urinary problems and B) workers with no urinary problems.

Among the workers, 42% were complaining from urinary problems such as burning micturation, sometime blood released in the urine (Fig.5.3). The concentrations of T-Hg, Me-Hg and I-Hg were higher in the biological samples collected from the workers suffered from urinary problems than the workers with no urinary problems (Langworth et

al. 1992; Sahu et al. 2014). T-Hg concentrations in the RBCs, plasma, urine, hair and nails of workers suffered from burning micturation were 32.9 µg/L, 12.4 µg/L, 172.6 µg/L, 4.16 µg/g and 5.95 µg/g, respectively, Me-Hg concentrations were 29 µg/L, 3.26 µg/L, 13.8 µg/L, 3.81 µg/g and 5.27 µg/g, respectively and I-Hg concentrations were 2.65 µg/L, 8.74 µg/L, 154.7 µg/L, 0.27 µg/g and 0.47 µg/g, respectively (Fig. 5.3).

The concentrations of T-Hg in the biological samples collected from workers suffered from urinary problems were correlated with the years of work found significant correlations (r=0.68, p=0.003 for RBCs, plasma, urine, hair and and nails) which indicated that Hg exposure for long period of time resulted in many worst diseases including urinary problems (Anim et al. 2011; Ikingura and Akagi 1996; WHO 2008). Hg concentrations in the biological samples of workers suffered from urinary problems were correlated with the hours of Hg exposure and highly significant correlations for T-Hg concentration in RBCs (r=0.88, p=0.0008), plasma (r=0.92, p=0.0001), urine (r=0.92, p=0.0001), hair and nails (r=0.88, p=0.0008) were observed.

5.4.4 Exposure to Hg (time)

The working hours per day were calculated and ranged from 2-13 hrs, which were not constant and dependent on personal ability because it is a private work. The time of Hg exposure per day was correlated with the diseases and Hg concentrations in the biological samples. The correlation of exposure to Hg in h/day with Hg concentration in the biological samples was significant. These correlation values were positive for RBCs and plasma (r=0.75, =0.01), urine (r=0.78, =0.008), hair (r=0.69, =0.03) and nails (r=0.7, =0.02). It means that vulnerability to Hg directly increased with increasing the Hg concentration in the body. A significant and positive correlation was obtained for working

h/day with the physical and mental diseases noted among the workers indicating that the diseases caused by high exposure of Hg. Among all workers the highest concentration of Hg was found in the RBCs, plasma, urine, hair and nails of workers with highest exposure time of 12 h and 30 years of work. These workers were found suffered from both mental and physical diseases such as stress, dementia, stomach problems, chest problems, tremor and burning micturation.

5.4.5 Experience (years of work) of workers

In this study, the experience length of the participated workers ranged from 2-40 years. The Hg concentration in the biological samples of workers with more years of work showed a higher concentration than the workers with less years of work. The highest years of work (40 y and 7 h/day) had led to highest concentration of Hg accumulation in the RBCs, plasma, urine, hair and nails (44 µg/L, 18 µg/L, 213 µg/L, 6.32 µg/g and 8.69 µg/g, respectively). These workers suffered from both mental and physical diseases (fatigue, chest problem, dementia and burning micturation). As the years of work and exposure time increased the number of diseases in the workers were also increased (Ritchie et al. 2002). Similarly, a worker with comparatively lower concentration of Hg in their RBCs (23.3 µg/L), plasma (9.10 µg/L), urine (144 µg/L), hair (3.19 µg/g) and nails (4.23 µg/g) among all the workers had no burning micturation, mental or physical diseases because that workers had years of work of 2 y and their exposure to Hg was 3 h per day. Hg concentrations in the biological samples were correlated with the years of work of the workers showed a significant correlation with RBCs ($r=0.54$, $p=0.03$), plasma ($r=0.66$, $p=0.006$), urine ($r=0.57$, $p=0.02$), hair ($r=0.66$,

p=0.005) and nails (r=0.61, p=0.01). These findings are consistent with those reported earlier (Ritchie et al. 2002).

According to the statistical analysis, the correlation between the demographic parameters (age and weight) and Hg concentrations in the biological samples of workers were found non significant (p>0.05).

The study showed that these workers were highly exposed to the toxic vapors of Hg through combustion. The Hg concentrations observed in all biological samples of the workers were 10 times higher than the permissible limit set for blood Hg concentration (5 µg/L), 42 times higher in the case of urine (4 µg/L), while 4 and 2 times higher than the permissible limit set for hair (1µg/g) and nails (5µg/g), respectively by the WHO and USEPA (WHO 2008, Harada et al. 1999). Due to complete unawareness of these workers about the toxicity of this metal, they used it openly without any precautionary measures. In Pakistan, the workers connected with this job were completely illiterate, therefore the effects were more pronounced among these workers. This process is not environmentally sound because the fume evolved may affect other people living in surrounding areas, therefore, this work should be kept away from residential areas.

5.5 Conclusion

The study observed that the goldsmith workers were exposed to extremely toxic levels of Hg**.** The concentrations of T-Hg, Me-Hg and I-Hg in the biological samples (RBCs, plasma, urine, hair and nails) were higher than the control. Both mental and physical diseases were noted in the workers, while the chest problems were highest as compared to other diseases. The strong and positive correlation was observed between the diseases with years of work and exposure time indicating that these parameters greatly

affect the health of the workers and their health problems increased with increasing Hg exposure. The workers were illiterate and completely unaware about the toxicity of Hg, therefore, it is suggested that their awareness should be raised about the protection from Hg through using gloves, masks and protective cloths. This amalgamation and burning processes should be shifted outside the residential area to protect the other people.

WHITENING CREAMS

CHAPTER-6

QUANTIFICATION AND EFFECT OF WHITENING CREAMS CONTAINING Hg ON THE BIOLOGICAL SAMPLES OF WHITENING CREAM USERS

6.1 Abstract

Asians are highly vulnerable to the I-Hg through the use of skin whitening creams. The aim of the work was the quantification of Hg in the skin whitening creams and in the biological samples of whitening cream users (WCU). The Hg concentration observed in the whitening creams was higher than the permissible limit (1µg/g) set by the US FDA .Similarly when the T-Hg concentration of these WCU was analyzed we found significantly ($p > 0.05$) higher T-Hg concentration in the plasma (3.61 µg/L), RBCs (9.83 µg/L), urine (21.2 µg/L), nails (1.76 µg/g) and hair (1.23 µg/g) samples of WCU than control plasma (0.57 µg/L), RBCs (1.77 µg/L), urine (3.04 µg/L), nails (0.45 µg/g), hair (0.37 µg/g). Similarly the Me-Hg and I-Hg found in the biological samples was found significantly ($p > 0.05$) higher than control. Among the whitening creams, Stillmans and White face creams have comparatively the highest Hg concentration of 63.3 µg/g and 57.6 µg/g respectively. The Hg concentration in the biological samples of the Stillmans and White face users were comparatively higher than other cream users. It was observed from the study that the use of whitening creams containing Hg is a health hazard and the people especially women should encourage not to use these creams due to high concentration of Hg. The people should enforce the health sector of Pakistan to regulate

this sector and increase awareness about the use of Hg in cosmetics and its hazardous effects.

Keywords: Whitening creams, Hg, Whitening creams users, Stillmans

6.2 Introduction

Hg an important ingredient of beauty because of its use in skin whitening creams, lotion and soap worldwide (Mahaffey, 2005). The Hg was invented by the Greek Philosopher in (371-287 BC) who powdered cinnabar with vinegar in copper pots. The Greek used Hg as cosmetic for skin whitening (Spangler, 2011). Skin whitening is a cause of concern for the women specially Asians because they have dark skin color (Cristaudo et al., 2013; Li et al., 2008a). Dark skin is considered the unattractive aspect of women in Asia which improving the philosophy of west “white is right” (Askari et al., 2013). The darkening of the color is due to the high production of the melanin by the melanocytes in skin due to environmental factors or inborn (ALqadami et al., 2013). The melanin protects the skin from the sun radiations harmful for skin as well as from the toxic drugs and chemicals (Askari et al., 2013). The high melanin production could be caused by the aging, pregnancy, sun exposure or weakness as well.

In some cultures white skin represents a higher social, economic status and success (Benz et al., 2011). Therefore there is worldwide demand for skin whitening creams. In most of the whitening creams in addition to other ingredients Hg is used as a skin whitening agent because the Hg act as a melanin inhibition agent causing whitening (Peregrino et al., 2011). The fig. 1 illustrate that how Hg inhibit the production of melanin.

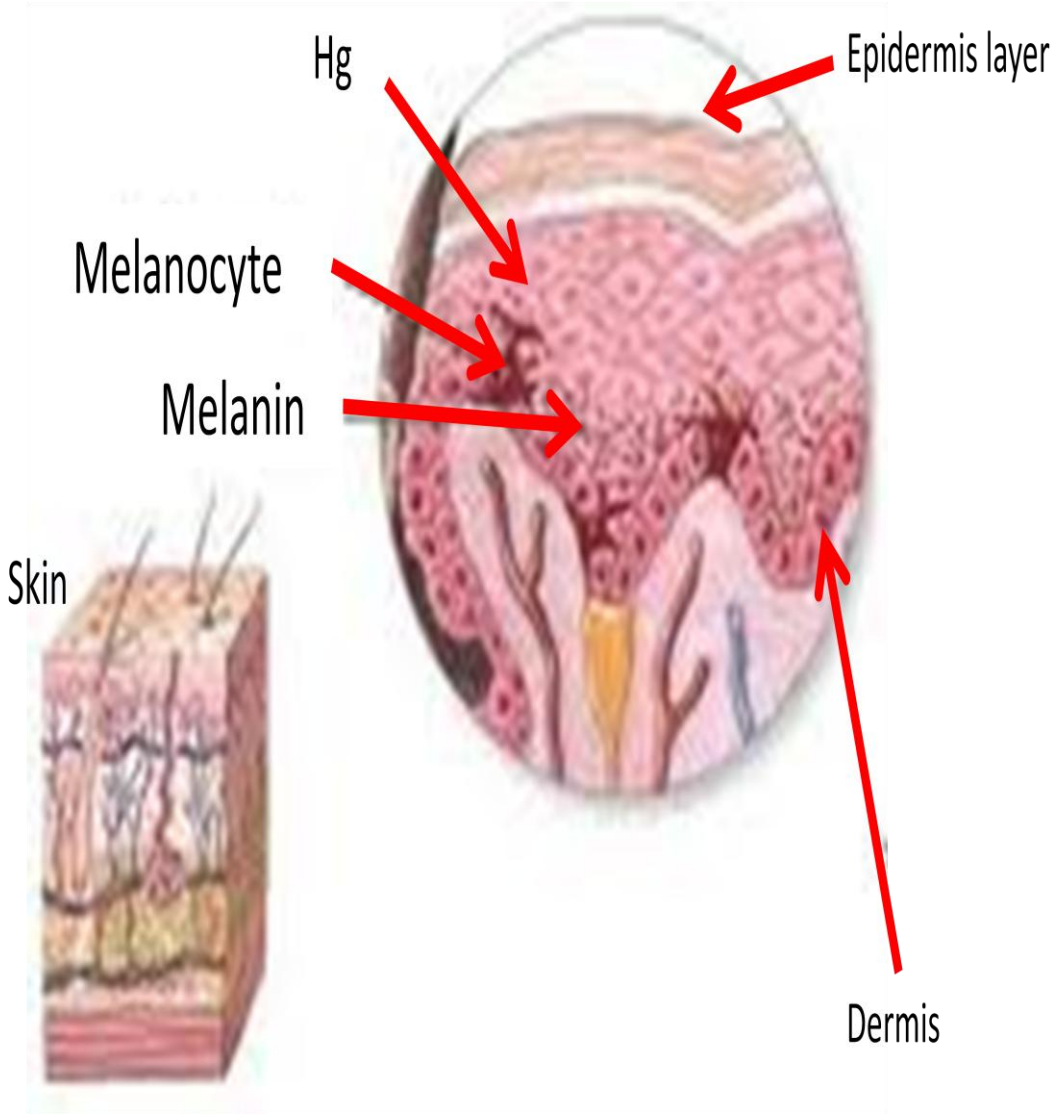


Fig. 6.1 Mechanism of action of Hg in skin

In cosmetics Hg is also used for preservation of creams; however Hg is a health hazard causing neurologic, urinary and skin diseases (Benz et al., 2011; Sin and Tsang, 2003). Due to the high toxicity of Hg its use in cosmetics was completely banned and the permissible limit set by the food and drug administration (FDA) for Hg in creams, cosmetics is less than 1ppm (Al-Saleh and Al-Doush, 1997; Hamann et al., 2014).

In spite of its potential toxicity whitening creams containing Hg is still available worldwide (WHO, 2012). The inorganic form of Hg (mercuric chloride, mercurous

chloride, ammoniated Hg) is used which induce neurologic and psychiatric symptoms (fatigue, weakness, acrodynia, insomnia) (Benz et al., 2011; Sin and Tsang, 2003). The Hg readily absorbed through the skin depends on skin variability and lipid solubility. The Hg could be ingested through the mouth by the application of cream near the mouth skin and through hands (Chan, 2011). After absorption the Hg is distributed to the body organs and eliminated through urine because major part of inorganic Hg excretion occurs through urine (Benz et al., 2011; Chan, 2011; Sin and Tsang, 2003). The use of whitening creams containing Hg is a health hazard for developing fetus causing neurologic and intellectual dysfunction (Drescher et al., 2013).

There are many cases raised by the exposure of inorganic Hg in skin whitening creams and lotions. however a study conducted on determination of Hg concentration in creams purchased from different countries including China, USA, Thailand, Japan etc found the high Hg concentration more than 200 and 1000ppm Hamann et al., 2014. Another study conducted by Al-Saleh and Al-Doush, 1997 found the highest Hg concentration in creams obtained from Thailand and England which was varied from 1,281-5,650 µg/g whereas the McRill et al., 2000; Peregrino et al., 2011; Sin and Tsang, 2003 and Fakour and Esmaili-Sari, 2014 found the highest Hg concentration in the biological samples (urine, blood, hair and nails) of the individuals used Hg containing whitening creams.

There are many papers related to the detection of Hg concentration in urine, blood, hair and nails but papers related to the detection of Hg concentration in creams and in biological samples of whitening cream users (WCU) are scarce. This study is distinct because of the detection of Hg concentration in whitening creams as well as species of

Hg (T-Hg, O-Hg, I-Hg) concentration in the biological samples of WCU in less studied area. The research objective is to study the association between the concentration of Hg in whitening creams and in biological samples of WCU. In Pakistan there are many cosmetics stores where the whitening cream containing Hg are available. The people in this area and even the seller are unaware of the use of Hg in cosmetics and its effects.

6.3 Methodology

6.3.1 Chemicals

Ultra-pure water was prepared by a Millipore ultra-pure water system (Milford, USA), Hg standard solution (1 mg/L in 10% HNO_3), H_2SO_4 (98%), $HClO_4$ (70 %), HCl (37%), HNO_3 (68%) obtained from Sigma–Aldrich® (via Analytical Measuring Systems, Karachi, Pakistan), Analytical grade acid (CH_3COOH) analytical-grade sodium borohydride ($NaBH_4$), ethanol (CH_3CH_2OH), acetic, tin chloride ($SnCl_2$), sodium hydroxide (NaOH), Hydroxylamonium chloride, cysteine ($HO_2CCH(NH_2)CH_2SH$), cadmium chloride ($CdCl_2$), potassium hydroxide (KOH) were obtained from both (via Musaji Adam & Sons, Karachi, Pakistan) and Sigma–Aldrich®, Merck chemicals (via Science Centre, Rawalpindi, Pakistan). These chemicals were available analytical purified therefore no need of further purification.

6.3.2 Sampling

Samples of blood, urine, hair and nails from the volunteers and the whitening creams from their homes were collected. Before starting the study an "*informed consent"* was obtained from the volunteers under study. A standard questionnaire regarding the detail data about demographic profile (age, weight, height etc.) type of cream use,

duration of cream use, education, work, fish consumption, health profile and dental amalgam filling was obtained from the WCU.

The study was approved by the world medical association declaration (WMA) of Helsinki and the research methodology was approved by the University of Peshawar, Pakistan.

6.3.3 Inclusion criteria

The WCU with no other occupational and environmental exposure to Hg except dental amalgam and fish consumption were included in the study. In cosmetics the samples of whitening creams only on the spot from the homes of the selected users were collected and included in the study.

6.3.4 Exclusion criteria

The individuals exposed to Hg through other cosmetics except whitening creams, occupationally and environmentally were excluded from the study. Cosmetics other than whitening creams were excluded from the study.

6.3.5 Collection and preparation of samples

Samples blood was collected in heparinized tubes and urine in polyethylene bottles. Blood was centrifuged for the separation of plasma and RBCs by means of centrifugation at 3000 rpm. The plasma was stored in eppendorf tubes while RBCs in heparinized tubes at -20C^{o}. In urine samples 1ml of 10% of HNO3 (15%) was added in each sample for the preservation of Hg and stored at 4C^{o}.

The samples of head hair and nails clipping were collected from the whitening cream users and stored in plastic bags for further analysis. Before analysis the hair and

nails samples were washed with distilled water and acetone separately then dried at 40Cº in oven.

6.3.6 Extraction

The Hg concentration was extracted from both whitening creams and biological samples of whitening cream users.

6.3.6.1 Quantification of Hg in whitening creams

The Hg was detected in the whitening creams by the reported method (Al-Saleh, 2011) with minimum changes. 0.2 g of whitening cream was weighed and transferred to the digestion tubes. The samples were predigested with concentrated H_2SO_4 (2ml) and HNO3 (2ml) heated upto 80Cº for 90 min. the predigested step dissolved the samples. After cooled the samples at room temperature, added 7ml of $KMNO_4$ (5%) and 5ml of HCl (3%) and heated for further 2 h at 95 Cº. cooled the samples at room temperature and added 3ml of Hydroxylamine solution for the reduction of excess $KMNO_4$ and diluted upto 50ml for further analysis by the AAS,700.

6.3.6.2 Quantification of Hg in biological samples

The detail procedure for the detection of Hg in the biological samples (plasma, RBCs, urine, hair, nails) of WCU is mentioned in the chapter 3.

6.3.7 Analysis

Prior to the analysis all the glasswares and containers were washed with distilled water and soaked in 40% HNO3 overnight and then dried at 70Cº.

Calibration standards of 10, 25 and 50 µg/L were prepared at the time of analysis from Hg standard stock solution of (1000 mg/kg). The samples of whitening creams and biological samples (RBCs, plasma, urine, hair, nails) of WCU were analyzed used Perkin

Elmer atomic absorption spectrometry (AAS Perkin Elmer 700, Waltham, USA) with Hg hydride system (MHS-15) connected with the electrodless discharge lamp (EDL) used for Hg detection. The instrument analysis was performed by the argon gas flow, with energy 62J, current 185MP and wavelength-253.7λ. The instrument setting was similar for the analysis of all biological samples. $NaBH_4$ was used as a reduction solution during analysis for the T-Hg, O-Hg while SnCl2 was used for creams and I-Hg reduction. 5% potassium permanganate and 1.5% HCl was added in the samples prior to the analysis. The laboratory standards and control were passed from the same processes of digestion and analysis.

6.3.8 Statistical analysis

The statistical analyses were performed by using sigma plot version 10, Graph pad version 5 and Xcel® softwares. The difference between the Hg concentration of whitening cream users and control was assessed by using unpaired t-test (Two tailed) at 95% confidence interval while a p value of ($p > 0.05$) was considered significant.

6.4 Results and Discussion

The whitening creams (n=10) and whitening cream users (WCU) (n=20) were recruited for samples collection. The users were only female and their blood, urine, hair and nails samples were selected for T-Hg, Me-Hg and I-Hg determination as well as from control (n=20). No male was selected in control because users have no male. The Table 6.1 represented the detail profile data of the WCU and control. The questionnaire data showed that the users have an average age of 30.8±4.9y quite older than control 25.7±4.3y with an average weight and height of (63.9±8.9 Kg, 5.7±0.3 feet for users) respectively and (61.6±7.8 Kg, 5.4±0.2 y for control) respectively. The users participated

were mostly post graduated (60%) while the control were young with under graduation (50%). The frequency of cream use is high in female workers and students because they are more conscious about fairness and beauty due to outdoor activities. 20% were teeth filled with amalgam filling and fish consumption frequency was also higher in users (50% 1/week) than control (70%). Samples were collected from volunteers worked as beautician because the beauticians using high doses of whitening cream either for themselves or for others. The users were used cream from long time with time range of 5month to 10 years. No chronic disease was noted except sometime dizziness, headache, skin irritation and dermatitis in 20% users only.

Table 6.1 Detail profile data of whitening cream users (WCU) and control

Parameters	WCU	Control
	Mean+S.D	**Mean+S.D**
Gender	Female	Female
Age (y)	30.8±4.9	25.7±4.3
Height (ft)	5.7±0.3	5.4±0.2
Weight (Kg)	63.9±8.9	61.6±7.8
Education (%)		
Post graduate	60	10
Under graduate	20	50
Under matriculation	10	30
Uneducated	10	10
Hg contact through whitening creams (%)	100	0
Work as beautition (%)	20	0
Teeth amalgam filling (%)	20	10
Oral breathing (%)	40	0
Fish consumption (%)		
Once/week	50	30
Once/month	50	70
Duration of cream use (years)		
1year	25	0
3 years	35	0
5 years	40	0
Mental/ physical problems (%)	20	0

Table 6.2 T-Hg, Me-Hg and I-Hg concentration in the biological samples of whitening creams users (WCU) and control

		WCU		**Control**			
		Mean+S.D	**Median**	**Mean+S.D**	**Median**	***P* value**	**CI**
RBCs (ug/L)	**Me-Hg**	8.63±3.3	8.05	1.6±0.7	1.66	0.001	95%
	I-Hg	0.8±0.4	0.67	0.13±0.07	0.13	0.001	95%
	T-Hg	9.83±3.7	9.19	1.77±0.8	1.82	0.001	95%
Plasma (ug/L)	**Me-Hg**	0.92±0.5	0.8	0.15±0.07	0.16	0.001	95%
	I-Hg	2.5±0.9	2.4	0.39±0.18	0.41	0.001	95%
	T-Hg	3.6±1.4	3.3	0.57±0.3	0.59	0.001	95%
Urine (ug/L)	**Me-Hg**	1.67±0.5	1.62	0.42±0.3	0.38	0.001	95%
	I-Hg	19.1±3.3	18.6	2.52±1.1	2.72	0.001	95%
	T-Hg	21.2±3.7	20.6	3.04±1.3	3.32	0.001	95%
Hair (ug/g)	**Me-Hg**	1.13±0.9	0.74	0.33±0.2	0.26	0.001	95%
	I-Hg	0.098±0.1	0.065	0.034±0.01	0.03	0.001	95%
	T-Hg	1.23±0.9	0.81	0.37±0.2	0.29	0.001	95%
Nails (ug/g)	**Me-Hg**	1.57±1.3	1.01	0.41±0.2	0.32	0.001	95%
	I-Hg	0.16±0.2	0.09	0.036±0.02	0.03	0.001	95%
	T-Hg	1.76±1.4	1.12	0.45±0.3	0.37	0.001	95%

Each value of mean ± S.D represents standard deviation (n=20)

The detail data of Hg concentration found in whitening creams and biological samples (plasma, RBCs, urine, hair, nails) of WCU were given in Table 2. The Hg concentrations in the biological samples of WCU were significantly ($p > 0.05$) higher than the control. The T-Hg mean concentration found in the RBCs, plasma, urine, hair and nails of WCU were (9.83 µg/L, 3.61 µg/L, 21.2 µg/L, 1.23 µg/g, 1.76 µg/g)

respectively while in control was (1.77 µg/L, 0.57 µg/L, 3.04 µg/L, 0.37 µg/g, 0.45 µg/g) respectively (Fig 6.3). Similarly the Me-Hg and I-Hg was also found higher in WCU than control as given in Table 6.2. It was observed from the study that the use of whitening creams add Hg to the biological samples of WCU or in other words the creams have high concentration of Hg (McRill et al., 2000).

The Hg concentration found among the 9 whitening creams were Stillmans, White Face, Golden Pearl, Melawhite, Ideal Face, Whitening Cream, Add Me, Garnier and Ponds. The creams were selected as 3 herbal products, 3 imported and 3 local. The highest concentration of Hg found in the Stillmans (63.2) and Golden Pearl (57.6) creams (Fig.6.1). It was observed that the night creams have high concentration of Hg used to reduce freckle (Sin and Tsang, 2003). Similarly the Golden Pearl and Melawhite creams also found high concentration Hg. The lowest concentration of Hg found in the Ponds (8 µg/ml) cream but all of the creams including Ponds found higher concentration than the permissible limit (1µg/g) (Al-Saleh and Al-Doush, 1997; Askari et al., 2013).

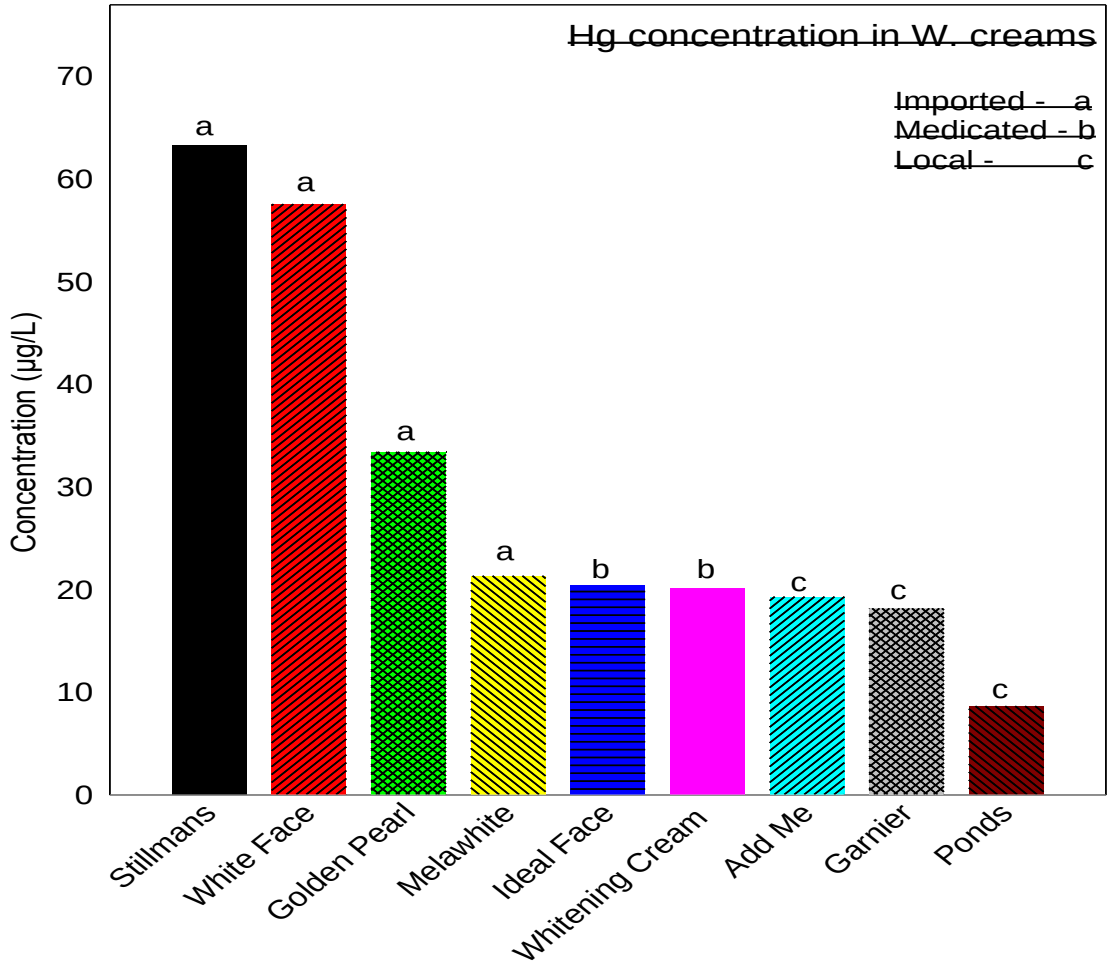


Fig. 6.2 Hg concentration in whitening creams

The Hg concentration observed in the biological samples of the WCU was analyzed and the cream utilization time was calculated which ranged from 1 to 5 years showed that the Stillmans and White Face user have high Hg concentration however the Melawhite and Ideal Face users found high Hg concentration than the Golden Pearl users as the Golden Pearl have high Hg concentration than these creams. The reason may be the Melawhite and Ideal Face cream users used the creams from longer period of time of 5 y while most of the volunteers involved in the use of Golden Pearl were used the cream

from less than 2 y. Some users of Ponds cream have the lowest Hg concentration however higher than the control (Fig. 6.3).

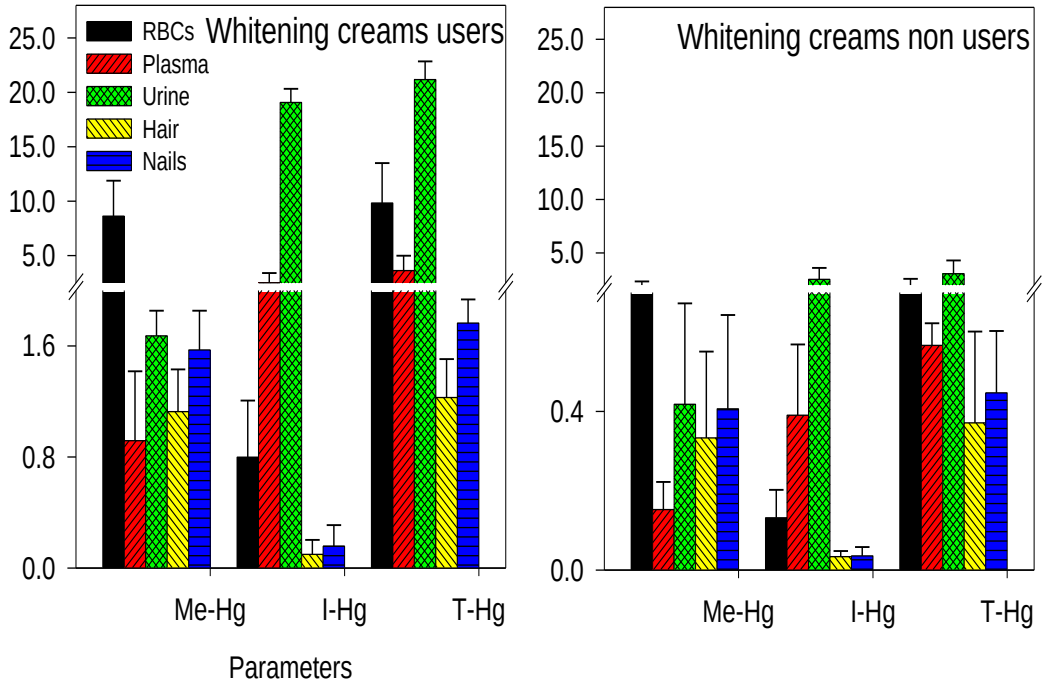


Fig. **6.3** Hg concentration in the biological samples (RBCs, plasma urine, hair, nails of whitening creams users and non users (control)

Dental filling was found to be the chief source of Hg in biological samples because the dental amalgam users were found to have high Hg concentration in their biological samples as their use of cream was the lowest. It was observed from the study that the duration of cream and dental amalgam use greatly influence the Hg body burden (Fakour and Esmaili-Sari, 2014) because dental amalgam used 50% of Hg and is a source of I-Hg concentration in the body (Sin and Tsang, 2003). This study is comparable with the conducted study (Fakour and Esmaili-Sari, 2014).

The weight of users have no effect on Hg concentration however the age affect to some extent that older women have high concentration of Hg in the biological samples (Fakour and Esmaili-Sari, 2014). It may assume that the older women used Hg from long time as Hg is accumulated with the passage of time (Agusa et al., 2005; Ngim and Ngim, 2013). Similarly the highest concentration of Hg was found among the biological samples of the WCU beautician because they were much more exposed to the toxic Hg by the application of Hg for themselves and for others (Fakour and Esmaili-Sari, 2014).

No symptoms of Hg toxicity were noted among the studied individuals except mild dizziness, headache, skin irritation and dermatitis (Drescher et al., 2013; WHO, 2012). It may assume that the WCU were young and they have strong immune system. The symptoms may appear in the late age as the Hg is accumulated (Ngim and Ngim, 2013). It was observed from the study that the mercuric chloride used in the whitening cream absorbed through the skin and distributed to the body organs and fluids therefore we found a high concentration of Hg in RBCs and plasma (Chan, 2011). The inorganic form of Hg used in the cream is excreted through urine because 90% of the Hg removed from the body through urine (Sin and Tsang, 2003).

6.5 Conclusion

The study indicated that the Hg in the skin whitening creams is a considerable source of Hg exposure in WCU. All the creams and WCU analyzed were found to have high Hg concentration than the permissible limit and control. The users and even the seller were unaware of the use of Hg in the cosmetics and about the toxic effects of Hg. Therefore there is a need of proper regulation in Pakistan health sector regarding

whitening creams and cosmetics. The women should strictly avoid the use of these creams and awareness campaign is necessary against the use of Hg in creams, cosmetics.

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