

Biomarkers of Occupational Manganese Toxicity

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Manganese (Mn) is an essential trace element that is required for maintaining normal function in the human body. However, excessive exposure to Mn has been associated with Parkinson-like symptoms of a disease called manganism. Mn exposure is occurred in different occupational settings such as mining, steel and alloy production, welding and dry cell battery manufacturing. Biological monitoring of chemical exposure in the workplace has become increasingly important in the assessment of health risk as an integrating part of the overall strategy in the occupational health and safety. This activity requires accurate sampling and analysis with correct interpretation of results. Biological monitoring is an important tool in the prevention of occupational diseases related to those exposed to chemicals such as manganese on a regular basis. Particularly, when there may be multi-route exposures (inhalation, skin and ingestion) or abnormal exposures. No reliable biomarker has been established to evaluate the effects of Mn exposure. A complete scientific understanding of the mechanism of Mn induced toxicity remains undiscovered. Over the past decade, extensive researches using animal models and human communities have led to several potential indicators of Mn exposure and biological effects. The present study aimed to identify reliable biomarkers of Mn exposure and toxicity. Using keywords such as biomarkers and manganese toxicity in the available databases, the relevant papers published from 1990 to 2014 were found and those having our criteria were selected. The results were obtained by analyzing the collected data. The biomarkers of Mn exposure such as Mn concentration in biological fluids (blood, urine and saliva) and some parts of body (hair and nail) were investigated. Mn of blood and urine are not good markers to evaluate the exposure. Although Mn in blood is different in group comparison of those having active exposure than those not exposed to it, it is not valid for the individuals. Biomarkers related to Mn poisoning effects including variation in Mn to Iron Ratio (MIR), variation in iron and regulatory proteins of iron, serum prolactin, auric acid (ROS) Reactive Oxygen Species, and MRI were investigated. Regarding criteria for a good biomarker, each investigated biomarker had a number of advantages and disadvantages. Therefore, further researches are required to introduce an appropriate biomarker for Mn exposure and effects to prevent manganism in workers exposed to Mn. Mn-exposed biological indicators such as Mn concentration in biological fluids (blood, urine and saliva) and some parts of body (hair, nail and bone) are studied. Mn of blood and urine are not good indicators to evaluate the exposure. However, Mn in blood for group comparison of current exposure is good but not for the discrimination of an individual. Biological indicators related to effects are Mn to Iron Ratio (MIR), variation in iron and regulatory proteins of Iron, serum prolactin, auric acid, (ROS) Reactive Oxygen Species, and MRI. Concerning the criteria for a good indicator, every mentioned indicator has a number of advantages and disadvantages. Thus, it is indispensable to conduct additional researches to introduce an appropriate indicator in order to prevent manganism in workers.

Key words: Biomarkers, Manganese, Occupational Exposure, Manganism

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To evaluate the risk of occupational exposure to chemical agents, quantifying the exposure levels is required to determine the risk levels of the workers. A toxic chemical agent can be determined by measuring its concentration in workplace ambient air and comparing the results with the allowed threshold limits recommended by organizations such as ACGIH and OSHA. Since the amounts of the allowed threshold limit are considered safe values, it can only determine the potential toxicity of a chemical. Due to different personal characteristics and sensitivities of workers, knowledge about exposure levels alone is not sufficient for risk assessment. Therefore, using biomarkers as an adjunctive tool for the exposure limits is necessary^{1,2}.

Biological indicators reflect the values of the chemical or its metabolites in tissues and biological fluids are measured.

For example, blood lead levels as a biological indicator is able to determine the boundary between safe and unsafe in confronting with the metal.

Although Manganese is an essential element in an indefinite status and is required for human exposure to excessive amounts can lead to diseases like Parkinson's disease, which is known as Manganism¹.

Manganese is an essential trace element in the diet of all animals

It is essential for many enzymatic reactions such as the synthesis of amino acids, protein and carbohydrates. [3]. It is found in many rocks and soils of the earth, and is vital to ensure the health and proper functioning of various body systems including the skeletal system, immune, nervous and reproduction⁴.

The major sources of dietary manganese are whole grains, fish, vegetables, nuts and beverages such as tea and coffee⁵.

The determined the recommended amount of manganese in the diet is difficult, but America National Research Council has determined 2-5 mg per day for adults as the safe enough magnesium in the diet⁶.

According to FDA (Food and Drug Administration) and NAS (National Academy of Sciences) the daily withdrawal amount of manganese is 2.3 mg for men and 1.8 mg for women⁷.

The average manganese air approximately is 0.02 micro-grams per cubic meter⁸.

In the U.S. the amount of manganese in the water is less than 0.05 million⁹.

Although manganese deficiency in the diet is rare, it can cause the effects. Manganese deficiency in animals is associated with growth disorders, reproductive system, testicular atrophy in male animals, skeletal disorders and imbalance in carbohydrate and fat metabolism¹⁰.

A few cases of manganese deficiency in humans has been reported that its symptoms are dermatitis, slow growth of hair and nails, reduction of serum cholesterol levels and protein values required for blood clotting^{7, 11, 12}.

The threshold limit of manganese is 0.2 mg/m³ ACGIH based on the latest book concerning threshold limit. Occupational exposure to high levels of manganese is mainly occurred in miners and welders. Long-term exposure to manganese can cause Parkinson-like disease that is called "Manganism"¹³. During recent years using biomarkers in different fields such as medicine, industry, and environmental sciences has been dramatically expanded¹⁴⁻¹⁷.

The use of biomarkers in risk assessment has grown considerably in the past decade.

These markers are visible tool show that the person will become sick.

This particular markers in the evaluation of chronic disease whose symptoms will appear after a long time after exposure, are important because at the time, the disease is not reversible¹⁸. The complexity of the nervous system does not allow a biomarker sensitive, specific and reliable to be determined for neurotoxicity easily and fast.

However, provided biomarkers are promising¹⁴. Determining a valid and reliable biomarker to identify manganese poisoning easily at an early stage before symptoms appear is necessary.

The aim of this study was to investigate the introduction of biomarkers in human and animal studies related to exposure to manganese.

MATERIALS AND METHODS

Using the keywords Biomarkers and Manganese, the relevant articles were searched in available databases and then the article, having

the intended criteria, were selected.

Human studies conducted in the workplace, exposure to manganese, published papers from 1990 to 2014, and the determination of the amount of manganese biological fluids were among the conditions.

In addition, it was attempted to select articles that were related to people with more experience and higher quality. Results were extracted by analyzing the collected data.

RESULTS

Manganese concentrations in body fluids as a biomarker of exposure

After inhalation exposure to manganese, this element is introduced into the bloodstream. Therefore, most studies on biomarkers primarily consider blood manganese.

Studies on rodents indicate that manganese concentration in the blood significantly increases after injection, oral or inhaled exposure^{2, 19-22}.

However, experimental studies on laboratory animals have reported similar results, but studies in human societies have reported conflicting results²³⁻²⁴. The use of manganese concentrations in the blood has been studied as a biomarker of exposure to manganese among welders, metal workers, miners and workers making dry batteries²⁵⁻³⁶.

In general, these studies revealed that the concentration of blood manganese as an acceptable index is also reflected in the comparison group in the face of active and current exposure and it is the most common indicator used to compare groups and the control group.

The low sensitivity of the use of blood manganese as biomarker is because half-life of manganese blood is shorter than its half-life in the tissue.

Toxicokinetic studies indicated that half-life of manganese in Rat's blood plasma is less than 2 hrs, while the level in its brain is over 50 hrs³³. The intracellular distribution of manganese ions underlies the mismatch between blood manganese and the actual concentration of manganese in the target tissue or organ, especially the brain¹¹. Manganese Urine (MnU) is considered as a non-invasive method. Since manganese is

primarily excreted through feces and urine manganese and Manganese is a small part of the body many of the changes have been observed between exposure and urine manganese and should not be used as a biomarker. In one study conducted by Hoet *et al.* (2012) a urine manganese concentration of 65% of welders was below the detection limit (LOQ)³⁴. The results of studies on the amount of manganese found in the urine of workers exposed to manganese are presented in Table 2-1^{26, 29, 35-42}.

The concentration of saliva manganese has been studied as a biological indicator of exposure in a number of studies⁴²⁻⁴⁴. In the study conducted by Dixing Wang *et al.* (2008) the use of saliva manganese in welders is studied as a reliable biomarker.

In this study, the Air Mn values for the control group, low exposure and high exposure groups were 0.01, 0.24 and 2.21 mg per cubic meter of air, respectively.

Manganese saliva was measured using ICP that manganese in welding saliva was significantly higher than the control group ($p < 0.01$) as well as changes in the saliva of the workers was related to workers' exposure to manganese⁴².

Fernando Gill *et al.* (2011) determined the concentration in the saliva of 178 patients who had occupational exposure to heavy metals.

The results indicated that those who had more experience had higher concentration in saliva and they concluded that saliva can be used as a biomarker of manganese excess or replace biological blood used in monitoring⁴³. Zhou Yz *et al.* (2010) determined a biomarker for job exposure in order to identify air manganese samples in exposed individuals. Then manganese concentrations in saliva, plus a number of other fluids and tissues were determined.

Results showed that the manganese concentration in saliva can be used as a biomarker in patients exposed to manganese⁴⁴.

Mn hair concentration is considered due to ease of access and as other non-invasive method. Several studies have emphasized that with increasing experience, hair manganese concentrations increases as a biomarker⁴³⁻⁴⁵.

Bader *et al.* (1999) found that compared with the exposed group controls, manganese of the exposed armpit hair was 3 times than that of

control group⁴⁵.

It is likely that this difference is caused by secondary pollution.

Nails and fecal Mn has been studied as a biomarker, but a lot of changes are shown in the results.

For example, the range between 0.07 to 15.9 micrograms manganese per gram of feces and fecal 0.02 to 14.7 micrograms per gram of nails was observed among exposed welders. Unfortunately, this study lacked control group⁴⁶.

The properties of manganese allow non-invasive method Magnetic Resonance Imaging

(MRI) to determine the amount of manganese. For this purpose, the Pallidus Index (PI) has been used.

The index is the ratio of signal intensity in the subcortical white matter in the anterior base of the axial T1 is at 100 dead¹¹.

In one study, by Yueming Jiang *et al.* (2007) used MRI as a biological indicator of manganese in metal smelting workers and drawn the conclusion that the mean PI scores among workers with low and high levels were 4.7% (P <0/05) and 1.16% (P <0/01), respectively that manganese exposure increased compared with the control group⁴⁷.

Table 1. Results of studies on blood manganese as a biomarker

Row	Author(s)	The number of control subjects	Studied occupation	Blood manganese concentration of control subjects (µg/l)	Air manganese concentration (mg/m ³)
1	Luncchini <i>et al.</i> (1997)	61	Alloy production, ore crushing, melting	9.8	0.3
		87		6.8	
2	Bast Pettersen <i>et al.</i> (2004)	100	Alloy production, ore smelting, furnace	10.4	0.3
		100		9.2	
3	Blond <i>et al.</i> (2007)	92	Metallurgy	10	-
4	Chia <i>et al.</i> (1993)	17	Mining	25.3	1.59
		17		23.3	
5	Elingsen <i>et al.</i> (2008)	96	Production of heavy machinery, shipbuilding and welding	8.6	0.12
		96		6.9	
6	Luncchini <i>et al.</i> (1999)	35	Mining	9.7	0.19
		27		6.8	
7	Mergler <i>et al.</i> (1994)	73	Mining	11.3	0.23
		73		7.2	
8	Myers <i>et al.</i> (2003)	101	Metallurgy	13.6	0.77
		67		6.4	
9	Roles <i>et al.</i> (1992)	92	Dry cell battery manufacturing	8.1	0.95
		101		6.8	
10	Roles <i>et al.</i> (1987)	92	Production of manganese oxides and salts	13.6	0.94
		101		5.7	
11	Sjogren <i>et al.</i> (1996)	12	Rail building, welding	8.5	-
		39		7	
12	Wang <i>et al.</i> (2006)	82	Welding, automobile building	20.5	0.25
		51		12.5	
13	Yuan <i>et al.</i> 2006	56	Machining, welding	48.4	0.14
		34		19.2	

MRI features of this technique potentially are a valuable method to detect exposure to manganese.

The increase of Manganese is in the bilateral and symmetric forms.

The signals among workers who have been exposed but do not show symptoms can be detected.

Signal strength with P indicator and Manganese cells of blood have significant relation with each other.

Not only high signal intensity in the basal area but nigra striatum has been observed in the workers with Manganism.

Abnormally high exposure to manganese may disappear after 6 months¹¹.

Manganese toxicity biomarkers or biological indicators of effectiveness: Biochemical, physiological changes, behavioral or other in an organism occurs after exposure to manganese that can determine the potential effects

Table 2. Results of a number of studies on urine manganese as a biomarker

Row	Author(s)	The number of control subjects	Studied occupation	Manganese concentrations in urine of control subjects ($\mu\text{g/l}$)	Air manganese concentration (Mg/m^3)
1	Roels <i>et al.</i> (1992)	92 101	Battery manufacturing	0.84 0.09 (per gram of creatinine)	0.948
2	Lucchini <i>et al.</i> (1999)	61 87	Alloy production	1.53 0.4 (per gram of creatinine)	0.0542
3	Laohaudomchok <i>et al.</i> (2011)	27-	Welding	1.5 (ng/ml)	0.0128
4	Lucchini <i>et al.</i> (1995)	58-	Alloy production	2.27	0.671
5	Järvisalo <i>et al.</i> (1992)	58	Welding	0.31	0.26
6	Hua and Huang (1991)	-	Alloy production	132	2>
7	Teresa <i>et al.</i> (1997)	-	Alloy production	0.6 (per gram of creatinine)	0.43
8	Hassani <i>et al.</i> (2012)	118 37	Welding	7.58- 0.77	0.3
9	Apostoli <i>et al.</i> (2000)	94 87	Alloy production	3.8 0.7	0.15

of poisoning or disease. Therefore, on the basis of hypothetical mechanisms of Mn neurotoxicity is presented.

The changes in the iron and iron regulatory protein

Manganese and iron compete for places of the same link. Data from human studies indicate that iron levels in individuals exposed to Mn are reduced and the results are shown in the experimental studies on laboratory animals. Cowan *et al.* (2009) in a study of 95 people exposed to manganese in a metal smelter (high exposure) and 122 administrative employees of the same factory (face down) and 106 subjects in a control group

not exposed to concentrations determined iron and manganese in saliva, plasma, erythrocytes, urine and hair With the increase of manganese concentrations in biological materials, metal concentration in plasma and erythrocyte significantly reduced in exposed workers compared to the control group. For this reason, the authors suggest that manganese to iron ratio can be used as a biomarker to distinguish exposed and unexposed ones⁴⁸.

The biomarkers of oxidative stress

Oxidative stress occurs when free radicals are produced in large quantities that their production mechanism is reviving oxygen. One of

the potential sources of oxidative stress is excessive exposure to manganese.

In mammals, manganese tends to accumulate in areas that are rich in dopamine in the ganglion Bashdbvzh basis⁴⁹. Another manganese mechanism in the production of oxygen species (ROS) is involved through the impact on the molecules within the cell.

Several enzymes that produce ROS such as Superoxide Dismutase (SOD), Malondialdehyde (MAD), Glutamine Synthetase (GS), Metallothionein (MT), or Glutathione (GSH) are known as the markers of systemic oxidative stress¹¹. A number of researchers have used these markers as biomarkers of exposure to manganese.

In one study, Chtourou *et al.* (2014) investigated the protective role of silymarin (as an antioxidant) in the prevention of oxidative stress resulting from exposure to manganese in rats. They found that it plays a significant role in the development of oxidative stress caused by exposure to manganese⁴⁹.

Furthermore, Prolactin been studied as a biomarker effects of manganese toxicity^{50,51}. In one study, Sergio Montes *et al.* (2008) describe the relationship between blood manganese and prolactin as a biomarker in people living near mining areas in Mexico who were exposed to manganese. Multiple regression analysis indicated that manganese levels in blood is an important factor in the value of serum prolactin⁵¹.

In the study conducted by Kim *et al.* (2007) the effect of occupational exposure to manganese on prolactin in 251 welding jobs and 100 administrative employees who were matched for age and sex, as the control group was analyzed. The results of study indicated that with the increase of exposure to manganese prolactin levels increased⁵⁰.

Moreover, a biomarker of uric acid in urine is examined. In a study conducted by SUN XiaoMing *et al.* (2011) on 65 welders, the results showed that the amount of uric acid in urine of people exposed to manganese was significantly lower than that of control group⁵².

Considering approximately 40% of manganese accumulating in the bone⁵³. A method is also provided with X-ray spectroscopy that small amounts of various metals such as lead and manganese can be measured and a number of

studies have been conducted on Manganese. Pejoviæ-Miliæ *et al.* (2009) using neutron activation analysis measured the bone manganese of 30 welders and 10 persons as the control group. The results indicated a significant difference between the control group exposed to manganese and manganese bone ($P < 0/05$). It seems that the amount of manganese can be used as a marker of bone⁵⁴ In addition to the work and experimental studies on laboratory animals, a number of studies are relevant to people exposed to manganese in the environment^{51, 55-62}.

In most of these studies, examined people's behavior is to be mentioned in a separate article.

DISCUSSION

It is important to emphasize manganese poisoning is usually progressive and irreversible because of the permanent damage to the neural structure.

The search for a reliable marker or a biomarker that can indicate the risk of manganese toxicity is essential in the studies of occupational health.

Scientific research is necessary to achieve such a biomarker. Numerous studies have been carried out on biomarkers related to manganese and manganese in biological fluids and tissues in the investigation so as to determine whether they can be used as a biomarker of the contract.

For a reliable indicator, it is desirable that the exposure of dose-response relationship is correlated.

Since the measurement of external exposure dose is not usually available in workplaces, manganese exposure is unknown.

Measuring blood and urine manganese problems and their relationship with external exposure are unknown.

Mn in blood or urine as a biomarker studies are presented.

Manganese has a short half-life in blood and the amount of time sampling and a short time before sampling are measured.

Regarding Manganese in urine, it should be noted that it is exerted mainly through the feces and only a small amount is excreted through the urine.

In sum, based on the articles, blood and urine manganese often can be used to show the difference between exposed and unexposed groups, but due to the change of the results, these indicators cannot be introduced as a suitable biomarker.

The ratio of Manganese to iron is suitable for biological monitoring. From the perspective of assessing exposure to the ratio of manganese to iron in erythrocytes (eMIR) compared to the ratio of manganese to plasma iron (pMIR) has a higher value because it shows higher correlation with the amount of air manganese⁴⁸.

It seems that eMIR is more dependent on exposure and pMIR is related to the effects of manganese.

Manganese and other metals measured in saliva samples have several advantages including saliva secreted by the salivary glands and contain components of the extracellular body. Metal ions such as manganese are not essential only through the influence of non-active but also take active transport

Thus, theoretically, different chemical properties of saliva, serum and saliva make it appropriate to examine manganese.

Other saliva samples can be easily prepared and maintained and a non-invasive procedure is a better and more comfortable and can be stored and sent to the laboratory for analysis.

One of the limitations associated with the use of saliva is the intervention factors of diseases such as ulcers and dental infection.

However, in a number of studies, it is suggested that manganese saliva can be used as a biomarker^{42, 43}.

Indicators weighed T1 and pI in MRI still continue to be used as a non-invasive and sensitive biomarker for assessing exposure to manganese in workers⁴⁷.

The usefulness of MRI in studies also is approved^{11, 63-67}.

Only in one article of uric acid, urine is investigated as a biomarker of manganese⁵².

Therefore, further studies are necessary to be able to judge its usefulness.

In the articles that hair and nails are considered as biomarkers it has been mentioned that in this material there is the possibility of

secondary infection in addition to changes in the hair, such as painting, on the results of curled up why to be affect who used underarm hair.

Wash hair to remove external contamination without hair on content affect the basic and necessary step prior to taking samples of hair and nail magnetic. Magnetic imaging a useful tool for estimating occupational exposure to manganese assessing and measuring the signal strength indicator P. it is widely used because of its easy use.

Two indicators show the status of P and T, as well as exposure to manganese.

These indicators are useful for monitoring biological monitoring biological exposure with the use of P index as an indicator of a lack of communication due to the accurate dose - response is limited.

Direct measurement of T1 is regarded as an option for a person to assess the concentration. Various studies on the impact of manganese on prolactin have reported conflicting results, although many reports have shown that Prolactin manganese in workers exposed to significantly has increased compared with that of the control group⁶⁸. Unlike them, some studies have reported reverse results^{29, 69}.

This contradiction may be due to non-uniform sampling time or non-uniform distribution of age because age prolactin levels may also be affected.

The highest and lowest concentration of prolactin is during sleep in the waking hours.

In a study by Roels *et al.* (1992), blood samples from 5.8 to 18 hrs for a day shift and from 20 to 2.5 hrs were collected. Thus, different sampling time can be effective²⁹.

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