Full Length Research Paper

**Evaluation of Liver biochemical parameters in manganese miners**

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**Background and objective:** Manganese (Mn) related jobs may cause Manganism especially in miners. Side effects include neural and pathological disorders. In spite of liver is the main organ that filters Mn (99%) but few studies has performed about Mn toxicity in liver so no specific biochemical indicator is available. In this study, the relation between blood, urine and saliva Mn level and its hepatotoxic effects is evaluated.

**Materials and methods:** Blood, urine, saliva of 50 accidently selected miners collected in acid washed tubes for an experience study. Samples were used to evaluation not only biochemical parameters by pars azmoon kits but also Mn concentration by mass spectroscopy.

**Findings:** Miners Mn concentration in all samples in addition to blood AST, ALT, ALP increased significantly (P<0.001) related to controls. Miners with 10-15 years background had higher blood total, direct and indirect billirubin and ALP levels compared to others. Mn concentration in serum declined but in urine and saliva had no changes by working in mine. AST and ALT increased significantly in miners with 300µg/L serum Mn concentration. Mn concentration in various samples and serum AST&ALT level were higher in native miners than non-native but in both not related to background. Conclusion: Significantly higher levels of billirubin, AST and ALT in miners compared to controls revealed Mn hepatotoxic effects in them. Also significant ALP increasing showed cholestasis in miners that supported by AST, ALT level. Significant billirubin, AST, ALT, ALP in miners with 10-15 years background revealed the importance of this period in miners liver check up. Higher Mn levels in different sources of native miners can be due to more environmental contact. Higher AST, ALT and lower ALP level in native miners indicate more hepatastoxic and less cholestasis and therefore arthrosclerosis and Parkinson risk in these workers.

**Keywords:** Manganese, Hepatocyte, Billious tubes, Liver enzymes, Manganism

**INTRODUCTION**

Manganese is the 12th plenty element of the earth’s crust. It is also a rare and essential element in plants and animals, so that human body needs 3-9mg of it to grow properly (Santamaria, 2008; Gunnar et al., 2005). Manganese exists in the most of the body tissues like liver, pancreas, kidney and especially bones. Not only does manganese work as a cofactor for some enzymes like arginase, coline esterase, phosphoglucomutase, pyruvate carboxylase and most of phosphatases and peptidases, but also participates with vitamine B to make prothrombin. Its mineral form is used in Iron melting industries, alloys, steel, glass, dry batteries, ceramic and colors production and its organic form is used to prepare chemicals like pesticides, fuel additives, photography materials, MRI materials. To prepare Mn and make industrial use of it, should extract and inspissate the quarry (Gunnar et al., 2005; Charles and Aurelio, 1975). This operation leads to the production of dust containing Mn which can be dangerous for the health of the people who are expose to it (Bienvenu et al., 1963; Crossgrove and Zheng, 2004).

Although Mn for its less absorption and fast repelling, has less poisoning effects on human comparing with other metals and its acute poisoning is less observed, chronic entering Mn to the body more than usual amounts has some pathologic effects that are called Manganism (Lu et al., 2005; Lipe et al., 1999). Manganism is a forerunner and irreversible brain disease which its symptoms are like Parkinson (Rabin et al., 1993; Wennberg et al., 1991).

Thanks to its small dimension, Mn can pass through brain-spinal barrier and gathers in the mitochondria of
cells of some parts of the brain that controls movement actions, as their damage are predicted to be the result of oxidative effect of the free radicals produced by Mn. Because of this, the most manganism cases can be seen in the Mn miners (Wang et al., 1989; Levy and Nassetta, 2003; Bowler et al., 1999). Mn is repelled by faces, urine, milk, and sweat, but the main place to filtrate Mn is the liver (99%) so that after excretion in bile, it is finally repelled by feces (Cikrt, 1973; Davis, 1998; Malecki et al., 1996). In spite of the different researches which are done related to the repellence of Mn from body, this process is not clear yet (Zwingmann et al., 2003). Lots of studies are done about the neurotoxicity of the Mn and its effects on behavior changes (Rabin et al., 1993; Wennberg et al., 1991; Wang et al., 1989; Levy and Nassetta, 2003; Bowler et al., 1999), fertility (Charles and Aurelio, 1975; Elbetieha et al., 2001), humoral hemostasis (Soldin et al., 2007), calcium and iron uptake (Davidsson et al., 1989; Akoume et al., 2003; Amdur et al., 1957), serum lipoproteins(Bloodsworth et al., 2000; Jenkins et al., 1991; Kawano et al., 1987), lipoprotein lipase activity(26-29). In high concentrations, Mn makes some disorders on fatty acids elongation and saturation In the liver (Jenkins et al., 1991; Senturk and Oner, 1996). It also leads to increase the liver lipids peroxidation (Bloodsworth et al., 2000; Pleban and Pearson, 1979). Studies show that cholesterol accumulation in brain and serum lead to read decreasing (Senturk and Oner, 1996). On the other hand the low uptake of Mn leads to decreasing appoprotein and HDL synthesis in the liver (Senturk and Oner, 1996). Some other studies are done about the effects of Mn on it’s liver pathology but there is no specific biochemical indicator for prediction and diagnosis of Mn toxic effects yet (Cikrt, 1973).

In this research has been tried to assess the probable relation between the Mn concentration in blood, urine and saliva of miners related to hepatotoxic effects of its particles.

MATERIALS AND METHODS

Sample gathering

To do this experimental applicatory research, we selected 25 persons of miners randomly. In the morning and before working, for two successive days, workers blood, urine and saliva were collected.

At the same time we gave them a questionnaire to file, obtaining miners information such as age, background, pathologies, drugs etc. On the other hand the same samples of five health 25-40 years old men living in qom was collected and evaluated as control group. Samples were received in nitric acid and then distilled water washed glass tubes and transferred to Lab. in dry ice. Some of the prepared serum was used for biochemical parameters evaluation. The rest serum with urine and saliva samples were freezed in -20°C until used for Mn assay by atomic absorption. Liver biochemical functional indicators such as total, direct and indirect billirubin, AST and ALT were measured by autoanalyzer and PARS AZMOON kits.

Mn measurement

Mn was measured by VARIAN AA240 atomic absorption equipment (Taylor et al., 1999). Samples was thawed in Lab temperature, then for preparing a clear solution, 0.5cc serum were mixed with 1.5cc nitric acid in capped tubes and put in the microwave at 200°C for 10 minutes. Tubes were saved in 4°C. Standard curve was aligned by 0.02, 0.2, 2, 5 ppm Manganese chloride (Taylor et al., 1999; Pleban and Pearson, 1979).

Data processing; prepared information was analyzed by ANOVA test using SPSS 11.5 software.

FINDINGS

Total data obtained of this research are presented in table 1 as data average and limits. Also differential data presented in compare with normal men and native miners and miners back ground.

Mn and biochemical parameters concentration in biological fluids

According to results, no meaningful difference observed between serum total, direct and indirect billirubin concentration in control men and miners, although all values were higher in miners. Enzymes SGOT (serum glutamate oxaloacetate transaminase) (OT) and SGPT (serum glutamate pyruvate transaminase) (PT) showed meaningful (p<0.001) elevation in miners. Also serum ALP (alkaline phosphatase) level had no significant (p<0.001) difference in miners related to control (Figure 1). Liver injuries are supported by elevation of PT/OT. The higher Mn level in miners serum, saliva and urine observed in significant range (p<0.001) as was expected. Figure 1 shows this variations related to control men.

Biochemical parameters variation related to miners background

Total, indirect and specially direct billirubin elevation in serum of miners with 10-15 years background was more considerable than other miners. Also miners with 10-18 years background had higher serum alkaline phosphatase levels and miners with 13-15 years had significantly higher OT, PT and PT/OT level. Based on the results serum Mn level reduces with working in the
Table 1. Average and limits of collected datas

<table>
<thead>
<tr>
<th>Number of Workers</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean±SD 40.3958±2.4</td>
</tr>
<tr>
<td></td>
<td>Range 30 – 66</td>
</tr>
<tr>
<td>Background</td>
<td>Mean±SD 14.85714±0.73</td>
</tr>
<tr>
<td></td>
<td>Range 5 – 19</td>
</tr>
<tr>
<td>Manganese Serum(µg/L)</td>
<td>Mean±SD 307.6626±18</td>
</tr>
<tr>
<td></td>
<td>Range 105.6995 - 611.399</td>
</tr>
<tr>
<td>Manganese Saliva(µg/L)</td>
<td>Mean±SD 65.1295±5.8</td>
</tr>
<tr>
<td></td>
<td>Range 30.05181 - 67.87565</td>
</tr>
<tr>
<td>Manganese Urine(µg/L)</td>
<td>Mean±SD 45.4555±4.6</td>
</tr>
<tr>
<td></td>
<td>Range 20.72539 - 88.0829</td>
</tr>
<tr>
<td>TOTAL BILIRUBIN(mg/dL)</td>
<td>Mean±SD 0.946735±0.24</td>
</tr>
<tr>
<td></td>
<td>Range 0.4 - 2.8</td>
</tr>
<tr>
<td>DIRECT BILIRUBIN(mg/dL)</td>
<td>Mean±SD 0.26898±0.09</td>
</tr>
<tr>
<td></td>
<td>Range 0.11 – 0.53</td>
</tr>
<tr>
<td>INDIRECT BILIRUBIN(mg/dL)</td>
<td>Mean±SD 0.677143±1.5</td>
</tr>
<tr>
<td></td>
<td>Range 0.2 – 2.37</td>
</tr>
<tr>
<td>SGOT(AST) (IU/L)</td>
<td>Mean±SD 32.52083±4.4</td>
</tr>
<tr>
<td></td>
<td>Range 18 – 49</td>
</tr>
<tr>
<td>SGPT(ALT) (IU/L)</td>
<td>Mean±SD 40.58333±5.1</td>
</tr>
<tr>
<td></td>
<td>Range 14 – 97</td>
</tr>
<tr>
<td>ALT/AST</td>
<td>Mean±SD 1.212917±0.86</td>
</tr>
<tr>
<td></td>
<td>Range 0.46 – 3.13</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>Mean±SD 247.1667±35</td>
</tr>
<tr>
<td></td>
<td>Range 113 – 470</td>
</tr>
</tbody>
</table>

Figure 1. Serum, urine and saliva Mn levels of miners and normal men

mine but saliva and urine Mn level has no significant change. Also miners with 200-400 serum Mn have higher serum OT and PT levels.

**Manganese concentration and biochemical parameters differences between native and non-native miners:**

Figure 2 to 4 show native miners have higher urine and saliva and especially serum Mn levels related to non-natives but both of them have the same diagram of changes with working in the mine. OT, PT and PT/OT in native and ALP in non-native miners have higher degrees but both have the same levels of serum different billirubin forms.

**DISCUSSION**

Results showed not only the hepatopathologic effects of Mn but also cholestasis parallel to working in Mn mines. More spread hepatotoxic effects in miners with 10-15 years background and native miners on one hand and less cholestasis and therefore less artherosclerosis, nerupathologic conditions and parkinsonism in native miners on the other hand revrale the relation between Mn effects and background and living near the mine. Also
higher serum bilirubin, OT and PT levels in miners related to controls confirmed hepatopathologic effects of Mn as mentioned Davis, (1998). Higher ALP level in miners serum was similar to Davis’s results that emphasized on relationship between cholestasis and Mn accumulation in miners (Davis, 1998). Because cholestasis can lead to liver injuries in long terms, hepatotoxic effects of Mn and then high OT and PT levels are predictable.

Although various studies introduce Mn as a external inducer for lipids vacuole synthesis in hepatocytes, Roby, (1982); Kawano et al, (1987) reported hypocholesterolemia in order to lack of Mn. Amdur et al, (1957) has introduced Mn as inducer of mevalonate kinase and Benedict et al (1996) as inducer of farnesyl pyrophosphate synthetase and they mentioned these enzymes as the reasons for hypercholesterolemia after accumulation of Mn. On the other hand Zwingmann et al, (2003); Akoume et al, (2003), Jonkins and Kramer, (1991). Senturk and Oner, (1996) have shown Mn can lead to bile duct closing and inhibition of bile acids transfer to gall bladder and accumulation of them in the liver lead to hypercholesterolemia. On the other hand Senturk and Oner, (1996) suggested high cholestrol is the reason for toxic effects of Mn in brain. Also hypercholesterolemia can lead to higher artherosclerosis risk in the miners (Farrokhi). Patricia, (1979); Wang et al, (1989) sentenced normal ranges of Mn is 2-8 µg/dl in serum, 0.1-0.8 µg/dl in urine and 1.2µg/dl in CNF. As we expected and similar to Patricia results (31) Mn concentration in serum, urine and saliva of miners increased. Significant bilirubin increasing in miners with 10-15 years, ALP in 10-18 years and OT and PT in 10-15 years background as mentioned by Zwingmann et al, (2003) is in order to hepato-biliary injuries and cholestasis of Mn. Therefore hepato-biliary injuries in miners after 10 years working in Mn mines start and after 15 years reach to highest degree and then have a constant condition. Also although Mn concentration in urine and saliva had no changes, but eleated in serum with working in Mn mine. Wang et al, (1989) has stated there are no direct relation between duration of working in Mn mine and parkinsonism rate. Higher levels of Mn in native miners can be relation to long term approximation and Mn receiving by water. Although higher serum OT, PT and PT/OT levels in native miners indicate wider liver injuries in them lower ALP levels lead to lower cholestasis and artherosclerosis and therefore parkinsonism risk, a cocept the native people refer to it: native miners more resistance.

Based on results liver injuries and cholestasis are happened in miners specially after 10-15 years working and therefore not only periodically medical check up are recomended but also the place of miners working should be changed periodically. Also the evaluation of Mn effects on other organs such as kidney and bone marrow should keep in mind.

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REFERENCES


Figure 2-4: Serum, urine and saliva Mn concentration in native and non-native miners


