Serum Manganese, Serum Iron and Ferritin Concentrations among Welders of Shipyard of Arab Contractors in Ismailia City

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Abstract:
Background: Cutting and welding metal pieces in industry increase chances for welders to be exposed to manganese emitted in welding fumes. There is raising concern about the potential neurotoxic effects of manganese inhalation in welders. Interactions between manganese and iron occur and impact the toxicokinetics of iron with subsequent alteration in blood levels of iron and iron regulating proteins. **Aim:** To assess the burden of manganese exposure on serum level of manganese and on iron metabolism for early detection of manganese toxicity among welders. **Methods:** The study was carried out in the shipyard of Arab contractors in Ismailia on 30 welders and 30 office workers. Blood samples were collected for measuring serum concentration of manganese, iron and ferritin in all subjects. **Results:** Mean ± SD of serum manganese in welders and control group were 1.4 ± 1.3 µg/dl and 0.7 ± 0.6 µg/dl respectively, while mean ± SD of serum iron in welders and control were 69.5 ng/dl ± 17.5 and 77.8 ± 22.6 ng/dl respectively (P= 0.118). Serum ferritin was significantly higher among control than among welders (P = 0.000). Both serum iron and serum ferritin showed significant negative correlation with serum manganese. Multiple linear regression analysis showed that serum manganese significantly predicted serum ferritin. **Conclusion:** Exposure to welding fumes among welders disturbs serum homeostasis of manganese, iron, and the proteins associated with iron metabolism. Serum manganese may serve as a reasonable biomarker for assessment of recent exposure to airborne manganese.

Key words: welding fumes, manganese, iron, ferritin.

Introduction

The welding fume generated during the welding process are a complex mixture composed of different metals. Manganese (Mn) is present in most welding fumes [1]. Welders are known to be at risk, particularly for chronic exposure to airborne manganese, which is one of the major coating materials in welding products [2].There exposure [4,5], resulting in a rare neurodegenerative disorder clinically known as ‘‘manganism’’ a motor syndrome similar to, but partially distinguishable from idiopathic Parkinson’s disease (IPD) [6,7].

Although the precise mechanism by which Mn induces neurotoxicity is poorly understood, several reports have is raising concern about the potential neurotoxic effects of Mn inhalation among welders [3]. In particular, occupational studies have documented adverse effects on psychological and neurological function following long term exposures in adults, with progressively worse symptoms persisting long after cessation of suggested that Mn neurotoxicity may be associated with its interaction with other essential trace elements, including iron [8]), zinc, copper and aluminum [9].

Chronic exposure to Mn is associated with altered iron (Fe) concentrations in blood and in CSF, presumably due to Mn–Fe interaction at certain iron–

sulfur-containing proteins, which regulate Fe homeostasis [10]. Both Mn and Fe are transition elements adjacent to each other in the periodic table, and share similar valence charges and ionic radius. These chemical similarities allow Mn to compete directly with Fe at the molecular level [2, 8, 10]. This would have significant impact on serum concentrations of iron and certain proteins associated with iron regulation and metabolism [11].

Over the past decade, extensive research using animal models and human populations has led to several potential indicators of Mn exposure and biological effect. However; no reliable biomarkers have been established to evaluate the effect of Mn exposure because the complete scientific understanding of the mechanism of toxicity remains undiscovered [12].

SUBJECTS AND METHOD

A) General Objective: To assess the burden of manganese exposure for early detection of manganese toxicity and better prognosis among welders.

B) Specific Objectives: To assess the effect of manganese exposure on serum level of manganese and on iron metabolism. This was achieved by assessing the relation between serum manganese and serum iron and ferritin.

In this cross sectional study, the required sample size was determined by EPI-INFO program version 7, using 95% confidence level and 80% power. The mean Mn concentration ± SD of welders was 3.40 ±1.85 years while that of control group was 1.09 ± 2.58 based on previous study (11). A sample size of 23 welders, which increased to 30 welders, is required. A comparable non-exposed office worker of similar size was included in the study. Eligible participants after exclusion criteria constituted our sampling frame in each group, from which the required number of participants was selected by the simple random method for each group.

All participants were males currently employed in the Shipyard with at least 2 years duration of employment. Subjects in both groups had no reported exposure to other toxins, radiation therapy, chemotherapy or substance abuse at the time of interview. Subjects were excluded from the study if they were subjected to any dietary restrictions, special medications, which would interfere with iron metabolism, herbal medication and repeated blood transfusions or diagnosed with cardiovascular disorders, liver disease, renal dysfunctions, hematological disorders or respiratory diseases. Office workers must not be working near welding department or ever welders.

Study objectives were achieved through:

- Structured Interview Questionnaire enquired about personal data, occupational and medical history.
- Laboratory assessments: Blood samples were obtained from all participants; 10 ml blood was withdrawn from each participant cubital vein with disposable syringes, collected into coded tubes after applying disinfectant measures. The collected blood samples were allowed to clot and centrifuged at 4000g for 10 minutes. Sera were separated and stored at -20ºC until analysis [13].

Then the following tests were carried out:

- Assessment of serum manganese level by graphite furnace atomic absorption spectrophotometer [13].
- Assessment of serum iron level using Guanidine/ FerroZine method[14].

- Assessment of serum ferritin level by using ELISA method [11].

All statistical analyses were performed using the SPSS software. Descriptive statistics were applied in mean ± SD for quantitative data and number and percent for qualitative data. Student t-test, Mann-Whitney test were used for continuous variables. Statistical significance was determined at 95% level of confidence ($P < 0.05$). Receiver Operating Characteristic (ROC) curves were performed to test the predictivity of serum ferritin and manganese criterions for manganese exposure. The area under the curve (AUC) was calculated for the ROC curve in order to measure the overall accuracy of the test.

Ethical Considerations

An approval from the Arab contractors was obtained, the purpose of the study was explained to the workers and a verbal consent was obtained from them. Confidentiality and anonymity were maintained according to the regulations mandated by Research Ethics Committee of Faculty of Medicine Suez Canal University.

Results

The mean age ± SD of welders was 36.4 ± 9.2 years while that of control group was 41.00 ± 11.7 years. The difference was statistically insignificant. The mean duration of employment among welders was 12.1 ± 8.9 years and the mean weekly working hours was 45.2 ± 3.4 hours. The mean duration of employment among control group was 14.5 ± 10.6 years, and they all worked for 48 hours weekly (Table 1).

The most common type of welding was Shielded Metal Arc Welding (SMAW), other types of welding as carbon welding; gas tungsten arc welding and plasma welding were performed when needed. Welders usually work outdoors but sometimes they work in confined spaces for maintenance and repair operations. The welded materials are mild steel and stainless steel. Personal protective equipment (PPE) was used by 73.3% of welders, while 26.7 % of welders weren't using PPE. The main barrier to PPE use among welders was unavailability (87.5%) (Table 2).

This study revealed that the mean serum concentration of manganese among welders was 1.4 ± 1.3 µg/dl, more than that of control group; (0.7 ± 0.6 µg/dl) but with no statistically significant difference. Mean serum concentration of iron among welders was 69.5 ± 17.46 ng/dl, less than that of control group (77.8 ± 22.6 ng/dl) and the difference was statistically non-significant. Furthermore the present study revealed that the mean serum concentration of ferritin of the studied welders was 54.2 ± 21.0 ng/dl less than that of the control group which was 86.06 ± 34.3 ng/dl) with high statistical significant difference between the two groups ($p < 0.001$) (Table 3).

Weak negative correlation was found between serum level of manganese and serum level of iron and this was statistically significant ($p = 0.034$), while moderate negative correlation was found between serum level of manganese and serum level of ferritin and this $p < 0.001$ was statistically significant ($p < 0.001$). Weak positive correlation was found between serum level of ferritin and serum level of iron which was statistically insignificant ($p = 0.056$) (Table 4 and figure 1).

Multiple linear regression was conducted to investigate the best predictor of serum concentration of ferritin level. It was noted that occupation, serum level of manganese and serum level of iron significantly predict serum concentration of ferritin level ($p < 0.001$). The adjusted R^2 value was 0.30 this means that 30 % of the variance of serum concentration of ferritin level was explained by the model (table 5).

ROC curves were performed to test the predictivity of serum ferritin (figure 2) and manganese (figure 3) criterions for manganese exposure. In comparison of ROC curves of serum manganese and ferritin for manganese exposure prediction, the overall diagnostic performance of serum ferritin was significantly better than serum manganese (p = 0.025), reflected by a larger AUC in serum ferritin compared to serum manganese (78.7% versus 62.0% respectively), difference between areas was found to be 16.7% (p = 0.025) (table 6 and figure 4).

Discussion

Results of this study support the hypothesis that exposure to manganese in welding fumes is associated with alterations in serum concentrations of manganese, iron, and ferritin. The current study indicated that the mean serum concentration of manganese among welders was 1.4±1.3 µg/dl, more than that of the control group (0.7±0.6 µg/dl) but with no statistically significant difference between the two groups (p = 0.109). The relatively elevated Mn levels among control group (normal serum Mn concentration is <0.5 µg/dl) could stem from the fact that they actually don't stay inside their offices all the time, instead, they keep wandering outside among their colleagues beside or even inside the shipyard workshops. In addition, they could be exposed to environmental pollution which could be attributed to the relatively short distances between their offices and the welding workshops (ranging from 28 to 68 m).

The current finding is in agreement with a study conducted by Abd-Elhady (2009) [15] in one of the private factories of iron and steel industry of the tenth of Ramadan city, Egypt. The study was conducted on 50 welders and 30 work officers and revealed that the mean serum level of manganese among welders was 3.92±2.30 µg/dl, more than that among control group; (2.98±1.08 µg/dl), with no statistically significant difference between the two groups (p > 0.05). In the contrary, study conducted in China by Lu et al. (2005) [11] on 97 welders and 91 controls to investigate serum manganese concentrations among welders and controls revealed statistically significant difference (higher concentration of manganese among welders than controls (3.40±1.85 µg/dl and 1.09± 2.58 µg/dl respectively, p < 0.001). This discrepancy may stem from the different industrial process in the study as it was carried out on welders of vehicle factory characterized by its intensive, day-to-day indoor welding practice in the production of vehicles.

The current study revealed that the mean serum concentration of iron among welders was 69.5 ±17.46 ng/dl, less than that of control group which was 77.8±22.6 ng/dl with no statistically significant difference between the two groups (p = 0.118). Smith and Ahmed (2013) (16) found strong relation between blood manganese and iron deficiency (r= 0.182) while at the present study, this relation showed weak negative correlation (r = - 0.274) which was statistically significant (p = 0.034). Considering the above mentioned studies, shows that exposure to manganese, have had effects on the factors related to blood iron.

Furthermore the present study revealed that the mean serum level of ferritin among welders was 54.2±21.0 ng/ml less than that among control group which was 86.06±34.3 ng/ml with statistically significant difference between the two groups ($p < 0.001$). Significant moderate negative correlation ($r = -0.478$) was found between serum concentration of manganese and serum ferritin ($p < 0.001$).

These observations were in agreement with the findings of Cowan et al., (2009) [17] who conducted a study in China on 323 participants classified as control (n = 106), power distributing and office workers (n = 122), and manganese exposed ferroalloy smelter workers (n = 95) and found that iron concentration in plasma and erythrocytes was significantly lower in Mn-exposed workers than in controls.

This is also supported by Ellingsen and his group (2003) [18] who stated that Mn overexposure causes a decrease in blood Fe content due to the up-regulation of transferrin and the resultant compartmental shift of Fe from the blood to the CSF.

The present findings corroborate the findings of Wang et al., (2006) [19] who showed that Mn exposure alters iron homeostasis by increasing the transcription and translation of transferrin and Divalent metal transporter-1 (DMT-1) iron transporters creating a net influx of Fe from blood into CSF with a resultant decrease of blood Fe content.

Dysfunctional iron metabolism has been seen in manganism patients. Serum parameters associated with iron metabolism, such as ferritin, transferrin, and total-iron-binding capacity are significantly altered. The excess accumulation of Fe in neurons may consequently produce the cellular oxidative stress that leads to neuronal damage [20, 21]. High levels of total iron, decreased ferritin, iron-associated oxidative stress, and abnormal mitochondrial complex-I have been repeatedly reported in the postmortem substantia nigra of manganism patients [22].

This alteration of Fe metabolism following overexposure to Mn is linked to neurodegenerative disease and has been observed *in vivo* and in human epidemiological studies [5, 10, 19].

Noticeably, the current results disagree with data suggested by Lu et al., (2005) [11] which revealed that welders had significantly higher serum levels of iron as compared to control subjects. They explained their results by that the altered serum iron levels among welders could be attributable to co-exposure to airborne iron during welding and thus inhalation of iron oxide emitted in welding fumes may lead to a higher serum iron concentration among their welders. When the serum iron concentrations were plotted against serum manganese concentrations, no significant correlation between these two metals was observed ($r = 0.36$, $p = 0.362$), suggesting that variations in serum iron appear to be independent of serum manganese level. Lu et al. (2005) [11], also found that serum ferritin levels among welders were (202.7 ±111.5) insignificantly higher than that of the controls (188.4 ±104.4) ($p= 0.468$) with no observed significant correlation between the serum concentrations of manganese and ferritin.

Simultaneous multiple regression analysis was conducted to investigate the best predictor of serum concentration of ferritin. Results revealed that occupation, serum concentration of manganese, and serum concentration of iron, significantly predict serum concentration of ferritin level when all five variables (age, occupation, duration of employment, serum manganese level µg/dl, and serum iron level µg/dl) were included. The adjusted R^2 value was 0.30 this means that 30 % of the variance of serum concentration of ferritin level was explained by the model.

CONCLUSION

Exposure to welding fumes among welders disturbs serum homeostasis of manganese, iron, and the proteins associated with iron metabolism. There was significant weak negative correlation between serum manganese level and serum iron level and moderate negative correlation between serum manganese level and serum ferritin. Serum manganese may serve as a biomarker for assessment of exposure to airborne manganese.

Recommendations

Environment levels of manganese fumes and other heavy metals should be monitored. The applied control measures should be assessed. Accurate medical evaluation of nervous system and respiratory system as well as biological monitoring of blood levels of manganese among welders should be assessed regularly.

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Table 1. Distribution of the study groups according to age & working parameters.

	Welders (n=30) mean ± SD	**Controls** (n=30) mean ± SD	**Statistical test**	**p value**
Age (years)	36.4 ± 9.2	41.0 ± 11.7	-1.731[a]	0.093*
Duration of employment (years)	12.1 ± 8.9	14.5 ± 10.6	390.5[b]	0.378
Working hours /weak	45.2 ± 3.4	48 ± 0.0	255.0[b]	0.000*

[a] Student t-test
[b] Mann-whitney test (Non parametric Data)
* Statistically significant at p-value < 0.05.

Table 2. Description of welding process among welders (n = 30)

<table>
<tr><td colspan="4">Description</td><td>N</td><td>%</td></tr>
<tr><td rowspan="2">Welding types</td><td colspan="3">Shielded Metal Arc Welding</td><td>24</td><td>80</td></tr>
<tr><td colspan="3">Other types</td><td>6</td><td>20</td></tr>
<tr><td rowspan="4">Use of PPE</td><td rowspan="2">Yes</td><td rowspan="2">22(73.3%)</td><td>Full shielded mask face</td><td>14</td><td>63.6</td></tr>
<tr><td>Mask</td><td>8</td><td>36.4</td></tr>
<tr><td rowspan="2">No</td><td rowspan="2">8 (26.7 %)</td><td>Not available</td><td>7</td><td>87.5</td></tr>
<tr><td>Not comfortable</td><td>1</td><td>12.5</td></tr>
</table>

Table 3. Serum concentrations of Manganese, Iron and Ferritin among welders and controls.

		Welders n = 30	Controls n = 30	Statistical test	p value
S. Manganese (μg/dl)	Mean ± SD	1.4 ± 1.3	0.7 ± 0.6	U= 342.0[b]	0.109
	Range	0.2 - 4.6	0.1-2.5		
S. Iron (ng/dl)	Mean ± SD	69.5 ± 17.5	77.8 ± 22.6	t = -1.586[a]	0.118
	Range	36 - 101	42-142		
S. Ferritin (ng/ml)	Mean ± SD	54.2 ± 21.0	86.1 ± 34.3	t = 4.414[a]	0.000*
	Range	27 - 115	35-170		

[a] Student t-test
[b] Mann-whitney test (Non parametric Data)
* Statistically significant at p-value < 0.05.

Table 4. Correlation of serum level of manganese (μg/dl), ferritin (ng/ml) and iron (μg/dl) among the studied groups (n=60).

	Manganese level μg/dl	Ferritin level ng/ml
Iron level ng/dl	-0.274*	0.248
Ferritin level ng/ml	- 0.478**	

* Statistically significant at p- value <0.05 ** p-value < 0.01

Table 5. Multiple regression analysis for the predictor variables of serum concentration of ferritin ng/ml (n=60)

	Coefficient	Adjusted OR	(95 % CI)	p-value
Constant	58.7		16.1 - 101.2	0.008
Occupation	26.4	0.408	10.9 - 41.8	0.001*
Serum manganese µg/dl	- 8.3	- 0.274	- 15.5 - - 0.9	0.027*
Duration of work				0.754
Serum iron ng/dl				0.266
Age				0.668

Note: R^2= 0.300, F (5.00) = 6.048, p < 0.001

Table 6. Comparison of ROC Curves of serum Manganese & Ferritin for Manganese exposure prediction

	AUC %	95% CI	Difference between areas	*p* - value
Mn	62.0	0.485 - 0.742	16.7%	0.025 *
Ft	78.7	0.662 - 0.882		

*Statistically Significant at p-value < 0.05

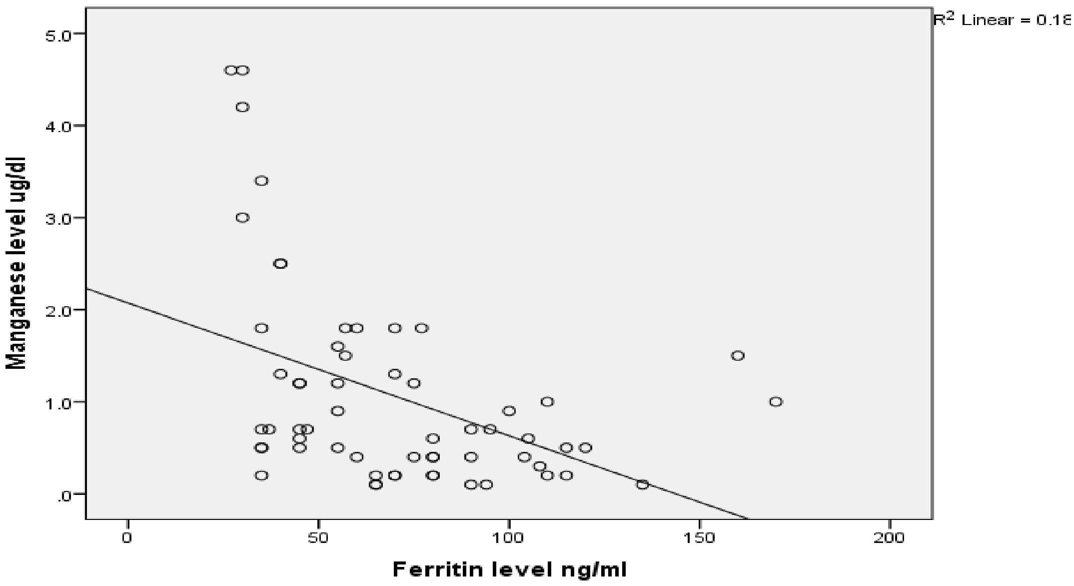

Figure 1. Shows moderate negative correlation between level of serum manganese and serum ferritin level of the studied groups (n=60) (r = -0.478, p = 0.000).

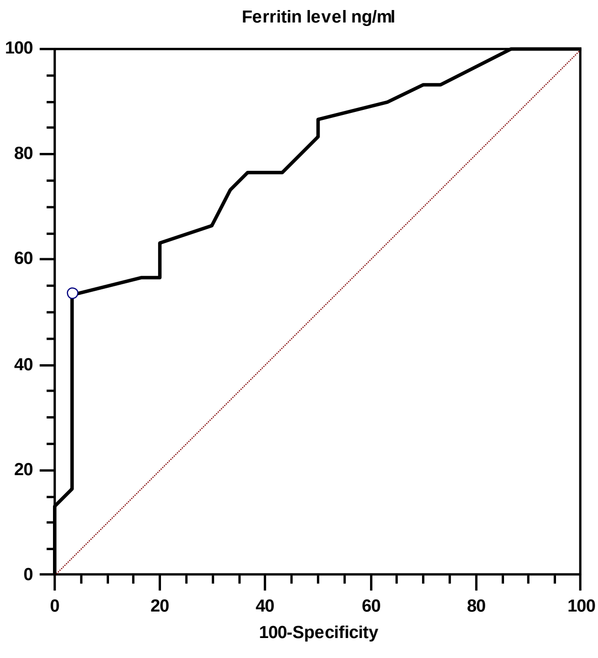

Figure 2. ROC Curve of serum ferritin criterions for Manganese exposure prediction; Area under the curve (AUC) = 78.7% (*p*<0.001)**

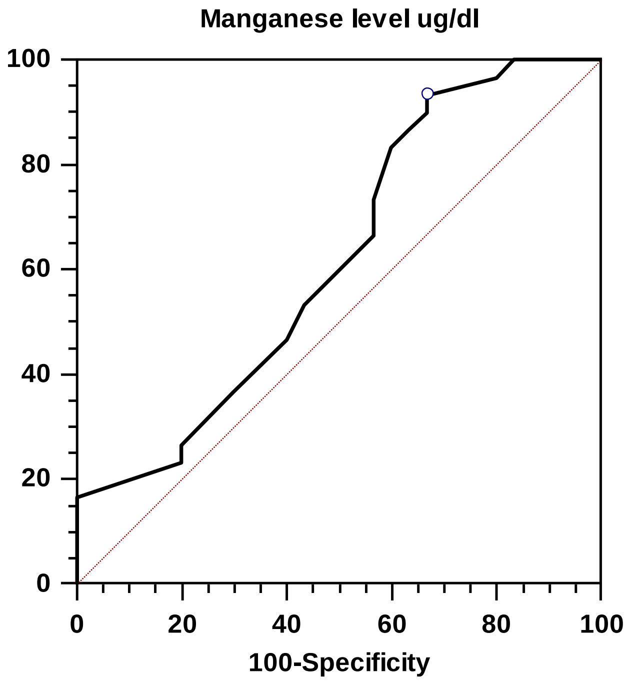

Figure 3. **ROC Curve of serum Manganese criterions for Manganese exposure prediction;Area under the curve (AUC) = 62.0% (*p* =0.101)**

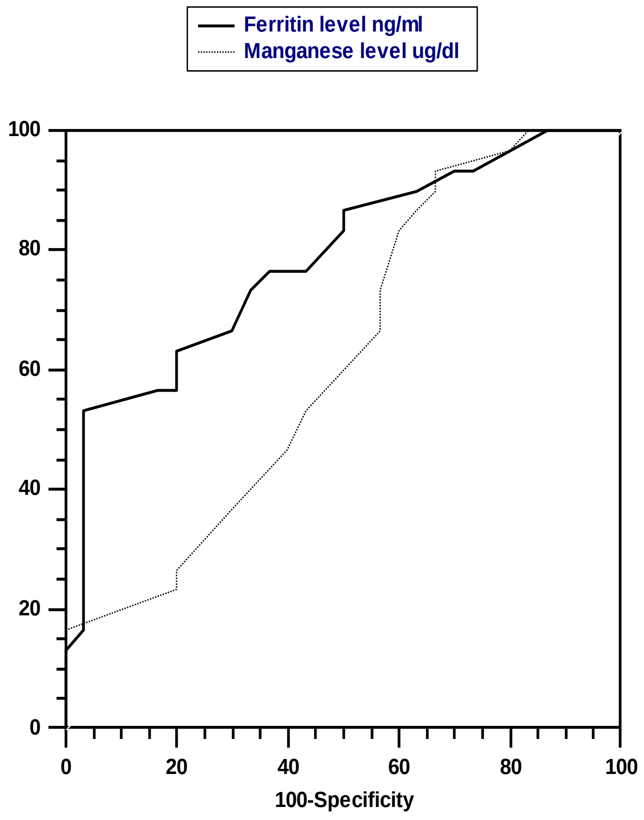

Figure 4. Comparison of ROC Curves of serum manganese & ferritin for manganese exposure prediction