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The association between PTX3 and serum manganese levels of welders in comparison with controls: An application of anti-inflammatory biomarker

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ABSTRACT

Aim: The purpose of this study was to compare serum PTX3 levels of manganese-exposed welders with non-exposed controls to evaluate the nature of the manganese-induced inflammatory response.

Material and Method: Overall, we collected 103 research samples (Mn-Exposed Welders Group:51 and Non-Exposed Controls: 52). PTX-3 levels were analyzed in the serum samples by the ELISA method, while Mn levels in whole blood specimens were quantified by the inductively coupled plasma (ICP-MS) method.

Results: The mean values of manganese and Pentraxin-3 of the control group were found to be significantly lower than those of the exposure group (Mn levels: 5.04±2.32 µg/L vs. 11.54±3.09 µg/L; PTX-3: 36.96±24.20 ng/mL vs. 48.29±27.13 ng/mL; $p<0.05$).

Conclusion: This hypothetical and observational investigation highlighted the relationship between Mn levels and PTX-3 for the first time.

Keywords: Manganese, exposure, pentraxin-3, inflammation

INTRODUCTION

Welding fumes contain Manganese (Mn), which may lead to Mn accumulation responsible for adverse effects in the central nervous system. Prolonged exposure often results in a Parkinson-like syndrome, called manganism (1). Generally, Mn exposures and ensuing toxicities occur in a variety of environmental mediums, dietary sources, contaminated food, infant formula, and natural or artificial contaminations in water, soil, and air (2). Mn is a solid and silvery metal primarily used as an industrial alloy, especially with iron in stainless steel (1). It is also an essential element found in bones predominantly. Mn is functional in bone formation, nutrient metabolism, and antioxidant defense system (3,4). Mn plays an important role as a cofactor in several enzymes and numerous vital processes, including nerve and brain development and cognitive functioning (5). Overexposure to Mn causes toxicity on the central nervous system and affects motor activities disrupting dopaminergic functions (6,7). In the brain, the target sites of Mn are the striatum, globus pallidus, and substantia nigra (6,8). People exposed to manganese may develop clinical signs and symptoms resembling Parkinson's disease (2).

One of the mechanisms that play a role in manganese toxicity is the increase in reactive oxygen species and, in turn, increased oxidation (9). N-acetylcysteine, an antioxidant, is known to protect the striatum, hippocampus, and hindbrain against Mn toxicity (10).

Pentraxin-3 (PTX3) from the pentaxin family, mainly produced by endothelial and dendritic cells, fibroblasts, and macrophages, is an essential acute-phase protein in initiating the innate immune response (11). PTX3 is known to be responsible for the pathogenesis, exacerbation, or control of many diseases, including

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psoriasis, juvenile idiopathic arthritis, and inflammatory rheumatic diseases (12–14). It was previously reported that Mn might have cardioprotective and atheroprotective properties in cardiovascular diseases and be a related marker in acute and chronic kidney injury as it correlates with the severity of the damage (14,15).

Ultimately, the objective and novelty of this paper were based on a comparison of serum PTX3 levels of Mn-exposed welders with those of non-exposed controls to assess the nature of Mn-induced inflammatory response for the first time.

MATERIAL AND METHOD

This study included 51 manganese exposed welders and 52 controls with no history of toxic metal exposure, including Mn. We extracted all sociodemographic characteristics, including alcohol consumption, smoking, and employment history. Yet, we had to exclude twelve subjects having coronary vascular disease, hypertension, diabetes mellitus, accrue upper respiratory tract infection, and cancer. All procedures followed the 1964 Helsinki Declaration and its later amendments. All subjects in the control group were healthy with no acute and/or chronic disease and selected among non-smokers and alcohol users. This study was conducted with the approval of Ankara Keçiören Training and Research Hospital Ethics Committee (Decision No:22.02.2012- B.10.4.ISM.4.06.68.49).

Collection of Blood Samples for PTX-3 Analysis

We analyzed PTX-3 levels in serum samples obtained with centrifugation of blood samples at 1500 rpm for 10 mins and transferred to 2 mL Eppendorf tubes to be saved at -20°C until analyses. The corresponding ELISA kit was used for PTX-3 assays, and samples were prepared according to the manufacturer's kit instructions. We then placed the samples on microplates and analyzed them using a CLARIOstar ELISA plate reader. (BMG LABTECH, Ortenberg, Germany). The wavelength was set at 450 nm. We found the r2 value of the calibration curve to be 0.9996.

Collection of Blood Samples for Mn Analysis

For Mn analysis, we put 1 mL of whole blood sample in Teflon tubes. Then, 5 mL of 65% nitric acid and 5 mL of ultrapure water were added to the tubes and resolved in a Milestone microwave digestion unit. The digested samples were then transferred to 50 mL polypropylene tubes with ultrapure water to obtain the total volume of 20 mL and stored at +4°C until analysis (16). We determined Mn levels with inductively coupled plasma mass spectrometry (ICP-MS). The operating parameters of ICP-MS were set as follows: RF power=1550 W, nebulizer gas=0.90 L/min, plasma gas=0.80 L/min, nebulizer pressure=3.1 bar, dwell time=0.01, and spray chamber temperature=3.3°C. We washed the sampler probe between injections by rinsing with ultrapure water for 30 s, followed by washing with 2% HNO_3 for 45 s and rinsing with ultrapure water for 45 s. Afterward, the instrument automatically ran the next sample. The r2 value of the calibration curve was 0.9999, and the interval of the calibration was set at 0.1–1000 µg/L manganese. We repeated the sample and standard of measurements three times and performed method validations using CRM Seronorm™

We ran Seronorm Whole Blood L-2 CRM 5 times on the same day and different days. Moreover, we used the average of the repeated measurements to validate the method whereby the relative standard deviation (RSD) of the values did not exceed 5%. We found the coefficient of variation (CV) and recovery to be 2.92% and 101.12%, respectively. On the other hand, the ICP-MS method for Mn analysis provided the limit of detection (LOD) and lowest limit of quantification (LOQ) equal to 0.029 and 0.146, respectively.

Statistical Analysis

We utilized the SPSS 20.0 software in statistical analyses. The Kolmogorov Smirnov test was performed to check whether the parameters showed a normal distribution. We found the data distributed normally; therefore, we used parametric tests. We presented continuous variables as mean±standard deviation. The differences between the groups were evaluated using the T-Test, while we deployed Pearson's correlation analysis to explore the relations between the variables.

RESULTS

In this study, we investigated a total of 103 employees, 51 of whom were exposed to manganese and 52 were volunteers working in the same workplace. **Table 1** presents the relationships between the main parameters and groups. Mean ages were found to be 35 and 39 years for the control and Mn-exposed workers, respectively. However, there was no difference between the exposed and control groups by age ($p>0.05$). The mean values of manganese and Pentraxin-3 of the control group were found to be significantly lower than those of the exposure group, respectively (Mn levels: 5.04±2.32 µg/L vs. 11.54±3.09 µg/L; PTX-3: 36.96±24.20 ng/mL vs. 48.29±27.13 ng/mL; $p<0.05$) (**Table 1**).

Table 2 shows the correlations between the continuous variables. Accordingly, we found a negative correlation between manganese and WBC levels ($r= -0.215$; $p<0.05$) (**Figure 2**), while manganese was positively correlated with Pentraxin-3 levels ($r=0.202$; $p<0.05$) (**Figure 1**). Finally, we reached a negative relationship between age and Pentraxin-3 levels ($r= -0.247$; $p<0.05$). (**Table 2**).

Table 1. The relationships between the main parameters and groups (n=103).

	Groups	N	Mean	SD	t	p
Mn levels (µg/L)	Control	52	5.04	2.32	12.101	<0.001**
	Mn-Exposed	51	11.54	3.09		
Age (years)	Control	52	35.42	8.86	1.988	0.06
	Mn-Exposed	51	39.18	10.24		
BMI (kg/m^2)	Control	52	27.10	3.02	0.396	0.693
	Mn-Exposed	51	27.32	2.57		
WBC (µl/ml)	Control	52	7.95	1.90	1.389	0.168
	Mn-Exposed	51	7.45	1.75		
HGB (g/dL)	Control	52	15.31	1.49	0.336	0.738
	Mn-Exposed	51	15.22	1.39		
HCT (%)	Control	52	45.47	3.78	0.381	0.704
	Mn-Exposed	51	45.73	3.29		
PLT ($10^3/\mu L$)	Control	52	242.67	59.85	1.275	0.205
	Mn-Exposed	51	228.65	51.37		
ALT (IU/L)	Control	52	25.21	14.58	0.24	0.811
	Mn-Exposed	51	25.92	15.41		
AST (IU/L)	Control	52	20.40	5.65	0.01	0.992
	Mn-Exposed	51	20.39	5.81		
Pentraxin-3 (ng/mL)	Control	52	36.96	24.20	2.237	0.027*
	Mn-Exposed	51	48.29	27.13		

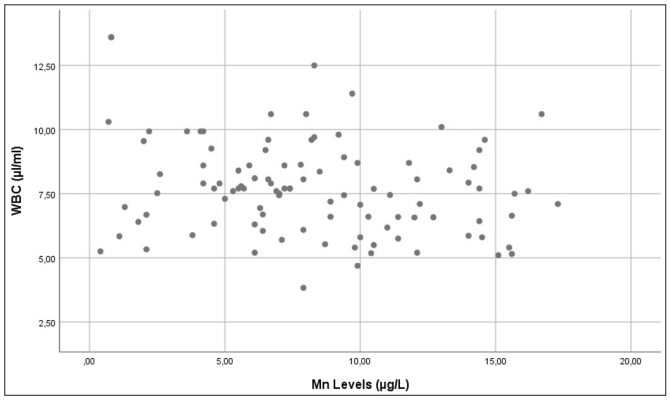

Figure 2. The relationships between Mn and WBC levels.

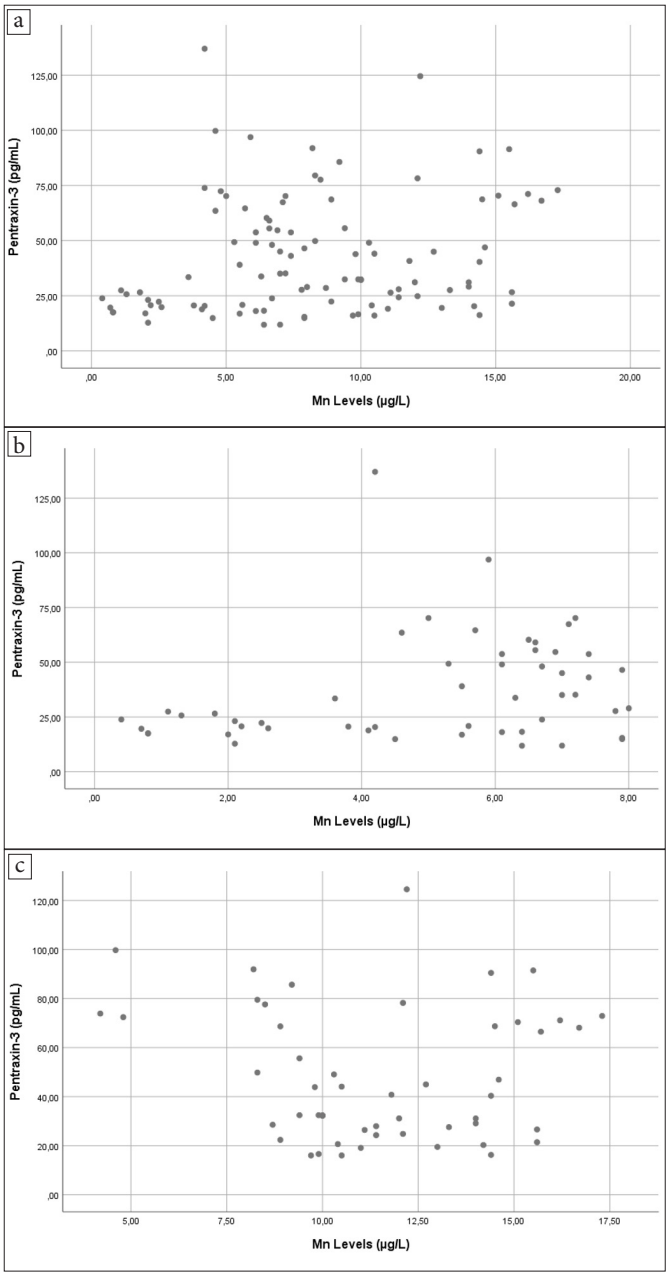

Figure 1. The relationships between Mn and PTX-3 levels (**a:** all groups; **b:** control group; **c:** Mn-Exposed group).

Table 2. Pearson correlations of continuous variables

	Mn levels	Age	BMI	WBC	HGB	HCT	PLT	ALT	AST
Age	0.165	1							
BMI	0.136	.349**	1						
WBC	-.215*	-0.055	-0.186	1					
HGB	0.096	-0.009	-0.029	0.075	1				
HCT	0.089	-0.063	-0.075	0.078	.882**	1			
PLT	-0.139	0.054	-0.104	.275**	-0.01	-0.179	1		
ALT	-0.048	-0.117	0.145	0.024	.209*	.229*	0.165	1	
AST	-0.069	-0.083	0.106	0.004	0.157	0.145	0.133	.710**	1
Pentraxin-3	.202*	-.247*	-0.165	0.029	-0.156	-0.048	0.065	-0.043	-0.139

*p<0.05; **p<0.01

DISCUSSION

In this study, we investigated the relationship between manganese exposure and the inflammatory biomarker pentraxin 3 (PTX-3). The results revealed that increased manganese level was associated with increased PTX-3 levels. Biological screening in toxicological research is important for the assessment of the risk of metal exposure for human health. Metal exposure in humans was previously found to be associated with adverse health effects such as neurological and cardiovascular harms, diabetes mellitus, skin lesions, and skin, lung, kidney, and liver cancers (6,16,17–22).

Studies showed that manganese triggers inflammatory processes through various pathways and shows neurotoxic effects. In a study examining neurotransmitter and metabolite levels after manganese exposure in the mouse brain, the researchers found increased dopamine, DOPAC (3,4-dihydroxyphenylacetic acid), and homovanillic acid (HVA) levels in the striatum (23). In another study, increases in norepinephrine and serotonin levels were observed in addition to these neurotransmitters (24). In a study on cell culture, exposure to manganese was shown to play an important role in the development of manganese-induced oxidative stress, inflammation, and apoptosis in microglia with the LRRK2 molecule. In the present study, the effect on inflammation was realized by the increase in manganese-induced TNF-α production (25).

Although the liver is the second most crucial storage organ following the brain, manganese-related hepatotoxicity in the liver has not been extensively studied so far (2). In a study with mice, hepatic accumulation was observed after manganese exposure, but no histopathological damage was observed (26). In our study, we could not find a difference in liver enzyme levels between the groups. On the other hand, like hepatotoxic effects, renal toxic effects of manganese have not been appropriately investigated. In a study conducted with predialysis patients, chronic renal failure was found to be associated with increased levels of manganese (27). However, in our study, there was no association between manganese levels and renal function tests.

We could not find a study examining the relationship between PTX-3 and manganese exposure in the literature. Yet, studies show that pentraxin 3 is related to neuroinflammatory processes in the brain (28). The correlation between manganese elevation and PTX-3 in our study suggests that a pathological process may progress through the PTX-3 molecule in the toxic effects of manganese in the brain.

Limitations

The lack of clinical endpoints is one of the most important limitations of our study. However, the observational nature of the research and the absence of a temporal relationship may have prevented the release of a causal relationship between the variables. Since blood or urine manganese levels are not clinically correlated, separating groups according to manganese levels may not coincide with clinical outcomes.

CONCLUSION

In conclusion, we suggested the relationship between manganese levels and PTX-3 for the first time in this hypothetical observational study. Accordingly, this study highlights the need for further studies to investigate the pathophysiology of both neurotoxic and other systemic toxic effects of manganese. Also, it is needed to perform animal or human studies with clinical outcomes that may provide evidence of causality.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was conducted with the approval of Ankara Keçiören Training and Research Hospital Ethics Committee (Decision No:22.02.2012- B.10.4.ISM.4.06.68.49).

Referee Evaluation Process: Externally peer-reviewed.

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