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GENETIC SUSCEPTIBILITY AND HEALTH EFFECTS OF OCCUPATIONAL EXPOSURE TO NITROAROMATIC COMPOUNDS IN AMMUNITION INDUSTRY

By

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Abstract

Introduction: Nitroaromatic compounds have been used in multiple applications and are the main constituent of explosives in ammunition industry. **Aim of work:** To evaluate health hazards among workers exposed to nitro aromatic compounds in the ammunition industry, and the possible role of oxidative stress in its mechanism of action. **Materials and methods:** The study was conducted on 40 workers as an exposed group comprising all workers engaged in ammunition factory in Helwan area, South of Cairo. The study also involved a control group composed of 40 workers never occupationally exposed to nitroaromatic compounds. All participants were subjected to a detailed specially prepared occupational and medical history questionnaire with full clinical examination, slit lamp examination and ECG scanning. Laboratory investigations were done and included: liver and kidney functions (ALT, AST, Bilirubin, blood urea, Serum Creatinine) complete blood picture, serum 8 hydroxyguanosine level and blood lead level. **Results:** Clinical examination revealed higher frequency of anorexia, nausea and vomiting, metallic taste, flushing, work accidents, cataract, diminution of vision, contact dermatitis, skin discoloration, bronchial hypersensitivity, reproductive troubles, cardiac ischemia, headache and hypertension among exposed workers compared to control group. Among carried investigations exposed workers showed elevation in mean blood urea while decrease in the mean level of ALT and AST compared to the control group. Mean serum 8 hydroxyguanosine level showed a highly significant statistical elevation in ammunition workers (706.72±355.84 U/ml) compared to their referent control group considerable adverse health effects including cardiac, respiratory, ocular, dermatological and reproductive effects. The highly significant elevation of the mean level of serum 8 OHG in the exposed workers compared to the control group identifies that oxidative stress is a possible mechanism for adverse effects in ammunition workers. **Conclusion and recommendations:** Ocular, dermal, respiratory, haematological, reproductive and cardiovascular clinical manifestations are to be properly assessed in pre-employment and periodic medical examination. Risk evaluation and management of explosive hazards is very important in ammunition industry. The possible utility of 8 hydroxyguanosine serum level as a marker of DNA oxidative damage may be considered. Workers supplementation with antioxidant medication is highly recommended.

Key words: Ammunition workers, Nitroaromatic health hazards, TNT (trinitrotoluene) and 8hydroxyguanosine.

Introduction

Nitroaromatic compounds constitute a large group of chemicals that are characterized by the presence of one or more nitro groups. Nitroaromatic compounds have been used in multiple applications as pharmaceuticals, antimicrobial agents, food additives, pesticides, explosives, dyes and raw materials in several industrial processes (De Oliveira et al., 2010).

Trinitrotolune (TNT) is the main chemical substance found on the ammunition production line because of its low melting point, its stability and low sensitivity to impact friction or high temperature (Preklang and Chantanakul, 2012). During the course of war when very large amounts of high explosives were consumed, primarily TNT. Thousands of TNT poisoning cases occurred with hundreds of mortalities (Bodeau, 1993). Most workplace exposure results from breathing TNT dust or vapor and contact with dust on the skin (ASTDR, 1995). Ingestion of food handled with contaminated hands or dust is another source of toxicity at the work place (Padda et al., 2003).

In factory workers, exposure to nitroaromatic compounds has been linked to many adverse health effects, including aplastic anemia, toxic hepatitis, cataracts, hepatomegaly, and liver cancer. Indeed the primary targets of ammunition nitrocompounds toxicity include the hematopoietic system, the cardiovascular system (CVS), the nervous system (muscular weakness, headache, dizziness, nausea, insomnia, and tingling pains in the extremities) and the reproductive system (reduction of sperm counts, alteration of sperm morphology, and aspermatogenesis) (ATSDR, 1995).

The U.S. Environmental Protection Agency (EPA) classified TNT as a possible human carcinogen (EPA, 2014 and Mallon et al., 2014). Indeed a significantly increased number of chromosomal aberrations (stable and unstable aberrations) were detected in workers exposed to TNT (Verdorfer et al., 2001).

8 hydroxyguanosine (8 OHdG) is the form of oxidized guanine. It is the most commonly studied critical biomarker of oxidative DNA damage (Rossnerova et al., 2011 and Fan et al., 2012).

It is assumed that ammunition nitroaromatic compounds mechanism of action is through the formation of reactive oxygen species (ROS) (ATSDR, 1995).

Aim of work

To evaluate health hazards among workers exposed to nitro aromatic compounds in the ammunition industry, and the possible role of oxidative stress in its mechanism of action .

Materials and methods

- **Study design:** Case control study.
- **Place and duration of the study:** this work has been conducted in in a military ammunition factory in Helwan area, South of Cairo from June 2014 to Jan 2016.
- **Study sample:** The exposed group comprised all 40 morning-shift workers (whole population) directly exposed to nitroaromatic compounds through engagement in ammunition production and maintenance line and fulfilling eligibility for inclusion in the study. The control group included 40 workers of both sex also randomly selected from the administration department in Kasr Al-Ainy hospital with no history of nitroaromatic compounds exposure but of comparable age, sex, and socioeconomic status to exposed group.

Inclusion criteria:

All workers exposed to nitroaromatic compounds in a military ammunition factory for at least the preceding 5 years.

Exclusion criteria for both groups were workers with current or treated viral hepatitis/ schistosomiasis, medical conditions as uncontrolled diabetes, alcohol consumption, on regular medication with nitrates, antioxidants supplements.

The studied groups were subjected to the following:

1-A self designed questionnaire about general health status, exposure history, smoking and alcohol consuming habits, previous medical record, and present symptoms

2. Clinical examination

- Vital signs (as blood pressure, pulse and respiratory rate),
- Ocular examination (Visual acuity and slit lamp examination for cataract),
- Cutaneous examination for discoloration and contact dermatitis,
- Cardiac, Chest, Abdominal, and Neurological examination.

3. Electro Cardio Gram (ECG) was done to detect arrhythmias and cardiac ischemia

4. Laboratory investigations

10cc of venous blood was taken from each subject through a venipuncture using a dry plastic syringe under aseptic conditions. The sample was then divided into three clean tubes; the first one contained EDTA for CBC, the second was heparinized for lead analysis, while the last was dry not containing any chemicals for the rest of investigations. All samples were transported in an ice box to the laboratory.

I. Blood lead level:

Tubes transported to the atomic absorption laboratory in the Occupational and Environmental Medicine Department at Kasr Al-Ainy Hospital for lead measurement. We used the flame atomic absorption spectrophotometry (model M6). Blood sample is taken from personnel on heparin, and then the RBCs are haemolysed using nitric acid to extract lead.2 standard solutions are prepared of concentrations 7 and 9 mg/L. A curve is drawn between 3 points of measurements of blank mineral water and the 2 standard settled. Samples are plotted on this curve and results are obtained (WHO, 2011).

II. Complete blood picture:

Complete blood count was performed by an automated analyser (cell-Dyn 1700). It has two main sensors, which were used, are light detectors and electrical impedance (Brown, 1993).

III. Kidney function tests:

Blood urea: by the Sclavo diagnostic method (Tietz, 1995). Serum creatinine: by Modified Jaffè's Kinetic method (Henry, 1974).

IV. Liver enzymes:

Test allows in vitro quantitative determination of human AST and ALT concentrations in serum employing sandwich enzyme-linked immune sorbent assay (ELISA) using antibodies and color changes. Here AST and ALT acts as antigen.

V. Human 8-OHdG Elisa kit:

ELISA kit 8-OHdG applies the quantitative sandwich enzyme immunoassay technique. The microtiter plate has been pre-coated with a monoclonal antibody specific for 8-OHdG. Standards or samples are then added to the microtiter plate wells and 8-OHdG if present, will bind to the antibody pre-coated wells (Witherel et al., 1998). In order to quantitatively determine the amount of 8-OHdG present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for 8-OHdG are added to each well to "sandwich" the 8-OHdG immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solutions are added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain 8-OHdG and enzyme-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm.

Consent

Authors declare that a verbal consent was taken from the studied group, confidentiality was maintained.

Ethical approval

The study protocol was approved by

the ethical committee of the Department of Occupational and Environmental Medicine, Faculty of Medicine, Cairo University.

Data management

Data was coded and entered using the statistical package SPSS version 15. Data was summarized using number and percent for qualitative data, Mean ± SD for quantitative data which were normally distributed, while median and interquartile range for quantitative data which is not normally distributed. Comparison between groups were done using Chi-square test for qualitative data, independent simple t-test for quantitative data which are normally distributed, while non parametrical Mann-Whitney test was used for quantitative data which were not normally distributed. Multivariate linear regression analysis was done to test for significant predictors of 8 OHG. P-value less than 0.05 and less than 0.001were considered statistically significant and highly significant, respectively.

Results

The mean age of the exposed group was 51.68 years (±3.67) and ranged (44-58). This matched the control group with mean age 51.6 years (±4.68) and ranged (45-59). The mean duration of work and exposure to nitroaromatic compounds in exposed workers was 31.55 years (±2. 68) and ranged (25 – 37). No significant statistical difference was found between the exposed and control groups as regards age, sex and smoking.

Table 1: Clinical manifestations among exposed and control groups.

		Exposed No=40		Control No=40		X^2	p
		N	%	N	%		
a. General	**Anorexia, Nausea, Vomiting**	8	20	0	0	8.889	<0.005*
	Metallic taste	18	45	0	0	23.226	<0.001**
	Bony aches	14	35	12	30	0.228	>0.05
	Flushing	18	45	6	15	8.571	<0.005*
	Memory troubles	11	27.5	11	27.5	0.000	>0.05
	Easy fatigability	12	30	15	37.5	0.503	>0.05
b. Work accidents		9	22.5	0	0	10.141	<0.005*
c. Eye	**Cataract**	19	47.5	9	22. 5	5.495	<0.05*
	Diminution of vision	25	62.5	10	25	11.429	<0.001**
d. Skin	**Contact dermatitis**	20	50	4	10	15.238	<0.001*
	Skin discoloration	20	50	0	0	26.667	<0.001**
e. Respiratory	**Bronchial hypersensitivity**	26	65	8	20	16.573	<0.001**
	Allergic rhinitis	18	45	6	15	8.571	<0.005*
f. Reproductive troubles		22	55	2	5	23.810	<0.001**

*Significant: ≤0.05 **Highly significant: ≤0.001.

Table 1 shows that all clinical manifestations studied were more prevalent among the exposed workers. Out of the general manifestations, significant statistical differences were found between both exposed and control groups as regards anorexia nausea, vomiting, metallic taste and flushing, with no significant statistical difference regarding easy fatigability, bony aches and memory troubles. Almost one quarter of the exposed workers had experienced accidents related to work (9/40, 22.5%). Half of the exposed workers were found to have cataract by slit lamp examination and almost two thirds complaint of diminution of vision (highly statistical significance, $p<0.001$). As regards skin manifestations (contact dermatitis and skin discoloration) were also significantly predominant among the exposed workers versus control group. Indeed, a statistically significant difference between exposed and control was found as regards allergic respiratory manifestations in the form of allergic rhinitis characterized with nasal itching, sneezing and discharge upon occupational exposure to dust and fumes and bronchial hypersensitivity characterized with repeated dyspnea ,cough or chest wheezes upon exposure to dust or fumes in work field. Also the table shows high statistically significant difference between exposed and control groups as regards reproductive troubles in the form of impotence reported by some male workers, menstrual irregularities, repeated abortions and unfavorable pregnancy outcome among female workers.

Table 2: Cardiovascular manifestations among exposed and control groups.

		Exposed No=40		Control No=40		Test value	p
		No	%	No	%		
IHD		17	42.5	7	17.5	5.952##	≤0.05*
ECG changes		16	40	4	10	9.6##	≤0.05*
Headache		22	55	10	25	7.500##	≤0.05*
Dizziness		7	17.5	4	10	0.949##	>0.05
HTN		17	42.5	5	12.5	9.028##	≤0.05*
SBP	Mean(SD)	130.13(±17.04)		121.51(±11.83)		2.629#	≤0.05*
	Range	110-180		100-150			
DBP	Mean	82.13(±9.67)		77.13(±7.84)		2.541#	≤0.05*
	Range	70-110		70-100			

IHD: ischemic heart disease,

HTN: Hypertension, cut off level of HTN is ≥140/90 (American heart association, 2014),
SBP: Systolic blood pressure (mmHg), DBP: Diastolic blood pressure (mmHg),
*Significant: ≤0.05 #: t test, ##: Chi square.

Table 2 showed that remarkable ECG abnormalities were detected among the exposed group in the form of bradycardia <60 b/m, tachycardia >100 b/m , arrhythmias and ischemic changes (statistically significant) except for dizziness.

Table 3: Liver, kidney function test and blood picture indices results among exposed and control groups.

		Exposed No=40	Control No =40	t test value	p
ALT	*Mean(SD)*	18.28(±5.11)	28.03(±11.12)	3.625	<0.001**
	Range	10-30	11-45		
AST	*Mean(SD)*	20.18(±10.66)	24.78(±8.16)	2.801	<0.005*
	Range	10-55	15-51		
Bilirubin	*Mean(SD)*	0.48(±0.35)	0.51(±0.24)	1.388	>0.05
	Range	0.22-1.62	0.21-1.10		
Urea	*Mean(SD)*	35.30 (±8.47)	28.15 (±5.36)	4.507	<0.001**
	Range	23-50	20-38		
Creatinine	*Mean*	1.04 (±.24)	0.97 (±0.32)	1.038	>0.05
	Range	0.6-1.7	0.6-1.8		
TLC	*Mean(SD)*	6.53(±2.04)	6.76(±1.94)	0.532	>0.05
	Range	3.20-10.70	3.70-10.10		
Hb	*Mean(SD)*	13.59(±1.37)	13.38(±1.41)	0.668	>0.05
	Range	10.30-15.90	10-15.50		
Platelet count	*Mean(SD)*	232.18(±66.17)	244.130(±60.48)	0.843	>0.05
	Range	128-376	122-206.5		

ALT: alanine transaminase (IU/L). AST: aspartate transaminase (IU/L).
Bilirubin: (μmol/L). Urea: (mg/dl).Creatinine: (μmol/L). HB: Haemoglbin
*Significant: ≤0.05. **Highly significant: ≤0.001.

Table 3 showed evident elevation in mean levels of urea among exposed versus control group ($p<0.001$). Liver enzymes (ALT, AST) were significantly lower among exposed workers with high statistical significance as regards ALT, however, no significant difference was obtained for creatinine or bilirubin between both groups.

Table 4: Serum Lead and 8OHdG among exposed and control groups.

		Exposed No =40	Control No=40	t test value	p
Lead (μg/dl)	Mean(SD)	12.77(±6.55)	12.92(±5.8)		>0.05
	Range	5-27	5-27		
8OHdG (U/ml)	Mean(SD)	706.72(±355.84)	109.32(±146.28)	7.017	<0.001*
	Range	159.1-1156	27.70-820		

8OHdG: 8hydroxyguanosine. *Highly significance: ≤0.001.

Table 4 shows that the mean serum OHdG level was significantly higher among the exposed group compared to the control (p<0.001). No significant difference between both groups was found as regards blood lead level.

Table (5): Predictors 8OHdG among all studied population.

	p value	Predictor coefficient(B)	95% Confidence Interval for B
Age	>0.05	-7.227	-22.645: 8.192
Sex	>0.05	-30.608	-169.539:108.323
Smoking	>0.05	27.047	-168.622:222.717
HTN	>0.05	129.035	-25.431: 283.500
Lead level	>0.05	0.182	-9.991: 10.355
GSTM	0.702	-33.378	-206.657: 139.900
GSTP	80.305	0.259	-60.378 220.989
GSTT	-91.512	0.173	-223.953 40.929
Exposure	**<0.001**	**565.477**	**430.474: 700.481**

8OHdG: 8hydroxyguanosine, Non significant: >0.05.
HTN: Hypertension GSTM: Glutathione-S-transferase M
GSTP: Glutathione-S-transferase P GSTT: Glutathione-S-transferase T

Regression was done to test for significant predictors of 8OHdG. Age, sex, HTN, lead level, exposure and polymorphism in GST were entered. Only occupational exposure to nitroaromatic compounds was found to be significant predictor. It was found that exposure group has 565.477 higher reading of 8OHdG than none exposed.

Pearson correlation was done (data not presented) between serum 8OHdG levels and some quantitative data in the exposed group (age, duration of work, smoking index, liver and kidney functions, and blood picture) the results showed a highly significant negative correlation between serum 8 hydroxyguanosine among the exposed group with total leucocytic count (TLC) and significant positive correlation with bilirubin level, while non significant correlations were detected with all other studied data.

Discussion

Ammunition industry is an old, world widespread one with many steps of production and exposures. Nitrocompounds are the most frequently used constituents of explosives especially TNT which is used in many countries and it is the main chemical substance found in the ammunition production line (Preklang and Chantanakul, 2012).

In this study, anorexia, nausea, vomiting, metallic taste and flushing showed significant statistical elevation among the exposed group compared to their referent control (Table 1). Anorexia, nausea and other gastrointestinal manifestations as constipation were reported in association with occupational exposure to TNT since 1946 in Davies study. It is not clearly explained why nitroaromatic compounds used in ammunition industry causes such general manifestations, however, anorexia, nausea and vomiting could be related to intestinal inflammation reported to occur in experimental animals (ATSDR, 1995).

Metallic taste is classified by early researches to be a neurotoxic effect of TNT (Morton et al., 1976). Similarly, several studies noted increased prevalence of bitter or metallic taste in ammunition workers (Letzel et al., 2003 and Glass et al., 2005).

Almost quarter of the exposed workers had experienced accidents related to work (Table 1). Explosions at site of work, together with hazard of movement of piston used to compress powder into ejector capsule were the major causes of accidents. In agreement to our study, it was reported that workers exposed to nitroaromatic compounds are in high danger of fire and explosion hazards (Ovesen, 2001).

With history taking and clinical examination, special concern was given to ocular effects in the form of cataract detected with slit lamb examination or diminution of vision by history. Both ocular effects show statistically significant difference between exposed and control groups (Table 1).As a potential mechanism behind the induction of cataract by TNT, one theory proposes that increased oxidative stress resulting from reductive activation of TNT may be involved (Kumagai et al. 2000).

Our study revealed high statistical significant elevation of dermal manifestations among the exposed group compared to control (Table 1). Letzel and colleagues in 2003 reported that in regularly exposed workers to ammunition nitroaromatic compounds, an increase in symptoms such as discoloration of the skin and hair were found. Indeed, workers engaged in filling or unloading TNT or in filling explosive charges are susceptible. TNT tended to incur dermatitis and contact allergies through increased temperatures or perspiration (DFG, 2014).

Workers in ammunition factories or other units in explosives and propellants production industry are exposed to chemical dusts, particulate contaminants and solvents. In this respect, bronchial asthma determined by history and clinical examination and allergic rhinitis in relation to exposure in ammunition factory were studied in our research. Our work shows statistical significant difference between exposed and control groups as regards allergic rhinitis, while highly statistical significant difference as regards bronchial hypersensitivity (Table 1).

High prevalence of asthma among exposed workers was found related to occupational exposure. The resulting effect is exaggerated by the definite lack of ventilation in the working area and the apparent reluctance of workers in using personal protective equipments. This is in agreement with results obtained by Cakmak and colleagues in 2004 that showed higher prevalence of asthma-related symptoms in chronic exposure to nitroaromatic compounds in a gun factory. Sahri and Widajati in 2013 reported TNT induced asthma in ammunition workers.

Regarding the toxicity mechanisms of ammunition nitrocompounds, there were few studies on human tissues especially the lungs. A possible reason of lack of research about pulmonary effects in ammunition workers could be security considerations (Shohrati et al., 2014). In some experimental research, Johnson and colleagues in 2000 concluded that TNT can induce oxidative-antioxidant system imbalance in the lung tissue of tiger salamanders. The possible etiology of development of this pattern in cases of exposure to nitroaromatic compounds could be gradual pulmonary parenchyma injuries, incomplete healing, persistent mild inflammation, and pulmonary fibrosis (Letzel et al., 2003 and Naderi et al., 2013).

More than half of the exposed workers complaint of reproductive troubles (55% versus 5% among control group, $p<0.0001$) (Table 1). Reproductive troubles were one of the most annoying complaints experienced by the workers themselves. The most prevalent reproductive adverse effect was impotence which was present in 15 out of 25 exposed male workers studied. Other reproductive troubles were recurrent abortion (reported by female workers and male workers regarding their wives, which was in one case reported in both of his wives), menstrual irregularities, bad pregnancy out come and congenital anomalies as cleft lip and cleft palate in offspring.

Similar to our results our results a study on male workers exposed to ammunition nitroaromatic compounds determined more sexual disorders such as impotence, prospermia, loss of libido and hypoaesthesia than in the control group (Liu et al., 1995).

Repeated abortions and stillbirth in wives of male workers revealed by our work could be explained by TNT induced oxidative testicular damage (Homma-Takeda et al, 2002), decreased spermatic motility and malformation of sperms affecting pregnancy outcome (Liu et al., 1995). On the other hand menstrual irregularities and bad pregnancy out come in the form of repeated abortions, stillbirth and congenital anomalies in offspring of female workers may be due to specific toxicity on female's reproductive system, which is neglected in almost all researches we reached.

Prevalence of CVS manifestations was higher among exposed workers compared to the control (Table 2). Studied CVS manifestations included IHD manifestations, ECG changes, headache, dizziness and hypertension (HTN).

High statistically significant elevated prevalence of ECG changes was found in exposed in comparison with the control. ECG changes were in the form of bradycardia in 5 cases, tachycardia in one case, other arrythmias in 3 cases and ischemic features in 7cases.

Similarly Tchounwou and his coworkers in 2003 recorded CVS toxicity especially IHD to be one of the toxicity targets in ammunition workers exposed to nitroaromatic. CVS manifestations are explained by causing myocardial dysfunction and increased vagal tone (Zakhari and Villaume, 1978). HuiPing in 2009 reported statistically significant higher prevalence of ECG changes in a group exposed to TNT. Sinus bradycardia, sinus tachycardia and sinus arrhythmias were the most prominent ECG changes.

We found difference between exposed and control group as regards headache and HTN which was significantly higher among the exposed workers, while both groups showed non significant difference as regards dizziness. Sun and co-workers (2005) in an experimental study reported elevation in blood pressure due to TNT exposure which they explained by inhibition of endothelial nitric oxide synthase.

About 55% of exposed workers were complaining of headache (statistically significant) (Table 2). This is in accordance with the study done by Preklang and Chantanakul in 2012 who mentioned that headache was the second most prevalent manifestation among ammunition workers with incidence of 67.5% in comparison with 47.5% in non exposed. The resultant throbbing headache is seemingly a primary headache. Metabolites of nitrocompounds cause activity alterations on different levels of the trigeminal system, which in turn results in arterial vasodilatation and possibly mast cell degranulation in the meninges (Messlinger et al., 2012). In earlier studies headache was explained by nitrocompounds irritant effect on nasal mucous membrane causing nasal obstruction with a consequence of headache (Zakhari and Villaume, 1978).

Adverse effect on liver and kidney functions was studied through comparing laboratory results between both exposed and control groups.

Blood urea level showed highly significant elevation, while significant lower levels of ALT and AST were detected among the exposed group, with otherwise no significant difference between both groups as regards bilirubin or creatinine levels (Table 3).

In agreement with our study Sabbioni and Rumler in 2007found that workers indulged in clean up of ammunition sites with biomonitoring indicating exposure to TNT and its metabolites showed significant higher levels of blood urea than control. The same study of Sabbioni and Rumler in 2007 and also earlier research of Dilley and co-workers in 1982 agreed with ours reporting significant lower ALT but not AST levels among TNT exposed workers.

As regards bilirubin level, similar to our results Sabbioni and colleagues, 2005 and Roberts' 1986 who found no significant correlation between bilirubin level and TNT in ammunition workers.

Complete blood count was determined to subjects in our research and revealed no significant difference between both groups (Table 3).In agreement with our results Naderi and colleagues in 2013 argued that TNT resulted in decrease in Hb, TLC or platelet counts. Similarly, no significant difference were found in Hb concentration and hematocrit value between exposed and control groups in Shinkai and co-workers study in 2015. In contrary to our study Sabbioni and Rumler in 2007 reported the presence of lower Hb and erythrocyte counts among ammunition workers exposed to TNT. Eddy in 1944 linked aplastic anemia in ammunition workers reported by his study with individual susceptibility that explain occurrence in few number of total exposed workers even with lower levels of exposure.

8OHdG is the form of oxidized guanine (a base that is most prone to oxidation). It is the most commonly studied critical biomarker of oxidative DNA damage. Our results showed highly significant statistical difference between exposed and control groups with higher values among the exposed group (Table 4). Mean level of 8OHdG in exposed workers was 706.72 (±355.84) U/ml compared to 109.32 (± 146.282.8) U/ml in the control group ($p<0.001$). Regression was done to test for significant predictors of 8OHdG in the studied population. Exposure to nitroaromatic compounds in ammunition appeared to be the only significant predictor and it was found that exposed group has 565.477 higher reading of 8OHdG than non exposed (Table 5).

This highly statistical significant elevation of 8OHdG in relation to ammunition nitroaromatic compounds exposure in ammunition workers could define its mechanism of action. It appears to be through development of oxidative stress. Oxidative stress results in deregulation of the homeostasis between the reactive oxygen species and the mechanisms of detoxification and repair.

Similarly, Johnson and colleagues in 2000 concluded that TNT can induce oxidative-antioxidant system imbalance in the lung tissue of tiger salamanders. The TNT metabolites damaged DNA, increasing the formation of 8OHdG and cleaving the DNA at sites with consecutive guanines (Homma-Takeda et al., 2002).

Though nitroaromatic compounds mechanism of action is not yet fully understood, it is, however, assumed that the formation of ROS induces lipid peroxidation in the liver and the formation of cataracts in the lens of the eye (ATSDR, 1995). The supposed resulting genotoxicity and mutagenicity as indicated with high elevation of 8OHdG is confirmed in Deutsche Forschungs gemeinschaft (DFG) study in 2014. It showed that inhalation exposure and presumably the simultaneous dermal exposure of workers to TNT results in a significantly increased incidence of chromosomal aberrations in the peripheral lymphocytes.

Conclusion

In our study exposure to nitroaromatic compounds in the ammunition industry was associated with considerable adverse health effects including cardiac, respiratory, ocular, dermatological and reproductive effects. Exposed workers showed multisystem affection. Anorexia, nausea, vomiting, metallic taste, flushing, work accidents, cataract, diminution of vision, contact dermatitis, skin discoloration, bronchial hypersensitivity, reproductive troubles, cardiac ischemia, headache and HTN. These manifestations showed higher incidence among ammunition workers than in control with a statistically significant difference.

The levels of liver enzymes ALT and AST showed statistically significant lowering while significant elevation of blood urea among the exposed group in comparison to control. Blood count indices as well as bilirubin level and serum creatinine showed irrelevant difference between both groups.

The highly significant elevation of the mean level of serum 8 OHG among the exposed workers compared to the control group identifies that oxidative stress is a possible mechanism for adverse health effects in ammunition workers.

Recommendations

More strict measures should be enforced at different levels of industrial hygiene. Substitution with less hazardous explosive compounds, automation and dust control are important engineering control measures. As regards work practices, education of workers about job hazards and importance of personal protective equipments could help in reducing the resultant adverse effects. Ocular, dermal, respiratory, haematological, reproductive and cardiovascular clinical manifestations are to be properly assessed in pre-employment and periodic medical examination. Risk evaluation and management of explosive hazards directed towards accident prevention and rehabilitation of work accidents victims are of special importance in ammunition industry. The possible utility of 8 hydroxyguanosine serum level as a marker of DNA oxidative damage may be considered. Workers supplementation with antioxidant medication is highly recommended.

Conflict of interest

Authors have declared that no conflict of interest exists.

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