Genetic Susceptibility and Health Effects of Occupational Exposure to Nitroaromatic Compounds in Ammunition Industry.

Thesis By

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Submitted for the partial fulfillment of MD Degree

In Clinical Toxicology

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2016

Background*:* Nitroaromatic compounds constitute a large group of chemicals that are characterized by the presence of one or more nitro groups. These compounds have been used in multiple applications and are the main constituent of explosives in ammunition industry.

Objectives: Our objective was to study health hazards in workers exposed to nitroaromatic compounds in the ammunition industry, the role of oxidative stress in the mechanism of action as well the role of polymorphisms of Glutathione S-transferase gene as an effect modifier.

Subjects and methods: The study was conducted on 40 workers as an exposed group comprising all workers engaged in ammunition production and maintenance line in a military ammunition factory in Helwan area, South of Cairo and who fulfilled inclusion criteria for participation in the study. The study also involved a control group composed of 40 workers randomly selected from administration workers in Cairo University Hospitals and never occupationally exposed to nitroaromatic compounds or genotoxic substances and matched the exposed group as regards age, sex, smoking and socioeconomic status. All participants were subjected to a detailed specially prepared occupational and medical history questionnaire with full clinical examination, slit lamp examination, ECG scanning and 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. Genetic study of GST gene polymorphism was done for the whole population. Multiplex PCR was performed for determination of GSTM1 and GSTT1 genes polymorphisms in the isolated DNAs, while PCR –Restriction Fragment Length Polymorphism (PCR- RELP) was performed for GSTP1 gene polymorphisms.

Results: Clinical examination of study population 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. Exposed workers showed elevation in mean blood urea (35.30 ±8.47mg/dl) compared to the control group (28.15±5.36 mg/dl) with a highly significant statistical difference. The mean level of ALT and AST showed marked decrease (18.28±5.11 and 20.18±10.66 IU/L respectively) among exposed versus control (28.03±11.12 and 24.78±8.16 IU/L respectively). No significant statistical difference was found between both groups as regards other laboratory investigations for liver, kidney functions and blood picture.

Mean serum 8 hydroxyguanosine level showed a highly significant statistical elevation in ammunition workers (706.72±355.84 U/cc)compared to their referent control group (109.32±146.28U/cc).

Regarding frequency distribution of GST genes, in the exposed workers, intact gene of GST (M, T) and the wild GST (P) genes were more prevalent (57.5%, 65% and 62.5% respectively). However, in the control group, the intact gene of GST (M) and the wild GST (P) genes were more prevalent (95% and 77.5% respectively). In the exposed group regarding GST genes polymorphism, null GSTM gene group were predisposed to flushing, lower levels of hematocrit value and Hb levels and blood urea. GSTP mutation group showed highly significant elevation of ALT and AST levels compared with intact gene group, while significant higher prevalence of ischemic heart diseases and level of ALT in GSTT null gene carriers on the other hand a significant higher mean level of creatinine and lower mean level of AST, was noted among exposed workers with GSTT nun null gene carriers.

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. 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. Moreover, genetic polymorphism in members of the GST gene family, may be considered as an effect modifier and as genetic susceptibility for some nitroaromatic compounds health hazards.

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 PPE, could help in reducing the resultant adverse effects. Ocular, dermal, respiratory, haematological, reproductive and cardiovascular clinical manifestations are to be properly assessed in preemployment 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.

Key Words
Ammunition workers, nitroaromatic health hazards, TNT, 8 hydroxyguanosine, GST genes, gene environment interaction.

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2,4DNT	2,4-dinitrotoluene
2,6DNT	2,6-Dinitrotoluene
2ADNT	2-amino-2,6-dinitrotoluene
2NT	2-nitrotoluene
4ADNT	4-amino-2,6-dinitrotoluene
8OHdG	8-hydroxy-2' –deoxyguanosine (8 hydroxyguanosin
A,N,V	Anorexia, nausea and vomiting
ACGIH	American Conference of Governmental Industrial H
ALT	Alanine transaminase
AST	Aspartate amino transferase
COPD	Chronic obstructive pulmonary disease
CVS	Cardiovascular system
CYP1A2	Cytochrome P450 1A2
DBP	Diastolic blood pressure (mmHg).
DNA	Deoxyribonucleic acid
ECD	ED electron capture detectors
ECG	Electro Cardio Gram
ED	Electrochemical detection
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FEV1	Forced expiratory volume 1
FISH	Fluorescent in situ hybridization
FVC	Forced vital capacity
G6PD	Glucose-6-phosphate dehydrogenase
GEI	Gene environment interaction
GINA	Genetic Information Nondiscrimination Act
GSH	glutathione GSH
GST	Glutathione-S-transferase
GSTA	GST classes alpha
GSTM	GST classes mu
GSTP	GST classes pi
GSTT	GST classes theta
Hb	Hemoglobin
HEV	Hydroxyethyl valine
HPLC	High performance liquid chromatography
HRP	Horseradish Peroxidase
HTN	Hypertension

HWE	Healthy worker effect
IHD	Ischemic heart disease
IHD	Ischemic heart disease.
Ile	Isoleucine (Ile)
LD	Lethal dose
LDH	Lactate dehydrogenase
LM-PCR	Ligation-mediated polymerasechain reaction
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean corpuscular volume
MRP1	Multidrug resistance-associated protein 1
MS	Mass spectrometry
NAT	N-acetyltransferases.
NIOSH	National Iinstitute for Occupational Safety and Health
NRC	National Research Council
Nrf2	Nuclear factor-erythroid 2 p45-related factor 2
NS	Non significant
OSHA	Occupational Safety and Health Administration
PBIs	Primary blast injuries
PCR	Polymerase Chain Reaction
PEF	Peak expiratory flow
PEL	Permissible Exposure Limit
PKU	Phenylketonuria
PPARgamma	proliferator-activated receptor gamma
Ppb	Part per billion
PPE	Personal protective equipements
ppt	Part per trillion
RBC	Red blood cells count
RDX	Red cell distribution width
RDX	Royal demolition explosive
REL	Recommended Exposure Limit
RNA	Ribonucleic acid
RNOxS	Reactive nitrogen oxide species
ROS	Reactive oxygen species
SBP	Systolic blood pressure (mmHg).
SGOT	Serum glutamic oxaloacetic transaminase
SNPs	Single nucleotide polymorphisms
SULT	sulfotransferases
TCE	Trichloroethylene
TLC	Total leucocytic count
TLV	Threshold Limit Value

TNP-BSA	2,4,6-trinitrophenol-bovine serum albumin
TNT	Trinitrotoluene
UV	Ultraviolet
Val	Valine
WHO	World Health Organization

Nitroaromatic compounds constitute a large group of chemicals that are characterized by the presence of one or more nitro groups ***(Oliveira et al., 2010).***

Nitroaromatic compounds have been used in multiple applications as pharmaceuticals, antimicrobial agents, food additives, pesticides, explosives, dyes and raw materials in several industrial processes ***(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 ***(Bečanová, 2010 and Preklang and Chantanakul, 2012).***

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 are the hematopoietic system (pallor, cyanosis, anemia, and leukocytosis), the cardiovascular system (CVS) (ischemic heart disease (IHD)), 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).*** A significantly increased number of chromosomal aberrations (stable and unstable abberations) were found in workers exposed to TNT ***(Verdorfer et al., 2001).***

8OHdG 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 ROS induces lipid peroxidation in the liver and the formation of cataracts in the lens of the eye ***(ATSDR, 1995).***

Despite the strong causal associations between certain occupational exposures and related health hazards, still there are differences in disease incidence between workers that cannot be accounted for by differences in exposures or work practices. Some of

these differences are likely due to genetic polymorphisms that should be included as relevant variables in study design and analysis ***(NIOSH, 2004).***

Glutathione S-transferase (GST) enzymes comprises a family of phase II metabolic enzymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates ***(****Atkinson and Babbitt, 2009),* thus preventing their interaction with crucial cellular proteins and nucleic acids ***(Hayes et al., 2005 and Josephy, 2010).***The conjugation to GSH serves to make the substrates more soluble and allowing them to be removed from the cell then excreted via urine or bile***(Josephy, 2010 andOakley, 2011).*** Moreover, they detoxify reactive chemical species by catalyzing their conjugation to GSH ***(Setiawan et al., 2000).***

GST, a supergene family of detoxification enzymes, appear to form a protection mechanism against chemical hazards ***(Cai et al., 2001).***The most well characterized GST classes have been named alpha (GSTA), mu (GSTM), pi (GSTP) and theta (GSTT). GST enzymes that belong to various classes have different, but sometimes overlapping, substrate specificities ***(Di Pietro et al., 2010).***

Being encoding for GST enzymes that have a prominent role in neutralizing the toxic effects of TNT and other nitroaromatic compounds, GST gene family polymorphisms thus, predispose exposed persons more to the toxicity of these compounds ***(Tamaki et al., 2011).***

Cataract which is one of TNT toxicity manifestations reported to be closely correlated with GSTM gene deletion proving that GSTM gene deletion may be one of the important hereditary factors for susceptibility to TNT hazards ***(Xuet al., 2001).***

It seems that workers carrying the GST polymorphic genotype were more susceptible to some nitrotoluene health hazards thus studying individual susceptibility (genetic polymorphisms of nitrotoluene metabolizing enzymes such as GST) is one of the variables of nitrotoluene biomonitoring paradigm ***(Sabbioni et al., 2006).***

This work aims at health promotion of workers occupationally exposed to nitroaromatic compounds in the ammunition industry and constructing a comprehensive program for pre-employment and periodic medical examination, through:

- Studying adverse health effects with emphasis on ophthalmic, dermal, respiratory, reproductive, cardiovascular, hepatorenal and blood effects.
- Moreover, evaluating the role of glutathione s-transferase gene polymorphism as an effect modifier in exposed workers.
- Also assessing oxidative stress as a possible mechanism of action of nitroaromatic compounds through measuring the level of serum 8 hydroxyguanosine which is one of the major oxidative adducts formed by radical induced damage of DNA.

Genetic susceptibility in relation to occupational diseases

Two major observations had significantly changed previous scientific principles and directed the sight to a new era of research, the first was that despite the fact that occupations relation to diseases has been well established, the question aroused is that why individuals with seemingly equal exposure to occupational hazards develop diseases or cancers in an unpredictable manner ***(Matic et al., 2014).***The second was that although traditional epidemiological and experimental data on certain diseases supported associations with familial genetic factors, growing evidence has shown the relevant role played by exposure to environmental chemicals in such diseases ***(Oddone et al., 2014).***

Accordingly the attention on simple mechanisms of genetic-environmental determination of disease has been strongly resized. Thereafter an introduction on basic components of this interaction (genetic- environmental) or genetic occupational, with emphasis on our research will be discussed below.

Genetic material

Every cell in the human body contains a nucleus, with the exception of red blood cells, which lose this structure as they mature. Within the nucleus are tightly coiled threadlike structures known as chromosomes. Every chromosome has a long arm and a short arm, with a pinch point known as a 'centromere'. Humans normally have 23 pairs of chromosomes, one member of each pair derived from the mother and one from the father. One of those pairs is the sex chromosomes with two X chromosomes determining femaleness, and one X and one Y determining maleness. The other 22 chromosomes are known as autosomes ***(ALRC, 2003).***

Each chromosome has within it, arranged end-to-end, hundreds or thousands of genes, each with a specific location, consisting of the inherited genetic material known as deoxy-ribo-nucleic acid (DNA). DNA is so called because it consists of a large acid molecule mainly found in the nucleus *(nucleic)* to which many sugar

groups (ribo) that are missing an oxygen molecule (deoxy) are attached ***(ALRC, 2003).***

There are many different definitions for a gene, but one of the most commonly accepted is that it is a locus or region of DNA that encodes a functional RNA or protein product and is the molecular unit of heredity. It contains all of the information required to determine the expression of a specific protein or a chain of amino acids (a polypeptide). Sometimes a polypeptide can form a complete protein on its own as in the case of insulin, but in most cases a number of polypeptides combine to create a single functional protein as in the case of collagen and globin ***(Slack, 2014).***

The human genome consists of DNA that is made up of four basic building blocks (nucleotides) (deoxyribose), a phosphate group and a base. There are four different types of bases; adenine (A), guanine (G), which are known as purines, and thymine (T), and cytosine (C) which are known as pyrimidines. These nucleotides link together to form long polynucleotide chains. A DNA molecule consists of two of these chains, linked together by hydrogen bonds, running in opposite directions. Linkage of the chains follows a strict rule, known as complementary base pairing.

- The base A can only pair with the base T, and vice versa, and
- The base G can only pair with the base C, and vice versa ***(de Jong, 2014).***

Watson and Crick in 1953 were the first to describe that there are over three billion of these base pairs of DNA making up the human genome. The two chains link together in a ladder-like shape, twisted into the famous double helix shape, with sugars and phosphates forming the sides or backbone of the ladder and the base pairs forming the rungs.

DNA contains a code that directs the expression or the production of proteins. Proteins are critical components of all cells, determining color, shape and function. Proteins can have a structural role (such as keratin, from which hair is made), or a functional role in regulating the chemical reactions that occur within each cell (such as the enzymes involved in producing energy for the cell). Proteins are themselves made up of a chain of amino acids. Within the DNA there is a code that determines which amino acids will come together to form that particular protein. The genetic

code for each amino acid, consisting of three base sequences, is virtually identical across all living organisms ***(Hawley and Mori, 1998).***

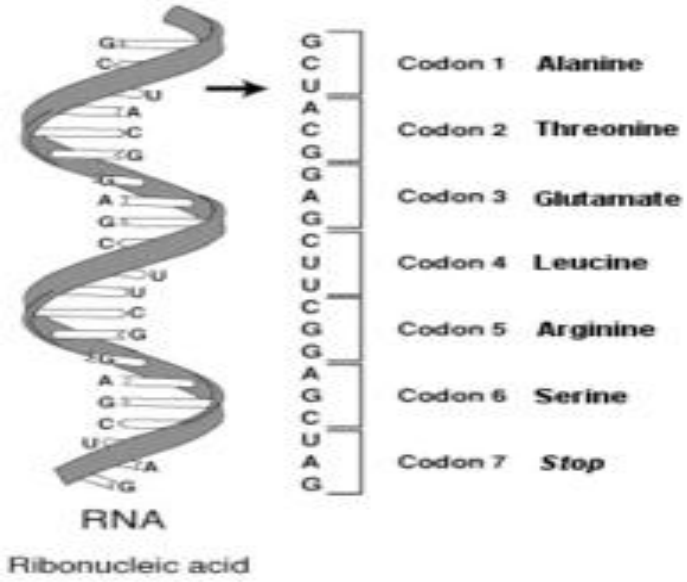

Figure (1): Schematic diagram of a single-stranded ribonucleic acid molecule illustrating the position of three-base codons ***(Hartwell et al., 2004).***

Although more than 99% of all DNA is similar between individuals, certain base pairs differ. These differences are called single nucleotide polymorphisms (SNPs). There are millions of SNPs in the human genome. Yet the functionality is only known for a small proportion of these variants. Functional SNPs may cause altered gene expression, altered protein structure or altered splice variants this alteration or difference then may result in modification in metabolism of chemicals managed by these genes reflected on different health hazards on exposure to such chemicals. Studying genetic susceptibility is important since it may provide novel insights into biological pathways leading to disease development ***(de Jong, 2014).***

Occupational risk

An occupational disease or illness is any disease or illness that is directly attributable to an exposure in the work environment. A disease or illness may be considered work-related if there is a preexisting condition that is aggravated by an exposure in the work environment ***(OSHA, 2001).***