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

By

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Abstract

Introduction: In factory workers, exposure to nitroaromatic compounds has been linked to many adverse health effects. **Aim of work:** To study the role of polymorphisms of Glutathione S-transferase (GST) gene as an effect modifier in relation to nitroaromatic induced health hazards. **Materials and methods:** The study was conducted on 40 workers as an exposed group comprising all workers engaged in ammunition production in a military factory in Helwan area, and a control group of 40 workers. All participants were subjected to an occupational and medical history questionnaire with full clinical examination, slit lamp examination, ECG scanning and laboratory investigations that included: liver and kidney functions, complete blood picture and serum 8-OHdG. 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- RFLP) was performed for GSTP1 gene polymorphisms. **Results:** 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:** 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:** Further studies are recommended on a larger scale to study the net effect of multiple contributing genes.
Key words: Ammunition workers, Nitroaromatic health hazards, GST genes and Gene environment interaction.

Introduction

Nitroaromatic compounds have been reported as toxic, mutagenic and carcinogenic. They are used in multiple applications as pharmaceuticals, antimicrobial agents, food additives, pesticides, explosives, dyes and raw materials in several industrial processes (Oliveira et al., 2010).

TNT is one of the most recalcitrant and toxic of all the military explosives used (Padda et al., 2003). It is used in military shells, bombs, and grenades, in industrial uses, and in underwater blasting (Oliveira et al., 2010). TNT has accumulated in areas of manufacturing, storage, and decommissioning over recent decades. Various government agencies, such as the EPA have listed TNT as a priority pollutant and have recommended that it should be removed from contaminated sites to prevent environmental and health problems (Van Aken, 2009).

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. Genetic polymorphisms account for some of these differences, thus should be included as relevant variables in study design and analysis (NIOSH, 2004).

Glutathione S-transferase (GST) enzymes comprise a family of phase II detoxifying 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). It is an important detoxifying mechanism ensuring more soluble substrates and allowing them to

be removed from the cell then excreted via urine or bile (Josephy, 2010 and Oakley, 2011). Moreover, they detoxify reactive chemical species by catalyzing their conjugation to GSH thus forms a protection against chemical hazards (Setiawan et al., 2000). The most well characterized GST classes have been named alpha (GSTA), mu (GSTM), pi (GSTP) and theta (GSTT) (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).

GSTM gene deletion may be one of the important hereditary factors for susceptibility to TNT hazards. Such deletion was reported to be closely correlated to TNT induced cataract (Xu et al., 2001).

Workers carrying the GST polymorphic genotype are supposed to be more susceptible to some nitroaromatic compounds health hazards thus studying individual susceptibility (genetic polymorphisms of nitroaromatic compounds metabolizing enzymes such as GST) is one of the variables of bio monitoring paradigm (Sabbioni et al., 2006).

Aim of work

To study the role of polymorphisms of Glutathione S-transferase (GST) gene as an effect modifier in relation to nitroaromatic induced health hazards.

Materials and methods

Study design: It is a case control study.

Study place and duration of the study: this work has been conducted in a military ammunition factory in Helwan area, South of Cairo from June 2014 to Jan 2016.

Study sample: The exposed group comprised of 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 Cairo University Hospitals with no history of exposure to nitroaromatic compounds

or genotoxic substances and matched the exposed group as regards age, sex, smoking and socioeconomic status.

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 and 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 four clean tubes; the first two contained EDTA one for CBC and the other for DNA study (incubated at -20), the third 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:

We used the flame atomic absorption spectrophotometry (model M6) (WHO, 2011).

II. Complete blood picture,

III. Kidney function tests (urea, creatinine)

IV. Liver enzymes (ALT, AST).

V. Human 8-OHdG Elisa kit:

ELISA kit 8-OHdG applies the quantitative sandwich enzyme immunoassay technique (Witherel et al., 1998).

VI. Determination of GSTM1, GSTT1 and GSTP1 gene polymorphisms:

1. DNA Extraction:

DNA extraction was done using Gene JETTM Genomic DNA Purification Kit- (Fermentas, Thermo Scientific, USA).

Principle:

The extraction of DNA from whole blood encompassed the lyses of proteins with proteinase K. Ribonucleic acid (RNA) is removed by treating the sample with RNase A. The lysate is then mixed with ethanol and loaded on the purification column where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

2. Amplification of the extracted genomic DNA by Polymerase Chain Reaction (PCR):

A- principle: PCR consists of repetitive cycles of DNA denaturation, primer annealing and extension by DNA polymerase. Each cycle produces complementary DNA strands to which the primers bind before the polymerase affects another extension in the next cycle of amplification. Accordingly, the products of each cycle are doubled, generating an exponential increase in the overall number of copies synthesized. This results in a highly sensitive method that allows multiple reactions from a very small number of initial DNA sequences (Serrano et al., 2004).

B- Multiplex PCR for determination of GSTM1 and GSTT1 polymorphisms in the isolated DNAs :

Multiplex PCR method was used to determine GSTM1 and GSTT1 polymorphisms in the isolated DNA as described before (Magno et al.,2009).

For GSTM1 polymorphism: Forward primer (5'-GAA CTC CCT GAA AAG CTA AAG C-3') and reverse primer (5'-GTT GGG CTC AAA TAT ACG GTG G-3') were used.

For GSTT1 polymorphism: Forward primer (5'-TTC CTT ACT GGT CCT CAC ATC TC-3') and reverse primer (5'- TCA CCG GAT CAT GGC CAG CA-3') were used.

As an internal control, a fragment of the human β-globin gene was also amplified using the forward primer (5'-GAA GAG CCA AGG ACA GGT AC-3') and the reverse primer (5'-CAA CTT CAT CCA CGT TCA CC-3').

The final reaction mixture consisted of 2μL DNA, 25μL Dream Taq Green PCR master mix (Fermentas, Thermo Scientific), 20pmol of each of forward and reverse primers, and was made up to 50μL with PCR quality water.

C- The PCR was carried out in thermal cycler (Perkin-Elmer).

The final PCR products from co-amplification of GSTM1 (157 bp) and GSTT1 (480 bp) were visualized on an ethidium bromide-stained 2.0% agarose gel. The subjects were classified as either (+), when at least one copy of the gene was detected, or (–) when they showed a null genotype. Heterozygous and homozygous individuals with GSTM1 (GSTM1+/– and GSTM1+/+) or GSTT1 (GSTT1+/– and GSTT1+/+) were reported to present similar enzymes activity and expression levels so they were grouped together (Bell et al., 1993).

D- PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) for detection of GSTP1 gene polymorphisms: PCR-RFLP is a sensitive measure of genetic variation between individuals. DNA is treated with a restriction endonuclease that cut at specific sequence or restriction sites. This produces a large number of restriction fragments of many different lengths.

GSTP1 polymorphism was detected by PCR-RFLP method as described previously (Zhong et al., 2006).

The primer sequences used were; forward primer: 5'-GAG GAA ACT GAG ACC CAC TGA G-3') and reverse primer: 5'-AGC CCC TTT CTT TGT TCA GCC-3').

The final reaction mixture consisted of 2μL DNA, 1.5μL Dream Taq Green PCR master mix (Fermentas, Thermo Scientific), 20pmol of each of forward and reverse primers, and was made up

to 25 µL with PCR quality water. The PCR was carried out in thermal cycler (Perkin-Elmer). The restricted PCR products were electrophoresed through 2% ethidium bromide stained agarose gel, and visualized by ultraviolet light.

The amplified fragment after digestion with Alw26I restriction enzyme can give rise to either two fragments at 329 and 107 bp which indicates the presence of wild-type GSTP1 (IIe/IIe), or two fragments at 222 and 107 bp which indicates the presence of the homozygous mutant type (Val/Val), or three fragments at 329, 222, and 107 bp, which indicates the presence of heterozygous mutant type (Ile/Val).

3. Detection of the PCR products:

A. Preparation of 2.0% agarose gel.

B. Samples preparation and loading: Samples were prepared for loading by adding 1 μl 1x loading buffer to 5μ1 of the PCR reaction mixture. Five μ1 of each prepared samples were slowly loaded into the sample wells.

C. Performing the electrophoresis: The lid was carefully placed on the gel electrophoresis apparatus then the gel apparatus was connected so that the negative electrode (cathode) is nearest to the sample wells at the start of electrophoresis at 150 volts and 250 milliamperes for 20 minutes.

D. Detection of the site of the bands in agarose gel using ultra-violet transillumination: The most convenient method for visualizing DNA on agarose gel is by staining with the fluorescent dye ethidium bromide. DNA- ethidium bromide complexes absorb ultra-violet light at 260 , 300 or 360 nm and emit at 590 nm in the red orange region of the visible spectrum .

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. p-value less than 0.05 and less than 0.001were considered statistically significant and highly significant, respectively.

Results

The mean duration of work and exposure to nitroaromatic compounds among exposed workers was 31.55 years (±2. 68) and ranged (25 – 37). No statistically significant difference was found between the exposed and control groups as regards age, sex and smoking.

Table (1): Frequency distribution of GST (M, P and T) polymorphism among exposed and control groups.

		Exposed No=40		Control No=40		X^2	p
		No	%	No	%		
GSTM	*Null*	17	42.5	2	5.0	**15.53**	**<0.001****
	Nun-null	23	57.5	38	95.0		
GSTP	*Mutant(Hetero)*	15	37.5	9	22.5	2.143	0.143
	Wild	25	62.5	31	77.5		
GSTT	*Null*	14	35	23	57.5	**4.073**	**<0.044***
	Nun-null	26	65	17	42.5		

Null: absent gene. Nun null: intact gene. Mutant (Hetero): Heterogenous mutation.

*Significant. **Highly significant.

Table 1 showed that among 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, among the control group, the intact gene of GST (M) and the wild GST (P) genes were the more prevalent (95% and 77.5% respectively).

Table (2): Frequency distribution of some clinical and cardiovascular manifestations among exposed workers with GSTM null and nun-null gene polymorphisms.

		GSTM null No =17		GSTM non-null No =23		X^2	p
		No	%	No	%		
a. General	**Anorexia, Nausea, Vomiting**	6	35.3	2	8.7	4.322	0.038
	Metallic taste	8	47.1	10	43.5	051	0.822
	Bony aches	6	35.3	8	34.8	0.001	0.973
	Flushing	11	64.7	7	30.4	**4.639**	**0.031***
	Memory troubles	7	41.2	4	17.4	2.774	0.096
	Easy fatigability	4	23.5	8	34.8	0.589	0.443
b. Work accidents		3	17.6	6	26.1	0.399	0.527
c. Eye	**Cataract**	9	52.9	10	43.5	0.351	0.935
	Diminution of vision	10	58.8	15	65.2	0.171	0.680
d. Skin	**Contact dermatitis**	9	52.9	11	47.8	0.102	0.749
	Skin discoloration	9	52.9	11	47.8	0.102	0.749
e. Respiratory	**Bronchial hypersensitivity**	10	58.8	16	69.6	0.496	0.481
	Allergic rhinitis	9	52.9	9	39.1	0.753	0.385
f. Reproductive troubles		8	47.1	14	60.9	0.753	0.385
g. Cardiovascular manifestations	**IHD**	9	52.9	8	34.8	1.319	0.251
	ECG changes	7	41.2	9	39.1	0.017	0.896
	Headache	11	64.7	11	47.8	1.125	0.289
	Dizziness	4	23.5	3	13	0.744	0.388
	HTN	8	47.1	9	39.1	0.251	0.616
SBP	*Mean(SD)*	132.06 (±18.63)		128.70 (±16.04)		0.612	0.544
	Range	110-165		110-180			
DBP	*Mean(SD)*	83.82 (±10.54)		80.87 (±9)		0.954	0.346
	Range	70-100		70-110			

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.

Table 2 showed significant higher prevalence of flushing among exposed workers with absent GST gene, while there was a non significant difference between exposed workers with GSTM null and nun null gene polymorphisms as regards the other manifestations.

Table (3): Complete blood picture, liver and kidney function, blood lead levels and serum 8OHdG results among exposed workers with GSTM null and nun null gene polymorphisms.

		Null No =17	Non null No =23	t value	p
TLC (c/cmm)	*Mean(SD)*	7.09(±2.13)	6.11(±1.92)	1.524	0.136
	Range	4.2-10.10	3.20-10.70		
RBCs (c/cmm)	*Mean(SD)*	4.87(±.0.51)	5(±0.41)	0.897	0.376
	Range	3.92-5.99	3.98-5.73		
Hb (g/dl)	*Mean(SD)*	12.96(±1.33)	14.05(±1.24)	**2.661**	**0.011***
	Range	10.30-15.20	11.30-15.90		
Hematocrit	*Mean(SD)*	38.75(±4.26)	41.22(±3.53)	**2.007**	**0.050***
	Range	30.80-46.40	33.70-48.60		
MCV (femtoliters)	*Mean(SD)*	79.79(±5.41)	82.88(±7.84)	1.398	0.170
	Range	70.30-88.70	67.20-95.10		
MCH (pictograms)	*Mean(SD)*	26.71(±2.19)	28.19(±2.9)	1.764	0.086
	Range	23.50-31.60	21.20-33.70		
MCHC (%)	*Mean(SD)*	33.46(±1.50)	34(±1.51)	1.131	0.265
	Range	30.20-36	30.80-35.90		
RDX	*Mean(SD)*	14.42(±1.60)	13.83(±1.55)	1.170	0.249
	Range	12-18.40	12.10-18.80		
Platelet	*Mean(SD)*	236.41(±68.51)	229.04(±65.77)	0.344	0.733
	Range	128-362	130-376		
ALT (IU/l)	*Mean(SD)*	19.24(±3.93)	17.52(±5.82)	1.442	0.149
	Range	13-25	10-30		
AST (IU/l)	*Mean(SD)*	23.82(±12.94)	17.48(±7.87)	1.773	0.076
	Range	10-55	10-33		
Urea (mg/dl)	*Mean(SD)*	31.12(±7.98)	38.39(±7.57)	**2.935**	**0.006***
	Range	23-49	25-50		
Creatinine (μmol/L)	*Mean(SD)*	0.96(±0.29)	1.10(±0.18)	1.807	0.079
	Range	0.6-1.7	0.8-1.7		
Bilirubin (μmol/L)	*Mean(SD)*	0.36(±0.13)	0.58(±0.42)	**2.089**	**0.037***
	Range	0.22-0.60	0.27-1.62		
8OHdG (U/ml)	*Mean(SD)*	744.98(±333.67)	678.45(±376.21)	0.493	0.622
	Range	222.7-1084	159.10-1156		
Lead(μg/dl)	*Mean(SD)*	12.19(±6.30)	13.19(±6.84)	0.438	0.661
	Range	5.20-26.20	5-27		

TLC: total leucocytic count (cells per cubic millimeter (c/cmm), RBCs: red blood cells count (c/cmm), Hb: hemoglobin level (grams per deciliter), Hematocrit: The ratio of the volume of red cells to the volume of whole blood, MCV: mean corpuscular volume (femtoliters), MCH: Mean cell hemoglobin (pictograms), MCHC: Mean cell hemoglobin concentration (%), RDX: Red cell distribution width, ALT: alanine transaminase (IU/L), AST: aspartate transaminase (IU/L), Bilirubin: (μmol/L), Urea: (mg/dl), Creatinine (μmol/L), Lead (μg/dl), 8OHdG: 8hydroxyguanosine(U/ml),

*: Significant.

Table 3 showed significant higher mean values of Hb, hematocrit, urea and bilirubin among exposed workers with GSTM nun null gene compared with those with null gene. Non significant difference were found as regards the other studied laboratory parameters.

Table (4): Complete blood picture, liver and kidney functions, blood lead levels and serum 8OHdG results among exposed workers with GSTP mutant and wild gene polymorphisms.

		GSTP Mutant No =15	GSTP Wild No =25	t value	P
TLC (c/cmm)	*Mean(SD)*	7.10(±1.92)	6.18(±2.08)	1.395	0.171
	Range	4.30-10.70	3.20-10.10		
RBCs (c/cmm)	*Mean(SD)*	4.92(±.0.50)	4.96(±0.44)	0.304	0.763
	Range	3.92-5.99	3.98-5.73		
Hb (g/dl)	*Mean(SD)*	13.50(±1.57)	13.64(±1.27)	317	0.753
	Range	10.30-15.80	11.40-15.90		
Hematocrit	*Mean(SD)*	40.09(±4.53)	40.22(±3.74)	0.102	0.919
	Range	30.80-48.60	33.40-48.00		
MCV (femtolitres)	*Mean(SD)*	81.62(±7.12)	81.54(±7.08)	0.036	0.971
	Range	67.80-88.80	67.20-95.10		
MCH (pictogram)	*Mean(SD)*	27.50(±2.69)	27.59(±2.75)	0.103	0.918
	Range	22.70-31.60	21.20-33.70		
MCHC (%)	*Mean(SD)*	33.67(±1.36)	33.83(±1.62)	0.317	0.753
	Range	31.50-35.70	30.20-36		
RDX	*Mean(SD)*	14.44(±1.84)	13.86(±1.39)	1.123	0.269
	Range	12.60-18.80	12-16.80		
Platelet	*Mean(SD)*	229.13(±59.07)	234(±71.21)	0.222	0.825
	Range	128-337	130-376		
ALT (IU/L)	*Mean(SD)*	21.87(±4.34)	16.12(±4.31)	**3.226**	**< 0.001****
	Range	16-30	10-23		
AST (IU/L)	*Mean(SD)*	27.20(±12.98)	15.96(±6.09)	**3.298**	**< 0.001****
	Range	13-55	10-28		
Urea (mg/dl)	*Mean(SD)*	35.07(±8.80)	35.44(±8.45)	0.133	0.895
	Range	24-49	23-50		
Creatinine (μmol/L)	*Mean(SD)*	1.07(±0.28)	1.02(±0.22)	0.582	0.564
	Range	0.80-1.70	0.6-1.6		
Bilirubin (μmol/L)	*Mean(SD)*	0.37(±0.10)	0.55(±0.42)	1.010	0.312
	Range	0.22-0.52	0.27-1.62		
8OHdG (U/ml)	*Mean(SD)*	640.28(±350.65)	756.59(±360.04)	1.356	0.175
	Range	159.10-1078	211.70-1156		
Lead (μg/dl)	*Mean(SD)*	12.75(±6.85)	12.78(±6.51)	0.028	0.978
	Range	5 -26.20	5-27		

Smoking index: number of packs per day× number of years, TLC: total leucocytic count (cells per cubic millimeter (c/cmm)), RBCs: red blood cells count (c/cmm), Hb: hemoglobin level (grams per deciliter), Hematocrit: The ratio of the volume of red cells to the volume of whole blood, MCV: mean corpuscular volume (femtoliters), MCH: Mean cell hemoglobin (pictograms), MCHC: Mean cell hemoglobin concentration (%), RDX: Red cell distribution width, ALT: alanine transaminase (IU/L), AST: aspartate transaminase (IU/L), 8OHdG :8 hydroxyguanosine (U/ml), SBP: Systolic blood pressure (mmHg), DBP: Diastolic blood pressure (mmHg), **Highly significant.

Table 4 showed high statistically significant elevation in mean ALT and AST among those with GSTP mutant gene than in those with wild gene. No significant difference of the rest of laboratory data was noted.

Also, non-significant difference between exposed workers with GSTP mutant and wild gene polymorphisms as regards studied clinical and cardiovascular manifestation (data not shown).

Table (5): Frequency distribution of clinical manifestations among exposed workers with GSTT null and nun-null gene polymorphisms.

		GSTT null No =14		GSTT non-null No =26		X^2	p
		No	%	No	%		
a. General	**Anorexia, nausea, vomiting**	5	35.7	3	11.5	3.324	0.068
	Metallic taste	6	42.9	12	46.2	0.040	0.842
	Bony aches	7	50	7	26.9	2.130	0.144
	Flushing	9	64.3	9	34.6	3.237	0.072
	Memory troubles	4	28.6	7	26.9	0.012	0.911
	Easy fatigability	5	35.7	7	26.9	0.335	0.563
b. Work accidents	**Accidents**	0	0	9	34.6	**6.253**	**0.012***
c. Eye	**Cataract**	7	50	12	46.2	.054	0.816
	Diminution of vision	10	71.4	15	57.7	0.733	0.392
d. Skin	**Contact dermatitis**	5	35.7	11	42.3	1.758	0.185
	Skin discoloration	9	64.3	11	42.3	1.758	0.185
e. Respiratory	**Bronchial hypersensitivity**	8	57.1	18	69.2	0.584	0.445
	Allergic rhinitis	6	42.9	12	46.2	0.040	0.842
f.Reproductive troubles		7	50	15	57.7	0.218	0.641
g. Cardiovascular manifestations							
IHD		9	64.3	8	30.8	**4.183**	**0.04***
ECG changes		6	42.9	10	38.5	0.073	0.787
Headache		8	57.1	14	53.8	0.040	0.842
Dizziness		2	14.3	5	19.2	0.154	0.695
HTN		8	57.1	9	34.6	1.890	0.169
SBP	*Mean(SD)*	133.93 (±17.12)	128.08 (±16.98)			1.037	0.306
	Range	110-165	110-180				
DBP	*Mean(SD)*	83.21 (±11.37)	81.54 (±8.81)			0.518	0.607
	Range	70-100	70-110				

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.

Table 5 showed that work accidents are more prevalent among exposed workers with nun null GSTT gene (P=0.012) and significant higher prevalence of IHD among exposed workers with null GSTT carriers. No otherwise statistically significant variation in distribution of studied clinical and cardiovascular manifestations.

Among laboratory investigations a significant higher mean level of creatinine and lower mean level of AST, was noted among exposed workers with nun null GSTT carriers (data not shown).

Discussion

Being one of the genes coding for xenobiotic metabolizing enzymes and an important line of defense in the protection of cellular components against reactive species (Di Pietro et al., 2010), GST(Glutathione S-transferase) intact genes appear to form a protection mechanism against chemical hazards (Cai et al., 2001). Accordingly, the current study investigated the associations between genetic variability in specific GST genes (GSTM1, GSTT1, and GSTP1) and susceptibility to nitroaromatic compounds health hazards. It may explain why individuals with seemingly equal exposure to nitroaromatic compounds develop hazards in an unpredictable manner. Recognizing role of individual susceptibility aiding in improving workers health through identifying those more susceptible and setting better protective strategies for them.

On the other hand although GST reaction generally results in detoxification, in selected cases GST mediated conjugation may lead to a more toxic or mutagenic metabolite (Moore et al., 2010).

Within GST identified allelic gene classes variations, GSTM1, GSTT1 and GSTP1 allelic genes variation received the most attention in genetic epidemiological studies (Di Pietro et al., 2010) and thus they are our study targets.

Our work recorded the results of the comparison between intact and polymorphic forms of GST genes (GSTM, GSTP and GSTT) as regards all studied health effects.

GSTM gene polymorphism was studied were GSTM null gene group is that gathering individuals homozygous for the GSTM1 thus exhibiting loss of GSTM1 enzymatic activity (Table 2-3).

In this study significant lower values of Hb, Hematocrit value, Urea and Bilirubin levels were detected among the exposed workers with null gene in comparison with nun-null gene group (Table 3). These findings suggest that GSTM enzyme metabolizes nitroaromatic compounds studied to another form more toxic on hematological system but less toxic on the kidney and the liver .

Regarding clinical manifestations, significant higher prevalence of flushing among exposed population with absent GSTM gene, while non significant difference of the rest of clinical manifestations (Table 2). Sabbioni and his colleagues in 2006 studied many health hazards as inertia, nausea, insomnia, somnolence, dizziness, and headache in relation to GST (GSTM, GSTP, GSTT) polymorphism. Workers with the GSTM1-null genotype also more often suffered from nausea and dizziness while those with a functional GSTM1 gene had a higher risk of suffering from headache and insomnia.

In order to detect the relationship between the activity of GSTs and susceptibility to Trinitrotoluene (TNT) cataract, Xu and his colleagues in 2002 studied the activity of GSTs enzymes among TNT cataract group in comparison with non TNT cataract and healthy non exposed groups. GSTs activity was significantly lower among TNT cataract group than in both other groups. Lowering in GSTs activity was correlated with GSTM gene deletion, concluding that TNT cataract and its stages are closely correlated with the activity of GSTs.

Xu and co-workers in 2001 examined the rates of GSTM gene deletion in 154 cases of TNT cataract patients, 41 cases of cataract patient by age or diabetes and 40 healthy persons. Results showed significantly higher rate of GSTM gene deletion in TNT cataract group than that of other two groups.

Close correlation of TNT cataract with GSTM gene deletion proving that GSTM gene deletion may be one of the important hereditary factors for TNT induced cataract susceptibility.

GSTP polymorphism was studied where GSTP mutant gene denotes single neoclotide polymorphism leading to amino acid substitution from Ile to Val changes catalytic activity of the GSTP1 enzymes (heterozygous genotypes as no homozygous mutant genotypes were found in our study). GSTP wild genotype denotes normal gene (Tables 4-5). Results showed significantly higher levels of liver enzymes (ALT, AST) and higher incidence of IHD among the group with mutant gene.

Among the exposed workers, chromosomal aberrations were more frequent among carriers of GSTP1 mutant allele than among wild gene group. These data suggest that GSTP1 variant allele may be associated with a lowered protection against reactive metabolites of TNT (Sabbioni et al., 2006).

The workers employed in a typical ammunition factory in China were examined as regards health effects as hepatomegaly, splenomegaly, cataract, mutagenicity and chromosomal aberrations and their relation to individual susceptibility. The genotypes polymorphism of GSTs (GSTM1, GSTT1, GSTP1) were determined. GST genotypes were found to be not associated with health effects (Sabbioni et al., 2007).

These results indicate that using a set of well-selected biomarkers may give more information regarding exposure and effect than routinely performed chemical measurements of pollutants in the air or on the skin (Sabbioni et al., 2006).

Personal susceptibility to occupational toxins in general and particularly nitroaromatic compounds is still a new era of research with very limited studies. Role of certain gene polymorphism on exposure to certain chemical couldn't be surely defined with one or few research studies. It needs a lot of supporting researches on the studied gene in relation to occupational exposures and in correlation with other xenobiotic metabolizing genes. It seems like connecting dots to define the figure clearly.

Conclusion

Despite the strong causal associations between certain occupational exposures and related health hazards, still there are differences in disease prevalence between workers that cannot be accounted for by difference 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.

Recommendations

Further studies are recommended on a larger scale including bigger number of ammunition workers to confirm the previous findings related to the role of individual susceptibility in nitroaromatic compounds toxicity and to study the net effect of multiple contributing genes.

Conflict of interest

The authors declare that no conflicts of interest exists.

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