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The interplay between the potential toxicity of inhaled volatile organic compounds and polycyclic aromatic hydrocarbons with detoxifying glutathione S-transferase and cytochrome peroxidase

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Abstract

Volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs) stemming from research labs represent potential threats to public health. This study aimed to identify the profile of organic air pollutants (VOCs and PAH) in research laboratories and the diversity of genetic polymorphism of detoxification enzymes (GSTP1, GSTM1, GSTT1, and CYP2E1) as well as serum immunoglobulins (IgE and IgA) among research workers. A descriptive cross-sectional study that included 75 workers from agriculture research labs (ARL) and chemistry research labs (CRL). The main study parameters comprised VOCs and PAHs in the air, serum immunoglobulins (IgE and IgA), and genomic DNA for glutathione-S-transferase (GST) and cytochrome peroxidase (CYP) genes. The concentrations of personal exposure of VOCs could be estimated from the prediction equation as 0.9*concentrations of VOCs at work environment in the same lab. The cumulative gas chromatography-8 working hours-∑ total VOCs concentrations were higher in CRL by about 13.5-32.4% than values in ARL due to different lab activities. Elevated but similar concentrations of benzene and toluene exceeding Egyptian threshold limit value were met in ARL and CRL. PAHs concentrations were within 7-11 ng/m^3. Those with null GSTT1, GSTM1 and GSTP1 AA genotypes have a double decrement in the activity of the detoxifying enzyme. The odds ratio of 0.2 times (protective) indicates that elevated IgA is 5 times less likely in present (GSTM1 &/or GSTT1) genotypes compared to the null subgroup, showing that GST genes were protective from elevated IgA. Workers carrying null GSTT1 & GSTM1 genotypes are at higher risk making them prone to oxidative stress-associated diseases and VOCs and PAHs induced carcinogenesis.

Keywords: detoxifying glutathione S-transferase gene; cytochrome peroxidase gene; immunoglobulin E; immunoglobulin A**;** volatile organic compounds, polycyclic aromatic hydrocarbons

1. Introduction

In pursuit of scientific advancements, research laboratories around the world play a pivotal role. However, various activities and experiments conducted in these labs might lead to unintended consequences. The emission of volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs) in research labs may impair indoor and outdoor air quality and pose potential threats to public health. VOCs are pollutants that are often associated with human activities. Indeed, VOC concentrations measured in the indoor air exceed outdoor air concentrations up to 10 times [1]. Most pollutants have proven carcinogenic and mutagenic properties [2]. The most widespread source of PAH is the inhalation of exhaust gases, tobacco smoke, and the usage of products and materials with PAHs [3]. Laboratories offer an example of special microenvironments in research centre buildings, in which specific pollutant concentrations may be elevated depending on the nature of the experiments conducted and the attitude of people working [4].

Most situations involve prolonged exposure to low concentrations of chemical agents that might impair health after relatively long durations of exposure. Therefore, it is not easy to explore causal relationships. Biomarkers of genotoxicity, whose analysis allows the assessment of genetic and cellular damage, are becoming promising techniques for toxicological and carcinogenicity studies. Genes involved in oxidative stress are candidates for studying air pollution versus gene interactions[2].

Exposure to organic solvents and other environmental pollutants produces free radicals, which, if present in excess, can harm tissue. The human body has, nevertheless, evolved defences against them. Antioxidants, which are classified as either enzymatic or non-enzymatic, are the constituents of these pathways. Enzymes including glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) are included in the first group. The second group consists of minerals (zinc, copper, selenium, manganese), polypeptides (thioredoxins, glutaredoxins, and sulfiredoxins), and low molecular weight antioxidants, such as ascorbic acid (vitamin C), glutathione (GSH), or metallothioneins (MTs) [5]. Understanding the contribution of GST genes may improve screening diagnostic assays for various cancers [6]. The key role of

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GSTM1 lies in detoxifying the electrophilic xenobiotics and environmental pollutants. GSTT1 isoform helps the conjugation of oxidized lipids and halogenated compounds. The safe removal of toxins by conjugation with glutathione saves cells from oxidative damage and DNA mutation, potentially altering the rate of cellular stability. A meaningful relationship is seen between the risk of cancer occurrence and xenobiotic metabolism enzyme gene polymorphism [7].

Cytochrome P450 (CYP) is one of the major enzymes responsible for the phase I metabolism of various environmental chemicals, carcinogens, and drugs. Cytochrome P450 family 2 subfamily E member 1 (CYP2E1) has attracted more interest than the other CYPs due to its role in the bioactivation of low-molecular-weight xenobiotics and their transformation into potential carcinogenic or hepatotoxic metabolites [8].

Immunoglobulin A (IgA) plays a pivotal role in mucosal homeostasis in the gastrointestinal, respiratory, and genitourinary tracts, functioning as the dominant antibody of immunity. It has a crucial role in protection against antigens. IgA production is greater than all other immunoglobulin subtypes, necessary for the multiple roles it plays systemically. Environmental changes may affect the IgA concentration. Due to the adverse effects of air pollution on health and considering the immunologic role of IgA, air pollution may cause changes in IgA secretion levels [9]. IgE is a marker of allergic reaction and is a key part of the immune response to allergens. Air pollution might have correlations with IgE-mediated allergic diseases [10].

This study aimed to identify the profile of organic air pollutants (VOCs and PAH) in research laboratories and the diversity of genetic polymorphism of detoxification enzymes (GSTP1, GSTM1, GSTT1, and CYP2E1) as well as serum levels of immunoglobulins (IgE and IgA) among research workers.

SUBJECTS AND METHODS

Environmental air assessment

VOCs

Individual VOCs were measured using charcoal tubes and low-volume sampler. VOC samples were collected according to a standard method developed by NIOSH using activated charcoal tubes (ORBOTM-32 activated coconut charcoal (20/40)) for the collection [11]. The collection tubes (Supelco, Inc., Bellefonte, PA) contained 150 mg of coconut charcoal sub-divided into two portions of 100 and 50 mg: the front portion of 100 mg was used to collect the VOCs, while the 50 mg backup section was intended to determine if solvent breakthrough occurred from the front section. Air was drawn through the charcoal tubes, using a low-volume sampler (calibrated vacuum pump) to draw 0.20 L/min. After sampling time, the charcoal tube was removed from the sampling train and two open sides were tightly closed using special caps to avoid any desorption.

During the sampling procedure, one charcoal tube was opened at the sample site and then the ends were capped, which served as a blank. The samples and blank tubes were put into special plastic bags that were tightly closed and kept in a freezer until processed not more than 15 days. Before analysis, all samples and blanks were taken from the freezer, contents of both sections of the adsorber tubes were transferred to glass test tubes containing 2 ml of distilled carbon disulfide (CS_2). The tubes were shaken by using a mechanical shaker and left sitting for at least 1 h to obtain the final sample solution. Immediately after this, a 2 ml aliquot was withdrawn from the samples, including the blank samples, and injected into a gas chromatography (GC).

GC assessed cumulative concentrations of 50 individual VOCs throughout 8-working hours in the work environment. The GC-8 working hours-sum of 50 individual VOCs (Cumulative GC-8h-∑TVOCs) were calculated. The average individual concentrations (mg/m^3) of four VOCs (benzene, toluene, ethyl benzene, and xylene) known as BTEX were calculated for agriculture research labs (ARL) and chemistry research labs (CRL). The concentrations of instantaneous total volatile organic compounds (ITVOCs) were monitored using Aeroqual's 500 portable air quality monitors to assess the work environment (middle zone of the lab) and the personal exposures in the respiratory zone of the researchers during work hours.

PAHs

Suspended Particulate Matter (SPM) samples were collected from research labs. The samples collected were used to figure out PAHs. Samples were collected through glass fiber filters of Whatmann GFA type (10 cm in diameter) by using a low-volume sampler. Loaded filters were weighed before and after sampling to evaluate the weight of suspended particulate matter. Samples were put in a glass vial and extracted with 10 ml of dichloromethane/n-hexane (1:1) in an ultrasonic bath for 10 min, three times at room temperature. The extract was transferred to a clean vial and concentrated to about 2 ml using a rotary evaporator. The concentrated extracts were fractionated by column chromatography.

A column filled with 2 gm anhydrous sodium sulfate and 10 gm silica gel (70 –230 mesh, ASTM bought from Merck) to remove water. Silica gel was activated at 120 C° for 8 h, 5% of water was added to deactivate the material. The PAH were collected in the second fraction and was eluted with 20 mL of n-hexane/dichloromethane (1:1, v:v). Qualitative and quantitative determinations of individual PAHs were done by Gas chromatograph (GC) [12,13]. GC using conventional packed columns can find the 16 EPA PAHs.

The concentrations of individual PAHs were calculated and expressed in ng/m^3. The GC was calibrated with a diluted standard solution of 16 PAH compounds (Supelco, Inc., Bellefonte, PA). The standard PAHs mixture (2000 µg/ml for each) containing: naphthalene (NAP); 2-bromonaphthalene (2-BNAP); acenaphthylene (ACY); acenaphthene (ACE); fluorene (FLU); phenanthrene (PHE); anthracene (ANT); fluoranthene (FLT); pyrene (PYR); benz[a]anthracene (BAA); chrysene (CRY); benzo[b]fluoranthene (BBF); benzo[a]pyrene (BAP); dibenz[a,h]anthracene (DBA); benzo[ghi]perylene (BGP) and indeno[1,2,3-cd]pyrene (IND). Gas chromatography-8-working hours-sum of polycyclic aromatic hydrocarbons (GC-8h-∑PAHs) of 16 PAH compounds were calculated for ARL and CRL.

Study design and population

The study was a descriptive cross-sectional study that included 75 research workers from the National Research Centre from 9 chemistry and 4 agricultural research laboratories. The workers are exposed to mixtures of small and diverse types of pollutants in their labs. Ethical approval of the study was obtained from the Medical Research Ethics Committee (20177). Written informed ethical consent was obtained from all participants before enrolling in the study. The study included males (n=39) and females (n= 36) aged 31-87 years with a minimum duration of exposure of 10 years. Workers with acute infections and history of diabetes were excluded. There were no significant differences between ARL and CRL groups as regards gender and age. The study protocol was approved by the Medical Research Ethics Committee - National Research Centre, Egypt no. 20177. The study was conducted according to principles set by the Declaration of Helsinki and all respondents provided informed consent

Data collection and laboratory assessments

A short questionnaire was filled by the research workers including personal, occupational, and medical data. Five milliliters of blood were withdrawn from all participants and prepared for immunology and genetic analysis (EDTA). Specimens were stored at -80 ºC.

Immunoglobulins

IgA was measured using a Human IgA ELISA kit, Sino Gene Clon Biotech Co., Ltd, China. IgE was measured using a Human IgE ELISA kit, BIOS Microwell Diagnostic systems, PerkinElmer Health Sciences, Inc, CA, USA.

Genetic analysis

Genomic DNA was isolated using QIAamp® DNA Mini and Blood Mini (Qiagen, Germany) cat no 51104 following manufacturer, protocol, and assessment of DNA yield concerning concentration and purity was performed via Nanodrop 2000 [Thermo Scientific, USA] with average conc. and purity at 260/280 of 25.8±16.1 ng/µl and 1.7±0.1, respectively.

The polymorphisms of GSTT1 and GSTM1 were investigated by multiplex polymerase chain reaction. The PCR reaction was carried out via Vforward and MAL Cycler (Applied BIOsystems, USA) in an ultimate volume of 25 µl containing 10 µl of a ready-made Master mix (Thermo Fisher Scientific) from each of the primers; forward and reverse 1.5 mmol/L along with an internal positive control (CYP1A1) were used and DNA yield at a concentration of 100ng/µl was added to the material. Amplification was performed through preliminary denaturation at 94 °C for 2 minutes, followed by f 35 PCR cycles at 94 °C for one minute, 59°C for one minute, and 72 °C for one minute, and a final extension step of 72 °C for 10 minutes. Amplified products were identified by electrophoresis in a 2% agarose gel and were stained with ethidium bromide. Product size was 480 bp, 215 bp, and 312 for GSTT1, GSTM1, and CYP1A1, respectively. The T1M1 genotype was specified by two bands of 480 bp for GSTT1 and 215 bp for GSTM1, the T1M0 genotype showed one band of 480 bp, while it was 215 bp for the T0M1 genotype. For the T_0M_0 genotype (deletion of two genotypes), no bands were shown, hence the use of CYP1A1as internal positive control was essential to diagnose the null genotype from aborted PCR responses.

GSTT1 FORWARD PRIMER: 5'- TTCCTTACTGGTCCTCACATCTC-3'
GSTT1 REVERSE PRIMER: 5'- TCACGGGATCATGGCCAGCA-3'
GSTM1 FORWARD PRIMER: 5'- GAACTCCCTGAAAAGCTAAAGC-3'
GSTM1 REVERSE PRIMER: 5'-GTTGGGCTCAAATATACGGTGG-3'
CYP1A1 FORWARD PRIMER 5' GAACTGCCACTTCAGCTGTCT-3'
CYP1A1 REVERSE PRIMER: 5'- CAGCTGCATTTGGAAGTGCTC-3

GSTP1 polymorphism (A 313 G) was figured out by a PCR-based restriction fragment length polymorphism (RFLP) technique. Purified DNA was used to amplify 176 bp fragment via VERITI Thermal Cycler (Applied BIOsystems, USA) using the following thermal profile; an initial denaturation step at 94 °C for two minutes, followed by f 35 PCR cycles at 94 °C for one minute, 59°C for one minute and 72 °C for one minute, and a final extension step of 72 °C for ten minutes

GST P1 FORWARD PRIMER: 5'- ACCCCAGGGCTCTATGGGAA-3'
GST P1 REVERSE PRIMER: 5'- TG AGGGCACAAGAAGCCCT-3'

A yield of 176 bp fragments was produced, this PCR product was subjected to restriction digestion by Alw261 and electrophoresed in 3% agarose gel. The presence of the restriction site revealed two fragments 91 bp and 85 bp denoting the G allele i.e., the presence of wild type (GG). The heterozygous genotype (AG) was characterized by 3 fragments consisting of 176 bp, 91 bp, and 85 bp. Homozygous mutant genotype (AA) was confirmed by a single 176 bp fragment.

CYP2E1 gene polymorphism was studied after DNA amplification using PCR- RFLP method. A DNA of 552 bp was obtained using the following primers and thermal profile of initial denaturation at 95 °C for two minutes, followed by f 35 PCR cycles at 94 °C for one minute, 57°C for one minute, and 72 °C for one minute, and a final extension step of 72 °C for ten minutes.

CYP2E1 FORWARD PRIMER: 5'-CCAGTCGAGTCTACATTGTCA-3'
CYP2E1 REVERSE PRIMER: 5'-AGACCTCCACATTGACTAGC-3'

Ten µl of the amplified 552bp PCR product was subjected to ten units of Rsal restriction enzyme in a final volume of 30 µl. The restricted product was analyzed by electrophoretic separation in 2.5% agarose gel and visualized by ethidium bromide. Rsal digestion showed three CYP2E1 genotypes; the homozygous c1/c1 (wild type) with two product fragments at 352 and 200 bp, and the resistant c2 allele showed the undigested band at 552 bp. finally, the c1/c2 type was identified by three fragments at 352, 200, and 552 bp.

Statistical Analysis

Statistical analysis was performed using SPSS-23 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, USA) and Microsoft Excel Sheets 365. The α-error was set at p-value<0.05 and a power 0.8. Chi-square tests were used to compare percentages. The odds ratio was used to report the strength of association between two variables or to estimate the risk. Linear correlation was used to measure the dependence between random variables. Linear regression analysis of P-ITVOCs as a dependent variable was used to predict concentration of P-ITVOCs from the concentration of W-ITVOCs.

Results

Table 1: Instantaneous P-ITVOCs and W-ITVOCs concentrations in the personal exposure and work environment respectively, and the cumulative GC-8h-∑TVOCs and Cumulative GC-8h-∑PAHs concentrations in the work environment in ARL and CRL.

ARL					CRL				
Lab #	Personal exposure	Work environment			Lab #	Personal exposure	Work environment		
	P-ITVOCs	W-ITVOCs	Cumulative GC-8h-∑TVOCs	Cumulative GC-8h-∑PAHs		P-ITVOCs	W-ITVOCs*	Cumulative GC-8h-∑TVOCs	Cumulative GC-8h-∑PAHs
	Conc. mg/m^3	Conc. mg/m^3	Conc. mg/m^3	Conc. ng/m^3		Conc. mg/m^3	Conc. mg/m^3	Conc. mg/m^3	Conc. ng/m^3
ARL1	35.5	54.6	-		CRL1	245.3	292.0	1348.5	8.2
ARL2	34.0	51.6	1142.8	9.1	CRL2	149.7	153.0	1469.1	7.0
ARL3	34.3	49.8	1109.4	9.03	CRL3	126.7	131.0	-	-
ARL4	26.4	40.6	-		CRL4	76.3	89.0	-	7.7
					CRL5	54.2	62.5	1262.0	-
					CRL6	49.5	51.0	1239.5	9.3
					CRL7	31.4	46.4	1362.6	11.0
					CRL8	29.9	38.7	1312.6	9.3
					CRL9	24.9	34.6	1262.0	8.0

ARL, agriculture research labs; CRL, chemistry research labs.
P-ITVOCs, personal exposure to instantaneous total volatile organic compounds (portable air quality monitor).
W-ITVOCs, Work environment instantaneous total volatile organic compounds (portable air quality monitor).
GC-8h-∑TVOCs, Gas chromatography-8 working hours-sum of individual VOCs (50 compounds).
GC-8h-∑PAHs, Gas chromatography-8-working hours-sum of polycyclic aromatic hydrocarbons (16 compounds).

There were marked variations in concentrations of P-ITVOCs and W-ITVOCs in CRL ranging from [34.6-292.0 mg/m^3] and [24.9 up to 245.3] mg/m^3 respectively. The cumulative GC-8h-∑TVOCs concentrations were higher in CRL by about 13.5-32.4% than values in ARL. ∑PAHs concentrations were within 7-11 ng/m^3 in the different agricultural and chemistry labs [Table 1]. Also, there was a strong positive correlation between concentrations of P-ITVOCs and W-ITVOCs (r=0.991_p< 0.001). Using linear regression analysis of P-ITVOCs as a dependent variable, it was found that P-ITVOCs can be predicted from W-ITVOCs in the same lab from the following equation: concentration (P-ITVOCs) = 0.9 * concentration (W-ITVOCs) (p-value< 0.001; constant was non-significant; R^2 =98.2%; F & p-value (ANOVA)=597.3, 0.000) . The significant predictor concentration (W-ITVOCs) explained 98.2% (R^2= 98.2%) of the model of the dependent variable (concentration (P-ITVOCs)) which is a very good fit.

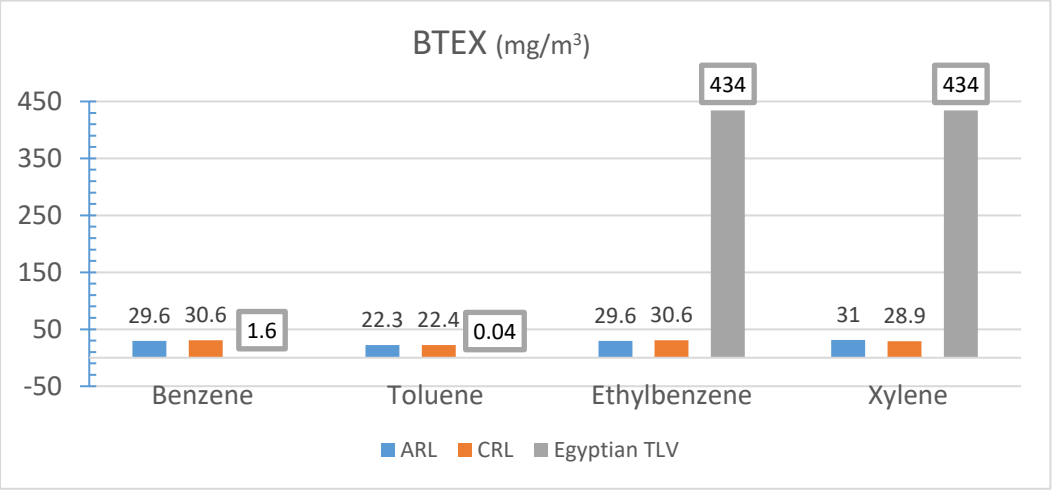

Figure 1: The average concentration of GC-8 working hours-of BTEX VOCs (mg/m^3) at the work environment in ARL and CRL compared to Egyptian threshold limit values (TLV) [14]

BTEX: 4 VOCs : benzene, toluene, ethyl benzene, and xylene; ARL, agriculture research labs; CRL, chemistry research labs

The average concentrations of BTEX were nearly similar in both ARL and CRL with values between 22.3 and 30.9 mg/m^3 [Figure 1].

Table 2: Percentages of genotypes of Glutathione S-transferases (GSTT1, GSTM1, and GSTP1) and human cytochrome P450 (CYP2E1) among ARL and CRL

	ARL N=47	**CRL** N=28		
	n (%)	n (%)	X^2	p-value
Males Females	29 (61.7) 18 (38.3)	10 (35.7) 18 (64.3)		NS
Age <50 years Age ≥50 years	17(36.2) 30(63.8)	16(57.1) 12(42.9)		NS
Genetic variants				
GST Genotypes				
GSTT1/GSTT1	18 (24.0)	17(22.7)	9.4	0.024*
GSTM1/GSTM1	4 (5.35)	2 (2.7)		
GSTT1/ GSTM1	17 (22.7)	9 (12.0)		
Null GST (T_0M_0)	8 (10.7)*	0 (0.0)*		
GSTP1 Genotypes				
AA	27 (36.0)	6 (8.0)	9.7	0.008**
GG	2 (2.7)	2 (2.7)		
AG	18 (24.0)	20 (26.7)		
G allele (presence vs absence)				
G (GG or AG)	20 (26.7)	22(29.3)	7.8	0.005**
Absence of G (AA)	27 (36.0)	6 (8.0)		
A allele (presence vs absence)				
A (AA or AG)	45(60.0)	26(34.7)	0.000	NS
Absence of A (GG)	2 (2.7)	2 (2.7)		
GSTT1, GSTM1 presence and null vs GSTP1 genotypes				
GSTT1/GSTT1 AA GG AG	 11/18 (61) 0 (0.0) 7/18 (38.9)	 5/17 (29.4) 0 (0.0) 12/17 (70.6)		
GSTM1/GSTM1 AA GG AG	 2/4 (50) 0 (0.0) 2/4 (50)	 0 (0.0) 0 (0.0) 2/2 (100)		
GSTT1/GSTM1 AA GG AG	 14/17 (82.3) 2/17 (11.7) 9/17 (52.9)	 1/17 (5.8) 2/9 (22.2) 6/9 (66.6)		
Null GST T_0M_0 AA GG AG	 6/8 (75) 0 (0.0) 2/8 (25)			
CYP2E1 genotypes				
C1/C1	39(52.0)	22(29.3)		NS
C1/C2	8(10.7)	6(8.0)		
C2/C2	0 (0.0)	0 (0.0)		

ARL, agriculture research lab; CRL, chemistry research lab; NS, non-significant (p-value ≥0.05)
GSTP1, Glutathione S-transferase pi 1 GSTT1, Glutathione S-transferase theta 1
GSTM1, Glutathione S-transferase mu 1 CYP2E1, Cytochrome P450 family 2 subfamily E member 1
GSTT1 allele = GSTT1/ GSTT1 + GSTT1/ GSTM1
GSTM1 allele = GSTT1/ GSTM1 + GSTM1/ GSTM1
GSTT1 or GSTM1 allele = GSTT1/ GSTT1 + GSTT1/ GSTM1 + GSTM1/ GSTM1
Null (M0T0) = neither GSTT1 nor GSTM1 alleles were present

The null genotype of GST is significantly higher in researchers in ARL (8, 10.7%) than those in CRL (0, 0%) (p-value= 0.024). In the context of GSTP1, genotype AA showed a statistically significant higher percentage in researchers in ARL than those in CRL (p-value = 0.008). In addition, the percentage of absence of the G allele is statistically significantly higher among those in ARL than those in CRL (p-value= 0.005). In ARL, workers carried GSTT1/GSTT1 genotype 61% harbor GSTP1 AA genotype, and 38.9% AG (the addition not =100), while in CRL, 29.4%, and 70.6%, respectively. Those carry GSTM1/GSTM1 in ARL workers, 50% harbor GSTP1 AA, and 50% AG while 100% in CRL harbor AG. Regarding GSTT1/GSTM1 in ARL 82.3% harbor AA, 11,7% GG, and 52.9% AG. While in CRL 5.8% AA, 22.2% **GG,** and 66.6% AG. Null genotype (T_0M_0) found in ARL workers only which harbor 75% AA, and 25% AG [table 2].

Table 3: Relation between immunoglobulin (IgE, IgA) serum levels with genotypes of Glutathione-S-transferase (GST) genes in the study research lab workers.

Immunoglobulin levels (n=75)			***Test	p-value OR [CI]
Mean serum IgE (µg/ml)* high IgE ≥ 150 IU/ml (Total) ARL (no./%) CRL (no./%)	250.0±271.1 [13.9 - 1117.7] 38/75 (50.7%) 22/ 47 (46.8%) 16/28 (57.1%)			
Mean serum IgA (µg/ml)* Low IgA < 0.5 µg/ml High IgA≥ 13.51 µg/ml	6.4±6.4 [0.48 - 24.7] 1 (1.3%) 14 (18.7%)			
Mean serum level IgA (µg/ml) in different subgroups (studied labs, and genotypes)	ARL workers (n=47) CRL workers (n=28)	8.8± 6.6 2.7±3.0	6.0	0.000**
	Null (T_0M_0) (n=8) GSTM1 or GSTT1 presence (n=67)	10.3±5.9 5.4±6.3	2.7	0.009**
Elevated serum level IgA ≥ 13.51 µg/ml	Null (T_0M_0) (n=8) GSTM1 or GSTT1 presence (n=67)	4(50.0%) 10(13.3%)	3.7	0.036* 0.18 [0.04-0.82]
	GSTT1 allele (n=61) Vs GSTM1/GSTM1, Null (n=14)	8(13.1%) 6(42.9%)	4.8	0.019* 0.20 [0.06- 0.73]
	GSTT1/GSTT1 (n=35) vs GSTT1/GSTM1, GSTM1 or null (T_0M_0) (n=40)	3(4.0%) 11(14.7%)		NS

ARL, agriculture research lab; CRL, chemistry research lab; NS, non-significant (p-value ≥0.05), * reference value for IgE and IgA is according to the used kit. ***(chi square/T test)
GSTT1 allele = GSTT1/ GSTT1 + GSTT1/ GSTM1
GSTM1 allele = GSTT1/ GSTM1 + GSTM1/ GSTM1
GSTT1 or GSTM1 allele = GSTT1/ GSTT1 + GSTT1/ GSTM1 + GSTM1/ GSTM1
Null (T_0M_0) = neither GSTT1 nor GSTM1 alleles were present

The mean serum levels of IgE among the study groups is (250.0±271.1 µg/ml). High IgE ≥ 150 IU/ml is found among 50.7% of all study participants. The mean serum level of IgA was significantly higher in researchers in ARL compared to CRL (p=0.000). The mean serum level of IgA was significantly higher in the null GST group (T_0M_0) than among those with GST presence (GSTT1/ GSTT1 + GSTT1/ GSTM1 + GSTM1/ GSTM1) (p= 0.009). Elevated serum IgA level (> 13.6 µg/ml) was less common in subjects with present GSTM1 &/or GSTT1 genotypes (GST) than in null genotypes (p=.036). The OR of 0.2 times (protective) means that elevated IgA is 5 times less likely in present (GSTM1 &/or GSTT1) genotypes compared to the null subgroup, showing that GST genes were protective from elevated IgA. Also, the OR of 0.2 times (protective) means that elevated IgA is 5 times less likely in present (GSTT1) allele compared to the other two subgroups (lacking GSTT1 allele), showing that the GSTT allele was protective from impaired IgA [table 3].

Discussion

The concentrations of W-ITVOCs can be used as a rapid and instantaneous guide to assess the emission of VOCs in the work environment. This can be a guide also to control elevation of ITVOCs above TLVs. There are no Egyptian TLV for ITVOCs or average GC-8h-∑TVOCs but the American reference value of TLV 60 mg/m^3 (CDPH, 2017) [14] are used. Environmental monitoring of W-ITVOCs can be used as a perfect assessment of personal exposure (P-ITVOCs). The P-ITVOCs concentration can be calculated as 90% of W-ITVOCs concentration as concluded from the prediction equation in our results. W-ITVOCs might be used, therefore, to estimate or substitute personal exposure in situations that requires saving money, effort and time. Environmental measurements are usually easier and less annoying to the workers than monitoring personal exposure. The Egyptian TLV of GC-8h-BTEX VOCs was markedly low for benzene (1.6 mg/m^3) and toluene (0.04 mg/m^3) and high for ethylbenzene and xylene (434 mg/m^3) [15]. Benzene and toluene concentrations exceeded the TLV in the present study which might indicate increased risk to the exposed workers. On the other hand, ethylbenzene and xylene concentrations were far below the TLV and consequently far below risk. There are no reference values available for PAH.

Benzene, toluene, and PAHs are universal environmental air pollutants. Presence of PAHs were attributed to laboratory combustion activities [16]. An earlier study showed that LMWT-PAHs originated from low-temperature combustion processes, while HMWT-PAHs originated from high-temperature combustion processes [17]. Kanu Priya et al., (2015) [18] found a high prevalence of null GSTT1 and GSTM1 genes in their study among the Indian group exposed to benzene, which agreed with our results. GST detoxifying enzymes engage in the metabolic detoxification of PAHs. Those people with null genotypes lack detoxification activity and are more prone to develop benzene, toluene, and PAH-induced diseases. The combined absence of the two genes in an individual is a major risk factor for the development of certain pathologies and renders him more prone to environmental carcinogenic compounds, having an increased risk for oxidative stress associated diseases [19]. In an Iranian study, the benzene exposed workers carrying null GSTT1 or both null GSTT1 and GSTM1 genotype were

more susceptible to benzene toxicity and had a higher risk of benzene-induced hematological disorders like leukemia, leukopenia, anemia, and thrombocytopenia [20].

Phase II enzymes such as GSTP1 (GG genotype) play an important role in protecting cells against damage induced by carcinogens [21]. In the present study, GSTP1, the wild type, was found only in two researchers in each study group. The researchers in CRL had better detoxifying enzyme activity compared to ARL group, as they harbored combined detoxifying activity of GSTT1, GSTM1, and GSTP1 compared to those in ARL. Moreover, those carrying GSTT1/GSTM1 showed better detoxifying activity. Hence, the researchers in ARL are more likely to be prone to diseases related to exposures to environmental pollutants as they had decreased GSTP1 detoxifying activity in addition to GSTT1 and GSTM1 decreased activity. This goes hand in hand with Hu *et al.*, [22], who previously mentioned that GSTP1 shows large inter-individual variations in the ability to detoxify the various active metabolites of PAH. They also added that GSTP1 (GG genotype) was the most active variant in addition to GSTM1 in the detoxification of the ultimate carcinogenic form of benzo[*a*]pyrene. GSTP1 and GSTM1 genetic polymorphism may be a crucial factor in differential susceptibility of humans for PAH-induced carcinogenesis. Moreover, in a Brazilian study, workers exposed to pesticides had higher frequency of genotype AA (52.6%) than controls (49.1%) [23], which agree with our findings. GSTs may change susceptibility in certain ethnic groups, showing ethnic dependent polymorphism. The frequencies of GSTM1 null alleles display race and ethnic variations, being highest in Europeans (42–60%) and Asians (41–63%) compared with that of Africans (16–36%) [24]. However, the frequency of GSTT1 null genotypes is less in Europeans (13.31%) compared with that of Africans (14–57%) and Asians (35–48%) [25]. A study done in Benin (West Africa), showed that the frequency of the null genotype T_0M_0 was higher among professional motorcycle drivers exposed to elevated levels of BTEX (63%) compared to non- professionals (33%) [26]. High prevalence of null GSTT1 and GSTM1 genes in an Indian study was noticed among the group exposed to benzene [27], in accordance with our results.

Cytochrome P-450 monooxygenases (CYPs) are Phase-I enzymes that function in the metabolic activation of PAHs and most VOCs [28]. It has been suggested that genetic polymorphism of the enzymes responsible for benzene metabolism such as CYP450 predisposes susceptible individuals to benzene toxicity [29]. As regards CYP2E1 in our study, the frequency of C1/C1 (wild) genotype was higher among researchers in ARL compared to CRL. This is in agreement with an earlier Brazilian study conveyed on workers exposed to pesticides which also found the percentage of C1/C1 genotype to be higher among exposed workers (98%) compared to the reference group (88.5) [23]. However, Nourozi et al., [20] found that genotype C1/C2 was higher in the benzene-exposed group compared to the reference group, although the difference did not reach statistical significance. Most studies also have failed to find any effect of CYP2E1 on biomarkers of exposure or effect. This may be due to the highly polymorphic nature of this gene which leads to a wide range of enzyme activity levels between individuals and considerable inter-individual variability in human CYP2E1 activity [23].

In the current study, the serum level of IgA was significantly higher among researchers in ARL compared to those in CRL as well as elevation of serum IgE among about 50% of the researchers in both studied ARL and CRL. Comparable results were seen in an earlier study done on bitumen workers exposed to PAHs, as elevated serum IgA level was seen among exposed workers compared to the control group, but this disparity was not significant [30]. Contrary to our results; an earlier Egyptian study that was done on fuel station workers exposed to benzene found a highly significant decrease in serum immunoglobulin IgA levels but similarly to our results they found an elevation of serum IgE among exposed workers compared to controls [31. Reduced serum levels of IgA were also seen in an earlier study which was done on coke oven workers exposed to PAH [32]. In agreement with our work concerning serum IgE, a previous Korean study which was done on adults exposed to PAH study showed that exposure to PAHs was associated with serum IgE elevation. They explained that absorbed PAHs are metabolized by Aldo-keto reductases, resulting in excessive generation of ROS; these ROS may affect the immune response and cause allergic reactions by enhancing IgE production [33].

Elevated serum level of IgA among ARL workers in the present study may be attributed to persistent exposure to environmental pollutants leading to chronic inflammation. Meirow and Baniyash [34] explained this previously as they mentioned that inflammation is a critical function of the innate immune system that protects against pathogens and consists of an immediate response to tissue damage caused by potentially harmful stimuli. Initially, inflammatory agents elicit an acute inflammatory response in which the immune system recognizes and removes harmful stimuli, but if the stimulus persists, chronic inflammation can occur, partly because of the activation of adaptive immunity. They added that environmental pollutants could induce chronic inflammation and then promote cancer development. As regards GST genotypes, our results showed that the presence of GSTM1 &/or GSTT1 was 0.18 times protective from elevation of IgA. Moreover, the GSTT1 allele only was found to be 0.2 times more protective from impaired IgA.

Conclusions

The results of this study may draw our attention to the researchers who lack full detoxifying GSTP1 activity. Those with null GSTT1 & GSTM1 and GSTP1 AA genotypes have a double decrement in the activity of the detoxifying enzyme. Elevated IgA is less likely in present (GSTM1 &/or GSTT1) genotypes compared to the null subgroup, showing that GST genes were protective from elevated IgA. Also, elevated IgA is less likely in present (GSTT1) allele compared to the other two subgroups (lacking GSTT1 allele), showing that the GSTT allele was protective from impaired IgA. Elevation of serum IgE among all the study groups suggests chronic inflammation and impaired detoxification due to frequent exposure to environmental pollutants. Those carrying null GSTT1 & GSTM1 genotypes (found in ARL) showed significantly elevated serum IgA compared to others carrying GSTT1 or GSTM1. This also confirms that they are at higher risk, making them prone to oxidative stress-associated diseases and VOCs and PAHs-induced carcinogenesis.

Conflicts of interest

There are no conflicts to declare.

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