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SOME LABORATORY PARAMETERS AND EPIGENETIC TESTING AMONG PRINTING WORKERS

By

El-Saka SF[1], Abou-ElWafa HS[1], Neamatallah MA[2], Elbestar SF[1]and Al-Wehedy A[1]

[1]Department of Public Health and Community Medicine, [2]Department of Medical Biochemistry, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Corresponding author**:** Abou-ElWafa HS: halasamir@mans.edu.eg

Abstract

Introduction: Printing workers are exposed to a variety of chemicals e.g. solvents, ink, that could exert potential health effects. **Aim of work:** to study some selected biochemical, hematological parameters, and epigenetic changes of four genes among Mansoura University Printing Press (MUPP) workers. **Materials and methods:** A cross-sectional study was conducted among 50 workers in (MUPP) and 50 administrative employees from January 1 to June 30, 2018. A questionnaire was used to study socio-demographic profile and some occupational characteristics, use of personal protection on duty, and hand hygiene facilities. Complete blood picture, some kidney function tests (serum creatinine, urea, and uric acid) and some liver function tests (serum albumin, bilirubin, and liver enzymes), and promoter regions methylation of four genes (P15, iNOS, CYP2E1, MAGE1) were investigated. **Results:** Both groups had nearly similar blood count. Printing workers had significantly higher serum creatinine ($p \leq 0.001$), uric acid ($p=0.02$), and liver enzymes ($p \leq 0.001$) however significantly lower serum albumin ($p=0.04$). Promoter regions methylation among printing workers was significantly higher for P15, iNOS, and CYP2E1 genes and MAGE1 promoter region un-methylation. There was no statistically significant association of promoter region methylation of P15 and iNOS genes with any of the socio-demographic and occupational characters of the studied groups. However, association between chemical exposure with methylation of CYP2E1 promoter region and association between age, duration of employment, and chemical exposure with un-methylation of MAGE1 promoter region were significant. **Conclusion:** Printing workers showed disorder of several laboratory parameters and some sort of promoter region methylation of studied genes in the form of significantly higher serum creatinine, uric acid, and liver enzymes while significantly lower serum albumin. They had significantly higher promoter regions methylation for P15, iNOS, and and MAGE1 promoter region un-methylation.

Key words: Printing workers, Solvent, Liver and kidney functions, Epigenetic, CYP2E1 genes and MAGE1 promoter.

Introduction

The printing industry is composed of many different types of business. Each printing process can be divided into 3 major steps: prepress, press, and postpress. Prepress operations include composition and typesetting, graphic arts photography, image assembly, and image carrier preparation (Decharat, 2014). Press refers to the actual printing operation. Postpress principally involves assembly of printed materials and consists of binding and finishing operations (PNEAC, 2013). The printing occupations have been linked to an increased mortality and morbidity from many diseases (Liu et al., 2002).

Workers in printing industries may be exposed to potentially hazardous levels of solvents, inks, adhesives, organic and inorganic pigments, polycyclic aromatic hydrocarbons, acrylates, lead, paper dust, and noise (IARC, 1996).

Workers in this industry are exposed to these agents through both inhalation and dermal contact (Decharat, 2014). Nearly, 20 % of these agents contain one or more products known or suspected carcinogens such as aromatic amines, toluene, lead, etc (Lynge et al., 1995). Potential health effects of chemicals used in printing include irritation of the skin leading to dermatitis, allergy and asthma (e.g. UV inks, laminating adhesives). Some solvent vapors can lead to dizziness, drowsiness and affect central nervous system and can also cause damage to internal organs (e.g. liver /kidney) if exposure is over a long period (HSE, 2014). An excess risk has been reported for several types of cancer sites among workers in the printing industry; however the findings were not consistent (Paganini-Hill et al., 1980; Leon, 1994; Leon et al., 1994).

In normal cells, the promoter region of p15 gene exhibits low or even no methylation and shows hypermethylation as the cell undergoes carcinogenesis process (Galm et al., 2006).

The MAGE family of genes belongs to a group of germ line-specific genes that are activated in different types of tumors. This family of genes was reported to direct the expression of a tumor-specific antigen that was recognized in a melanoma cell by cytolytic T lymphocytes. The MAGE-A1 gene has a CpG-rich promoter, which, unlike classical CpG-rich promoters, is methylated in all normal somatic tissues, except for the placenta and testis (Kim et al., 2006).

Nitric oxide synthase (NOS) gene is present on chromosome 17 and has been implicated in a wide variety of diseases. The nitric oxide synthase enzyme forms

nitric oxide that besides being a signaling molecule plays an important role in host immune response. Inducible nitric oxide synthase expression is regulated at the level of transcription (Qidwai and Jamal, 2010). Gene expression of human genes is controlled by DNA methylation (Tarantini et al., 2009).

The human CYP2E1 gene is functionally surprisingly well conserved compared with other cytochrome P450 enzymes active in drug metabolism, which suggests an important endogenous function in humans (Hu et al., 1996). CYP2E1 is a versatile phase I drug-metabolizing enzyme responsible for the biotransformation of most volatile organic compounds, including toluene. In the exposed workers, significant correlations between toluene airborne levels and CYP2E1 promoter methylation was found with subsequent gene repression (Jiménez-Garza et al., 2015).

Aim of work

To study some selected biochemical, hematological parameters, and epigenetic changes of four genes among Mansoura University Printing Press (MUPP) workers.

Materials and methods

Study design: This is a comparative cross-sectional study conducted upon printing workers.

Place and duration of the study: Mansoura University Printing Press (MUPP) located within the University campus during the period from January 1 to June 30, 2018.

Study sample: The study group (MUPP workers) comprised 50 workers with the longest duration of employment. The total number of Mansoura University Printing Press workers was 106; however, we included only 50 workers in the study due to cost limitations of assessment of DNA methylation. A comparison group, of 50 employees from administrative departments at faculty of Medicine, Mansoura University, was matched with MUPP workers in most of confounding factors apart from exposure to printing work environment.

Study methods: Each participant was subjected to the following:

1. **A self administrated questionnaire** was used to collect the following data: socio-demographic and occupational profile of workers, usage of personal protective measures, and facilities for hand hygiene.
2. **Laboratory investigations**: A 5 ml blood sample was withdrawn from each participant through venipuncture which was then divided into:

-1ml blood collected in a plastic tube containing EDTA for complete blood count (CBC) using Sysmex XP-300™ Automated Hematology Analyzer.

-2ml blood collected in dry plastic tube for liver function tests [including serum albumin, bilirubin and transaminases: Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)] and renal function tests [including serum urea, uric acid and creatinine]. Liver function tests were done using commercial kit according to manufacturer's instructions (ELITech, Puteaux, France). Renal function tests were determined colorimetrically using Randox assay kits, while serum creatinine was assayed by kinetic method using diagnostic kits (Human, Germany. Co) according to instructions of kit's manufacturer.

-2ml were collected in k2EDTA vacutainer tubes for DNA host analysis (for epigenetic testing) using Methylation Specific PCR (MSP) technique for promoter methylation status of P15, iNOS, CYP2E1 and MAGE1.The EDTA blood was then aliquoted and stored at -50^{O}C. The remaining blood sample was transported into plain tubes centrifuged at 4000 rpm for 10 min. Then the serum was separated and aliquoted into 250 µl volume and stored at – 50^{O}C until tested.

Consent

An informed consent was obtained from subjects who agreed to participate in the study before the start of work with assurance of confidentiality and anonymity of the data.

Ethical approval

The study was approved by Institutional Research Board (IRB) of Faculty of Medicine, Mansoura University. Approval of MUPP manager was obtained.

Data management

Data were analyzed using SPSS version 16. Qualitative data were described as numbers and percentages. χ^2test, Fisher's exact test, and Monte Carlo exact probability were used for comparison between groups, as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test and described as means (SD) or medians, as appropriate. In the normally distributed variables, independent sample t-test was used; while in non-normally distributed variables, Mann Whitney test was used for comparison between groups. Odds ratios and their 95% confidence interval were calculated. A statistically significant difference was considered at p value $\leq$0.05.

Results

Table 1: Socio-demographic and occupational profile of the studied groups.

Characteristics	MUPP workers No= 50		Comparison group No = 50		Test of significance
	No	%	No	%	
Age/ years					
< 40	13	26.0	19	38.0	χ^2=1.7, p=0.2
≥ 40	37	74.0	31	62.0	
Mean ± SD	44.4±8.6		41.4±10.8		t=1.5, p=0.14
Gender					
Male	22	44.0	26	52.0	χ^2=0.4, p=0.55
Female	28	56.0	24	48.0	
Level of education					
Illiterate/ read & write	11	22.0	6	12.0	χ^2=3.7, p=0.2
Primary up to secondary	31	62.0	29	58.0	
University and above	8	16.0	15	30.0	
Marital status					
Single	3	6.0	6	12.0	Fisher›s Exact test, p= 0.48
Ever married	47	94.0	44	88.0	
Residence					
Rural	13	26.0	17	34.0	χ^2=0.4, p=0.51
Urban	37	74.0	33	66.0	
Smoking habits##	6	12.0	8	16.0	χ^2=0.3, p=0.56
Duration of smoking /years Median (Min – Max)	17.5(11-32)		10 (2-41)		Z#=0.8, p=0.44
Duration of employment /years					
< 15	10	20.0	13	26.0	χ^2=0.5, p=0.47
≥15	40	80.0	37	74.0	
Mean±SD	21.4±7.1		19.3±6.04		t=1.6, p=0.11
Additional job	11	22.0	5	10.0	χ^2=2.7, p=0.1
Printing	8	72.7	0	0.0	
Other	3	27.3	5	100.0	
Previous job	21	42.0	11	22.0	χ^2=4.6, **p=0.03***
Printing	17	80.9	0	0.0	
Other	4	19.1	11	100.0	
Chemicals use at work	39	78.0	2	4.0	χ^2=56.6, **p≤0.001****
Machinery use at work	30	60.0	1	2.0	χ^2=39.3, **p≤0.001****
PPE use	0	0.0	2	4.0	χ^2=2.04, p=0.15
Workplace hand washing facilities					
Water and soap	32	64.0	50	100.0	χ^2=21.9
Water and detergent	15	30.0	0	0.0	MEP=**≤0.001****
Solvent e.g. gasoline	3	6.0	0	0.0	

#Z of Mann-Whitney test ##Cigarette and goza smokers
PPE: Personnel protective equipment MEP: Monte Carlo Exact Probability
MUPP: Mansoura University Printing Press
* : Statistically significant difference **: Highly statistically significant difference.

Table 1 showed that MUPP workers matched the comparison group in all sociodemographic and working profile except for having previous job (42% of MUPP workers) and the use of chemicals (78% of MUPP workers) and machinery at work (60% of MUPP workers). None of MUPP workers and 4% of the comparison group used PPE. The whole comparison group and 60% of printing workers wash hands with water and soap with statistically significant difference.

Table 2: Laboratory profile of the studied groups.

Investigations	MUPP workers No=50	Comparison group No=50	Test of significance
Complete blood count			
Hemoglobin (gm/dL)	12.6±1.4	12.7±1.6	t=0.4, p=0.68
RBCs count (million cells/mcL)	4.3± 0.5	4.4±0.5	t=1.3, p=0.2
WBCs count (x10^9/L)	7.4±2.14	7.6± 2.26	t=0.5, p=0.63
Relative lymphocytes (%)	35.1± 11.2	35.6±8.2	t=0.3, p=0.8
Platelets (x10^9/L)	235.3± 66.6	257.3±58.7	t=1.7, p=0.09
Renal function tests			
Serum creatinine (mg/dL)	1.2± 0.2	0.9±0.1	t=8.9, **p≤0.001****
Serum urea (mg/dL)	32.7±7.01	30.8± 7.7	t=1.3, p=0.2
Serum uric acid (mg/dL)	6.1±1.2	5.6±1.02	t=2.4, **p=0.02***
Liver function tests			
Serum albumin (gm/dL)	4.6±0.44	4.7±0.33	t=2.1, **p=0.04***
Serum bilirubin (mg/dL)	0.94±0.19	0.90±0.12	t=1.3, p=0.2
AST (U/L)	42.5 (10-151)	24 (7-52)	Z=5.3, **p≤0.001****
ALT (U/L)	46 (12-160)	9-52))25	Z=5.5, **p≤0.001****

Z: of Mann-Whitney test
cells/mcL = cells per microliter, gm/dL = grams per deciliter, U/L = units per liter
All parameters are expressed as Mean±SD except AST and ALT as median (min-max)
MUPP: Mansoura University Printing Press
AST: Aspartate Aminotransferase ALT: Alanine Aminotransferase
*: Statistically significant difference **: Highly statistically significant difference.

Table 2 showed that, both groups had approximately equal values of blood count (with statistically non-significant difference). MUPP workers had significantly higher mean values of serum creatinine and uric acid, significantly lower level of serum albumin, however, serum bilirubin was high (p=0.2). Liver enzymes (AST & ALT) were statistically significantly higher (exceeding the upper limit of normal range) among MUPP workers compared to the control group.

Table 3: Methylation status for the promoter regions of P15, iNOS, CYP2E1 and MAGE1 genes of the studied groups.

Gene No		MUPP workers No=50		Comparison group No= 50		Test of significance
		No	%	No	%	
P15	**Methylated**	11	22.0	2	4.0	χ^2 =7.2, **p=0.007***
	Un-methylated	39	78.0	48	96.0	
iNOS	**Methylated**	8	16.0	1	2.0	Fisher›s exact test, **p=0.01***
	Un-methylated	42	84.0	49	98.0	
CYP2E1	**Methylated**	15	30.0	3	6.0	χ^2 =9.7, **p=0.002***
	Un-methylated	35	70.0	47	94.0	
MAGE1	**Methylated**	6	12.0	9	18.0	χ^2 =0.7, p=0.4
	Un-methylated	44	88.0	41	82.0	

*: Statistically significant difference

Table 3 showed that the frequency of promoter regions methylation among MUPP workers were significantly higher than the comparison group for P15 (22% vs. 4%), iNOS (16% vs. 2%), and CYP2E1 (30% vs. 6%) genes. However, the frequency of MAGE1 promoter region un-methylation was higher among MUPP workers (88%) than the comparison group (82%) with statistically non-significant difference ($p \leq 0.05$).

Different combinations of gene methylation were found in 12% of MUPP workers, while, it was not detected in the comparison group with statistically significant difference ($p \leq 0.05$) (Data are not shown in tables).

Table 4: Association of promoter region methylation of genes and some socio-demographic and occupational characteristics of the studied groups.

Characteristics	P15 promoter region methylation		iNOS promoter region methylation		CYP2E1 promoter region methylation		MAGE1 promoter region un-methylation	
	MUPP workers No=11	Comparison group No=2	MUPP workers No=8	Comparison group No=1	MUPP workers No=15	Comparison group No=3	MUPP workers No=44	Comparison group No=41
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Age /in years								
< 40	3 (27.3)	2 (100.0)	3 (37.5)	0 (0.0)	3 (20.0)	1 (33.3)	13 (29.5)	21 (51.2)
≥40	8 (72.7)	0 (0.0)	5 (62.5)	1 (100.0)	12 (80.0)	2 (66.7)	31 (70.5)	20 (48.8)
Test of significance OR (95% CI)	Fisher›s exact test, p=0.1		Fisher›s exact test, p=1		Fisher›s exact test, p=1		χ^2=4.2, **p=0.04*** 0.4 (0.1 – 0.9)	
Gender								
Male	5 (45.5)	1 (50.0)	5 (62.5)	1 (100.0)	7 (46.7)	2 (66.7)	20 (45.5)	22 (53.7)
Female	6 (54.5)	1 (50.0)	3 (37.5)	0 (0.0)	8 (53.3)	1 (33.3)	24 (54.5	19 (46.3)
Test of significance OR (95% CI)	Fisher›s exact test, p=1		Fisher›s exact test, p=1		Fisher›s exact test, p=1		χ^2=0.6, p=0.4	
Smoking	3 (27.3)	1 (50.0)	2 (25.0)	0 (0.0)	2 (13.3)	1 (33.3)	6 (13.6)	7 (17.1)
Test of significance OR (95% CI)	Fisher›s exact test, p=1		Fisher›s exact test, p=1		Fisher›s exact test, p=0.4		χ^2=0.2, p=0.7	
Duration of employment/ years								
< 15	1 (9.1)	1 (50.0)	3 (37.5)	0 (0.0)	2 (13.3)	2 (66.7)	9 (20.5)	23 (56.1)
≥15	10 (90.9)	1 (50.0)	5 (62.5)	1 (100.0)	13 (86.7)	1 (33.3)	35 (79.5)	18 (43.9)
Test of significance OR (95% CI)	Fisher›s exact test, p=0.3		Fisher›s exact test, p=1		Fisher›s exact test, p=0.1		χ^2=11.5, **p=0.001**** 0.2 (0.1-0.5)	
Exposure to chemicals on assigned duty	9 (81.8)	0 (0.0)	5 (62.5)	0 (0.0)	14 (93.3)	3 (100.0)	34 (77.3)	1 (2.4)
Test of significance OR (95% CI)	Fisher›s exact test, p=0.07		Fisher›s exact test, p=0.4		Fisher›s exact test, p=0.005*		χ^2=49.1, **p≤0.001**** 0.007 (0.001 - 0.06)	

*: Statistically significant difference **: Highly statistically significant difference.

Table 4 showed that there was no statistically significant association of promoter region methylation of P15 and iNOS genes with any of the studied socio-demographic and occupational characteristics of the study groups. However, association between chemical exposure on assigned duty with methylation of CYP2E1 promoter region and association between age, duration of employment, and chemical exposure on assigned duty with un-methylation of MAGE1 promoter region were significant.

Discussion

Printing workers customarily have high occupational exposure to health hazards (Cherry et al., 2001; HSE, 2002; Rushton et al., 2008; Rushton et al., 2010). There are significant health risks, for instance dermatitis, musculoskeletal disorders, occupational asthma, deafness, eye damage, and other problems attributed to the use of solvents and other chemical compounds that need to be considered in the printing sector (HSE, 2002).

The results of the present study showed that both groups (exposed and control) had matched values of blood count and parameters ($p>0.05$). MUPP workers had significantly higher mean values of serum creatinine and uric acid, significantly lower level of serum albumin and high level of serum bilirubin compared to the control group. Liver enzymes were also significantly higher (exceeding the upper limit of normal range) among MUPP workers (Table 2)

Similarly, a study from Turkey showed no statistically significant difference between printing workers and a comparison group in fields other than printing with respect to WBCs and RBCs count, hemoglobin and MCV (Celik et al., 2013). Likewise, In Iraq, no statistically significant difference in the levels of hemoglobin and hematocrit was found, while levels of circulating total WBCs and RBCs were significantly lower among printing workers when compared to controls. Moreover, during shoemaking, workers are exposed to similar types of solvents (Abdulateef and Talib, 2016). In Pakistan, statistically significant difference between the shoemaking workers and the control group regarding total leukocytes count, platelets count, uric acid, creatinine and ALT levels was reported (Khan, 2013).

An Egyptian study (Kotb et al., 2013) reported a higher level of serum creatinine among printing workers than the clinically acceptable normal range, however, the level of serum ALT and AST was found to be within normal.

Another Egyptian study reported a higher mean serum urea level (33±9.6 mg/dl) among workers exposed to organic solvents than that in the control group (30±7.9mg/dl) with statistically significant difference. However, differences in mean values of serum creatinine and glomerular filtration rate were statistically non-significant (Hegazi et al., 2016).

A Finnish study evaluated the common laboratory tests among solvent-exposed workers, printing workers were included, their results demonstrated a higher level of serum ALT, AST and total bilirubin in solvent-exposed workers compared with the reference group, but with no statistically significant difference (Kaukiainen et al., 2004).

DNA methylation, one of the epigenetic mechanisms, that directly affect gene expression often occur in the Cytosine–Guanine dinucleotide (CpG) sites located in the promoter regions of the genes (Irizarry et al., 2009). In the present study, the frequency of promoter regions methylation among MUPP workers was significantly higher than the comparison group for P15, iNOS, and CYP2E1 genes. However, the frequency of MAGE1 promoter region un-methylation was higher among MUPP workers than the comparison group (p□0.05). Different combinations of gene methylation were found in 12% of MUPP workers, while, it was not detected in the comparison group with statistically significant difference ($p \leq 0.05$) (Table 3).

Similarly, previous studies reported that hypermethylation in p15 and hypomethylation in MAGE-1 were associated with benzene exposure (Bollati et al., 2007) and down-regulation of p15 and p16 expression was correlated with hypermethylation in benzene exposed patients (Xing et al., 2010). Methylation might have an effect on the development of benzene-induced hematotoxicity and carcinogenicity in a manner complementary to direct mutations of the DNA sequence (Zheng et al., 2017).

MAGE-1, which is frequently densely methylated in normal cells, shows lower methylation in individuals exposed to airborne benzene (Bollati et al., 2007).

In Mexico, significant correlation was found between toluene airborne levels and CYP2E1 promoter methylation (Jiménez-Garza et al., 2015).

Jiménez-Garza et al. (2017) compared promoter methylation status for genes involved in inflammation, nitrosative stress and xenobiotic biotransformation in workers exposed to low levels of benzene. They found that iNOS promoter methylation positively correlated with CYP2E1 promoter methylation and the cumulative time of exposure. The correlations between iNOS methylation with CYP2E1 methylation symbolize novel evidence about CYP2E1 epigenetic regulation and activity related with nitrosative stress, making promoter methylation status

of these genes a possible biomarker in early stages of oncogenesis.

Conclusion: Printing workers had significantly higher serum creatinine, uric acid, and liver enzymes with significantly lower serum albumin. Promoter regions methylation among printing workers were significantly higher for P15, iNOS, and CYP2E1 genes and MAGE1 promoter region un-methylation with non statistically significant association of promoter region methylation of P15 and iNOS genes with any of the socio-demographic and occupational characters of the studied groups. However, association between chemical exposure with methylation of CYP2E1 promoter region and association between age, duration of employment, and chemical exposure with un-methylation of MAGE1 promoter region were significant. Based on the study findings, printing workers operate at a potentially vulnerable state with unawareness of the risk of such chemical exposure together with lack of proper protection. **Recommendations:** Introduction of periodic medical checkup should be introduced for early detection of any health effects attributed to chemicals at printing industry as changes in laboratory profile, higher frequency of promoter region methylation of genes. Encourage the use of PPE at work to reduce exposure. Further larger scale studies combine with exposure monitoring should be carried out to explore the long term effects of printing chemicals exposure among workers.

Study limitations

This study had some limitations in the form of small sample size due to cost restriction; investigating DNA methylation by qualitative PCR; and finally lack of environmental monitoring of airborne chemicals.

Conflict of interest

None

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