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THE ASSOCIATION BETWEEN ENVIRONMENTAL TOBACCO SMOKE AND INFLAMMATORY MARKERS AMONG NON-SMOKER NURSES IN SHEBIN AL-KOM TEACHING HOSPITAL

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Abstract:

Background: Environmental tobacco smoke (ETS) can cause or exacerbate a wide range of adverse health effects, including cancer, respiratory infections and cardiovascular disease (CVD). There is limited and inconsistent evidence of an association between ETS exposure and inflammatory markers. **Aim of the work:** To examine the relationship of ETS exposure measured by urinary cotinine level with systemic inflammatory markers that included high-sensitive C reactive protein (hs-CRP), homocysteine, inter-leukin-6 (IL-6), and fibrinogen among non-smoker nurses. **Subjects and methods:** A cross-sectional study was conducted to study one hundred and forty eight non-smoker nurses at Shebin Al-Kom Teaching hospital. Participants were interviewed and a urine sample for quantitative determination of urinary cotinine level was collected. A blood sample was withdrawn for measuring hs-CRP, homocysteine, IL-6 and fibrinogen levels. **Results:** With increasing urinary cotinine level, hs-CRP, homocysteine, fibrinogen and IL-6 levels increased, reaching a significant level for hs-CRP and homocysteine not the others. Multivariate regression analysis after adjusting for age, education, BMI and duration of employment, revealed that nurses with ETS exposure had a significant higher levels of hs-CRP, fibrinogen and homocysteine. **Summary and recommendations:** Regular and repetitive exposure to ETS leads to relevant effects on inflammatory system rather than cytokine system. Longitudinal studies are necessary to determine the potential causal relevance of this

association and to test the clinical important effect on susceptibility to inflammatory disease.
Key words: Environmental tobacco smoke, hs-CRP, IL6, Fibrinogen, Homocysteine

Introduction

Environmental tobacco smoke (ETS), known as secondhand smoke (SHS), is a mixture of the side-stream smoke given off by the burning end of a cigarette, pipe or cigar and the smoke exhaled from the lungs of smokers. It is involuntarily inhaled by non-smokers (called passive smoking), and can cause or exacerbate a wide range of adverse health effects, including cancer, respiratory infections and cardio vascular disease (CVD) (Panagiotakos et al., 2002, Barnoya and Glantz, 2005, Californea Environmental Protection Agency, 2005, Centers for Disease Control and Prevention (CDC), 2006, Raupach et al., 2006, Eisner and Iribarren, 2007, Ho et al., 2007).

The Current Surgeon General's Report concluded that scientific evidence indicates that there is no risk-free level of exposure to secondhand smoke. Furthermore, short exposures to ETS can cause platelets aggregation, damage the lining of blood vessels, decrease coronary flow velocity reserves, and reduce heart rate variability, potentially increasing the risk of heart attacks (Taylor et al., 1992 and Department of Health and Human Services, U.S., 2006).

Several studies of systemic inflammatory markers, which have been associated with susceptibility to CVD, have shown positive relationships with active smoking (Blake and Ridker, 2002, Saadeddin et al., 2002 and Tousoulis et al., 2007). However, there is limited and inconsistent evidence of an association between ETS exposure and inflammatory markers (Cook et al., 2000, Panagiotakos et al., 2004, Venn and Britton, 2007, Clark et al., 2008 and Nagel et al., 2009). A study of adults in Greece (Panagiotakos et al., 2004) and another study of elderly British (Jefferis et al., 2010) without clinical evidence of CVD suggested that exposure to ETS is related to an increase in C-reactive protein (CRP) levels, whereas this association was not found among non-smoking adults in the U.S. general population (Venn and Britton, 2007 and Clark et al., 2008). These studies have used either self-reports or biomarkers to assess SHS exposure. A meta analysis found that non-smokers exposed to environmental smoke were 25 % more likely to have coronary heart disease compared to non-smokers not exposed to smoke (He et al., 1999). In adults, markers of low grade inflammation such as Interleukin 6 (IL6) is considered as

a risk factor for CVD (Gabay and Kushner, 1999). Of the hemostatic variables associated with CHD and with smoking, plasma fibrinogen (serum is clotted, there by containing no fibrinogen) (MacCallum, 2005) and homocysteine which is derived from protein and methionine metabolism (Perna et al., 2004), have been the most widely reported.

Nicotine is present in substantial concentrations in virtually all tobacco products and in insignificant amounts in some foods (Benowitz, 1996 and Siegmund et al., 1999). Nicotine is extensively metabolized, primarily in the liver, and its major proximate metabolite is cotinine: on average, 75% of nicotine is converted to cotinine, primarily by the liver enzyme cytochrome P450 2A6 (Hukkanen et al., 2005). Cotinine's half-life (t1/2), the time in which its concentration halves, is longer (average: 16 h) than nicotine's (2 h). Cotinine concentrations are more stable throughout the day, making it the preferred blood, saliva and urine biomarker for SHS. Blood's cotinine concentrations and saliva are highly correlated. Urine cotinine concentrations are in average four fold to six fold higher than those in blood or saliva, making urine a more sensitive matrix to detect low-concentration exposure (Benowitz et al., 2009 and Avila-Tang et al., 2012). In urine, values between 11-30 ng/mL may be associated with light smoking or passive exposure, while levels in active smokers typically reach 500 ng/mL (Foundation for Blood Research. 2007).

Aim of the work

To examine the relationship of SHS exposure measured by urinary cotinine with systemic inflammatory markers that included high-sensitive CRP (hs- CRP), inter-leukin-6 (IL-6), homocysteine and fibrinogen among non-smoker nurses.

Subjects and Methods

This cross-sectional study was took place in Shebin al-Kom Teaching hospital in Menoufiya governorate, Egypt, between June and December 2011. One hundred and forty eight non-smoker nurses working at in-patient and out-patient clinics with mean age of 33.09±5.77 (mean duration of employment was 12.34±3.58 years) were chosen as a study group from all nurses in the hospital (769 nurse), after exclusion of those who live with a smoker at home (husband, father or brother). They were divided into 2 groups according to urinary cotinine level: group A with urinary cotinine level below the median value (1.84 ng/mL), it included 99 nurse and group B with urinary cotinine level above the median value (1.84 ng/mL), it included 49

nurse. The Menoufiya Faculty of Medicine Committee for Medical Research Ethics reviewed and formally approved the study before it began. Approval of the manager was obtained, and all participants gave written informed consent before inclusion and all personal information collected was treated confidentially.

Participants were interviewed by trained investigators at the hospital during the day shift (between 8:00 am – 4:00 pm). At each visit, demographic data, smoking status, history of previous chronic diseases and detailed occupational history (duration of employment, name of the department, number of days worked/ month including night and day shifts and past occupations and their hazards) were gathered. In addition, the following measurements were made: A urine sample (25 ml) with a preservative, was collected from each nurse and stored at – 20oC until analyzed for quantitative determination of urinary cotinine, which was assayed using the Cozart EIA cotinine urine kit M155 u1 (in ng/mL) (Buckley, 1979). Five ml of venous blood were collected from all subjects, 2ml into a plain tube, let to stand to clot and serum was separated in aliquots after centrifugation and stored at -80° until analysis of high-sensitive CRP assay using latex enhanced immunoturbidemetric assay. Serum CRP causes agglutination of latex particles coated antihuman CRP, the agglutination of the latex particles is proportional to CRP concentration (Roberts et al., 2001) and total homocysteine assay by Enzyme Linked Immunosorbent assay (Frantzen et al., 1998). The remaining 3ml was put in a tube containing sodium citrate and centrifuged at room temperature at 3000 rpm for 10 minutes. Plasma then separated and immediately stored at -80° until analysis of IL6 using sandwich enzyme linked immunosorbent assay kits (Minneapolis, Minnesota) (De Rijk et al., 1996) and fibrinogen assay. This assay employs a quantitative competitive sandwich enzyme immunoassay technique that measures fibrinogen in less than 3 hours (Handley and Hughes, 1997).

Data management: Independent student's t test was used for continuous normally distributed variables while mann whiteny U test was used for non-normally distributed ones. Fisher exact test was used for categorical variables when the expected values were less than 5. To test the association between urinary cotinine level and inflammatory markers, partial correlation coefficient test was used. Logestic regression analysis was used to test relation between variables after adjusting the confounders' effects. Comparisons of

data were made with overall α error set at .05 (2-tailed). Analyses were conducted with SPSS v.19 software (SPSS Inc, Chicago, III).

Results

The mean values of inflammatory markers were significantly higher among nurses with urinary cotinine level above the median value. There was non-significant difference between non-smoker nurses according to their urinary cotinine level (above and below the median value) regarding demographic characteristics and medical conditions (table 1). The mean values of hs-CPP and IL-6 were observed to be significantly higher among older aged nurses, however, the mean value of hs-CRP and fibrinogen were significantly increased among nurses with more years of employment, both hs-CRP and homocysteine showed a significant higher mean value among obese nurses where only hs-CRP was increased among those with chronic rhinitis (table 2). With increasing urinary cotinine level, hs-CRP, fibrinogen, homocysteine and IL-6 levels increased, reaching a significant level for hs-CRP and homocysteine only (table 3). Multivariate linear regression analysis between ETS and studied inflammatory markers after adjusting age, employment years, BMI and history of medical conditions revealed that ETS exposure was a risk factor for elevated hs-CRP, homocysteine and fibrinogen and not for IL6 (table 4).

Table (1): Levels of urinary cotinine and inflammatory markers and characteristic of studied non-smoker nurses.

Variable	Studied nurses according to median urinary cotinine level		Total (NO=148)	P-value
	≤ 1.84 (ng/mL) (NO= 99)	> 1.84 (ng/mL) (NO= 49)		
Urinary cotinine (ng/mL):	1.52±1.21	2.04±1.16	1.69±1.22	0.000*
hs-CRP (mg/L):	1.19±0.61	1.81 ± 0.89	1.39±0.78	0.00*
IL-6 (pg/mL):	0.95 ± 0.36	1.13 ± 0.32	1.01±0.34	0.003*
Fibrinogen (g/L):	1.98 ± 0.97	2.32 ± 1.49	2.09±1.17	0.15*
Homocysteine (μ mol/l):	7.59±2.48	8.7±2.19	7.95±2.44	0.03*
Age (years):	32.68 ± 5.41	33.94 ± 6.4	33.09±5.77	0.21
Duration of employment (years):	9.18±2.7	10.0±2.9	12.34±3.58	0.09
BMI (kg/m²):	24.99±2.55	25.83±3.02	26.4±1.4	0.07
Medical conditions (NO & %):				
-Asthma/COPD.	4 4	3 6.1	7 4.7	0.69#
-Chronic rhinitis.	3 3	2 4.1	5 3.4	1.00#
-Aspirin use.	3 3	5 10.2	8 5.4	0.06#

*** Mann whiteny U test. # Fisher exact test.**

Table (2): Mean values of inflammatory markers by demographic data and medical conditions.

Variable	NO (148)	hs-CRP (mg/L)	IL-6 (pg/ mL)	Fibrinogen (g/L)	Homocysteine (μmol/l)
Age (years):					
≤30	79	0.96±0.52	1.27±0.77	2.0±0.29	7.86±2.18
>30	69	1.35±0.73	1.54±0.76	1.94±0.25	8.03±2.65
P-value		0.006*	0.04*	0.22*	0.17*
Duration of employment:					
≤12	124	0.93±0.44	1.01 ±0.35	1.94±0.28	8.0±2.4
>12	24	2.22±0.45	1.03±0.32	2.15±0.19	7.7±2.64
P-value		0.000*	0.69*	0.000	0.64*
BMI (kg/m2):					
Normal	90	0.89±0.43	1.04±0.36	1.98±0.31	7.62±2.51
Overweight/obese	58	1.53±0.74	0.97±0.3	1.96±0.20	8.47±2.24
P-value		0.000*	0.25*	0.84	0.01*
Asthma/COPD:					
No	141	1.98±0.66	1.02±0.34	2.31±0.75	8.01±2.46
Yes	007	02.3±0.32	0.79±0.29	2.53±0.59	6.87±1.64
P-value		0.25	0.08*	0.44	0.17*
Chronic rhinitis:					
No	142	1.84±0.46	1.00±0.34	2.1±1.19	7.95±2.48
Yes	006	2.42±0.52	1.20±0.29	1.9±0.11	7.99±1.29
P-value		0.006	0.13*	0.52*	0.93*
Aspirin use:					
No	139	2.01±0.41	1.01±0.35	2.01±0.16	7.97±2.48
Yes	009	1.80±0.12	1.06±0.23	2.10±.1.21	7.66±1.67
P-value		0.12	0.49*	0.52*	0.62*

*** Mann whiteny U test.**

Table (3): Pearson correlation coefficient between urinary cotinine level and inflammatory markers.

Inflammatory markers	Urinary cotinine (ng/mL)	
	r	P-value
-hs-CRP (mg/L):	0.30	0.01
-IL-6 (pg/mL):	0.13	0.26
-Fibrinogen (g/L):	0.11	0.22
-Homocysteine (μ mol/l)	0.27	0.03

Table (4): Multivariate regression analysis of inflammatory markers among studied non-smoker nurses adjusted for age, education, BMI and duration of employment.

Inflammatory markers	SE	β	p-value	OR (95% CI)
-hs-CRP (mg/L):	0.44	1.8	0.02	1.15 (0.33-0.62)
-IL-6 (pg/mL):	1.57	1.01	0.43	0.17 (0.73-5.06)
-Fibrinogen (g/L):	1.46	1.2	0.03	1.30 (1.15-1.64)
- Homocysteine (μ mol/l):	1.03	2.2	0.008	1.64 (0.04-0.93)

Discussion

In this study, although nonsmoker nurses showed a lower mean value of urinary cotinine level, it was positively associated with hs-CRP. This observation suggests that serum level of CRP may predominantly reflect the consequences of smoking on inflammatory diseases beside pro-inflammatory effects. Previous studies have examined the association between ETS and CRP with inconsistent results. Panagiotakos et al., 2004 reported a significant association between SHS and CRP among Greek non-smoking adults. Also, among nonsmoking elderly British, Jefferis et al., 2010 observed a significant association between serum cotinine and CRP in a linear regression model adjusted for age, sex, residence, health behavior, social class and BMI. Nagel et al., 2009 in a study among young children exposed to ETS in Germany showed a significant positive association with CRP. However in US general population, no significant association was observed between CRP and serum cotinine levels in never-smokers (Venn and Britton, 2007). Also, Clark et al., 2008 studied non-smoking adult workers without home secondhand smoking exposure where there was no correlation between serum cotinine level and CRP. Hammett et al., 2007 measured CRP before and 6 weeks after attempting smoking cessation in 138 healthy women, but there was no significant change in its plasma level. Moreover, high sensitive C-reactive protein (hs-CRP) was measured in eighteen male, non-smoking volunteers before and 12h after a 1-h SHS exposure with non-significant difference (Bonetti et al., 2011). The difference between the result in this study and others may be attributed to the analytic method for CRP used in this study which is high-sensitive immunoassay with a detection limit of 0.03mg/L, compared with 3mg/L and 0.1mg/L respectively in the previous studies. Also, CRP has a half-life of about 19 h (Koenig et al., 2003), similar to that of urinary cotinine 16h (Benowitz, 1996) used in this study.

In the current work, there was non-significant relationship between ETS depending on urinary cotinine level and IL6. To our knowledge ETS exposure and IL6 was investigated only in few previous studies. Chui et al., 2011 observed non-significant association between ETS and IL6 among non-smoker workers in truking industry in USA. Also, Nagel et al., 2009 reported that in the dichotomized model, IL-6 showed non-significant positive trend with ETS in the study among young children exposed to ETS in South-west Germany. IL-6 was measured in eighteen male, non-

smoking volunteers before and 12h after a 1-h SHS exposure with non-significant difference (Bonetti et al., 2011). On the other hand, Jefferis et al., 2010 in a study of elderly British, a multivariate adjusted linear regression showed a marginally significant positive association between IL-6 and serum cotinine. Moreover, Flouris et al., , 2009 reported that a 1-hour exposure to SHS at bar/restaurant levels is accompanied by significant increases in inflammatory cytokines, particularly in men and it remains elevated for at least 3 hours following SHS exposure in Greece. Animal data also support the hypothesis that the effects of SHS on the cardiovascular system are mediated in part through inflammation. After exposing mice to SHS from 2 cigarettes for 30 minutes/ day for 4 months, Zhang et al., 2001 noted an increase in interleukin-6, a pro-inflammatory cytokine. The difference in results between this study and others may be explained by the stability of IL6 which has a shorter half-life (2-6h) than urinary cotinine (Riches et al., 1992) and reflects a more immediate response.

In this study, other marker of inflammation, fibrinogen, was non-significantly associated with urinary cotinine level. This result is in agreement with that reported by Clark et al., 2008 and Jefferis et al., 2010. Also, Panagiotakos et al., 2004, found that adults breathing SHS for >30 minutes at least 1 day/week had higher leukocyte count and CRP, but not fibrinogen, than did unexposed adults (adjusted for several potential confounders). In contrast with this result, Venn and Britton, 2007 reported a significant association between ETS and fibrinogen among never-smoking adults in Nottingham, UK. Moreover, Hammett et al., 2007 studied 138 healthy women from them, 48 participants who stopped smoking, fibrinogen was significantly decreased. In the previous studies, serum cotinine was used to measure SHS and not the urinary one as this study did, this may be the explanation of difference in the results.

It was observed, in this study, that homocysteine was significantly associated with urinary cotinine level and, also, after adjusting for age, education, BMI and duration of employment, nurses with ETS exposure had a significant higher level of homocysteine. This result is in agreement with that reported by Panagiotakos et al., 2004. Moreover, Iso et al., 1996; Stavroulakis et al., 2000; Kiechl et al., 2002; Bazzano et al., 2003 who found that self-reported exposure to SHS was correlated with inflammatory markers, including CRP,

homocysteine, and white blood cell (WBC) count.

Conclusion: There was a positive relationship between ETS exposure, as measured by urinary cotinine level and the studied inflammatory markers, however after adjusting of confounders; hs-CRP, homocysteine and fibrinogen not IL-6 were still associated. Regular and repetitive exposure to ETS leads to relevant effects on inflammatory system rather than cytokine system. However, longitudinal studies are necessary to determine the potential causal relevance of this association and to test the clinical important effect on susceptibility to cardiovascular disease. In Egypt, implementation of smoke-free policies for hospitals is obligatory.

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