BIOMONITORING THE GENETIC EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE EXPOSURE IN RESTAURANT WORKERS

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BACKGROUND

Environmental tobacco smoke (ETS) is recognized as a health hazard by environmental and public health authorities worldwide[1]. Therefore, several countries have implemented legislation that prohibits smoking in the majority of public spaces. In Portugal, since 2008, legislation banned smoking in most restaurants and bars, but some except ions remained, namely in spaces where devices for ventilation and air extraction were installed. Nevertheless, whether those preventive measures are efficiently protecting the health of the workers that are continuously exposed to ETS at their workplaces is still an open question.

To address this issue, a recent work by our group showed that ETS pollution was higher in Lisbon restaurants and bars where smoking is allowed, comparatively to smoke-free restaurants and canteens and also that workers were indeed internally exposed to ETS at their workplaces [2,3]. Major concerns with the workers' health originate from the carcinogenic effects of ETS (IARC Group 1 carcinogen[1]). The use of biomarkers of early genetic damage for biomonitoring these workers can unravel genotoxic lesions that may be implicated in cancer development.

OBJECTIVES

This pilot study aimed at investigating the association between occupational exposure to ETS and the induction of genetic damage in somatic cells from employees from the previously studied restaurants, where smoking is still permitted, considering the individual susceptibility in the response to ETS components.

STUDY POPULATION

Characteristics	Non Smoking Workers		Smoking Workers
	Not Exposed to ETS	Exposed to ETS	
Number of Workers:	33	29	19
Age (years):			
Mean ± SD	45.18 ± 12.21	37.17 ± 10.77	39.05 ± 11.08
Range	*19 – 66*	*24 – 57*	*18 – 63*
Gender (%)			
Female	11 (33.3)	4(13.8)	6 (38.6)
Male	22 (66.7)	25 (86.2)	13 (68.4)
Smoking habbits			
No. of cigarettes per day	0	0	16.47 ± 7.25
Range	0	0	*3 – 30*
No. of Years of smoking	0	0	22.89 ± 10.88
Range	0	0	*3 – 49*
Second-hand smoke at home* (%)			
Yes	3 (9.1)	7 (24.1)	4 (21.1)
No	30 (90.9)	17 (58.6)	12 (63.2)
No Data	na	5 (17.2)	3 (15.8)
Exposure assessment**			
Cotinine concentration (Mean ± SD)	2.23 ± 4.31	7.98 ±7.26	1598.29 ± 806.97
Range	*1– 19.0*	*1 – 28.0*	*237.0 – 3125.0*

*Exposure outside the workplace (e.g., at home, means of transport, second job, etc.); ** Results presented in Pacheco et al., 2012; SD- standard deviation. Values of the cotinine concentration that where below the level of detection of the assay were assumed to be 0.1 for the calculation of the means and sd.

METHODS

Biomarkers of Genotoxicity	• **Sister Chromatid Exchanges**, SCEs[4] • **Comet assay** in blood leukocytes[5] • **Micronucleus (MN)** assay in lymphocytes[6] and buccal cells[7]
Cells early response to a genotoxic challenge	• **Comet assay following *ex vivo* exposure of leukocytes to an acute dose of an alkylating agent (Ethyl-methanesulphonate, EMS,32 mM)**
Biomarkers of susceptibility	• Polymorphisms in genes associate to **xenobiotic metabolism (*GSTP1*, *GSTM 1* and *GSTT1*) by PCR/RFLP.** • Polymorphisms in genes involved in **DNA repair (*hOGG1*, *XRCC1*, *XRCC3*,*NBS1*, *PARP1*) by PCR/RFLP.**

RESULTS

MICRONUCLEUS ASSAY

- The frequency of micronuclei in peripheral blood lymphocytes was significantly lower in ETS-exposed comparatively to non-exposed workers.
- No significant differences in the frequency of MN in buccal cells were observed between exposed and non-exposed workers.

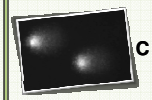
Non-Smokers

SISTER CHROMATID EXCHANGES

- Smokers could be distinguished from non-smokers by a significantly increased proportion of HFCs.
- No significant differences in SCE and HFC between ETS-exposed and non-exposed workers.

COMET ASSAY

- No significant differences observed in the basal level of DNA strand breaks in ETS-exposed *vs.* non-exposed workers.



CELLS EARLY RESPONSE TO A GENOTOXIC CHALLENGE

- Challenge with EMS resulted in a lower level of DNA damage in workers exposed to ETS, comparatively to non-exposed, irrespectively of the susceptibility biomarkers analysed.
- This effect is presumably due to an increased cells capacity to overcome the effect of an acute stimulus .

Adaptive response?

ASSOCIATION BETWEEN BIOMARKERS OF SUSCEPTIBILITY AND EFFECT

- The distribution of the common and variant alleles between the ETS-exposed and non-exposed groups did not show significant differences for any of the polymorphisms analysed ,except for NBS1 (p=0.047, Chi-square test).
- *GSTM1* null genotype carriers presented a non significant increase in the frequencies of HFCs associated with ETS exposure, suggesting an increased susceptibility to this environmental stressor.
- Likewise, *XRCC1*^{399} variant allele carriers presented a higher level of MN than the wild-type allele carriers, in response to ETS exposure (P=0.080).
- Finally, among the ETS-exposed subjects, those carrying the *hOGG1* variant alleles presented a lower level of SCEs (p= 0.087) and of *ex vivo* EMS-induced DNA damage (p=0.072), comparatively to the wild-type subjects suggesting a modified DNA repair capacity.

DISCUSSION

- In this pilot study, no clear association between occupational exposure to ETS and the induction of genetic damage was identified, as assessed through several biomarkers of genotoxicity.
- Interestingly, a differential response of leukocytes from ETS-exposed and non-exposed workers to an *ex vivo* acute genotoxic stimulus was observed, suggesting that ETS exposure positively modulates the DNA repair machinery and other cellular protection responses towards the restoration of cells stability.
- The observed response might be comparable to the adaptive response that has been mainly described for exposure to low doses of ionising radiation[8] and may be the result of the upregulation of DNA repair functions[9].
- Although an adaptive response may be beneficial because it results in an enhanced capacity to deal with stress, it has been suggested that an exposure that elicits an adaptive response can also produce toxicity with longer or higher exposures[10].

After the implementation of the Portuguese legislation that regulates smoking in public spaces, this study was aimed at biomonitoring the genetic effects in ETS-exposed workers from restaurants where smoking is still allowed. The most relevant effect detected in those workers was a modified early response to a genotoxic challenge, compatible with an adaptive response. It remains to be determined, however, whether the induction of this kind of response might have long term consequences to the health of ETS-exposed workers.

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Work supported by Fundação Calouste Gulbenkian, ACSS, INSA and F CT/Pluriannual funding.
The authors thank the restaurants'owners/managers and their workers for cooperating in this study.
Special thanks are extended to Bruno Alexandre, Nuno Charro, Fátima Vaz, Solange Pacheco and Filomena Gomes for their assistance and to Eleonora Paixão for the statistical analyses.