

**Title:**

**Disclosing effects of tobacco smoke on occupationally exposed workers at Lisbon restaurants**

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**Abstract:**

Tobacco smoke is increasingly recognized as an occupational hazard to workers. Partial smoking restriction in Portuguese hospitality venues, such as restaurants, does not sufficiently protect these workers against Second Hand Smoke (SHS), whatever protective measures are used. Therefore, they are at increased risk for developing chronic respiratory diseases such as chronic obstructive pulmonary disease, asthma and lung cancer. The aim of the present work was to study by proteomics the biological effects of SHS on the plasma proteome collected from occupationally exposed workers.

After Indoor Air Quality (IAQ) assessment of a group of restaurants located in Lisbon, 96 workers of these places were evaluated for acute exposure to SHS, genotoxic lesions and respiratory function. To investigate global changes in the plasma proteome, plasma were prepared from the same blood sample used in the genotoxic study and pooled according to average age, level of genotoxic lesions, smoking habits and SHS exposure. There were four conditions in study, each represented by three biological replicates: Smokers and Non-smokers exposed, or not, to SHS. Samples were then depleted of the 14 most abundant serum proteins (serum-14) using a multiple affinity removal system (MARS).

Depleted fractions were analysed by 2-D Fluorescence Difference Gel Electrophoresis (DIGE) approach. Labelled proteomes were submitted to isoelectric focusing in 24cm-IPG strips pH4-7 (GE Healthcare) prior to SDS-PAGE electrophoresis. Acquisition and analysis of gel images were carried out on a typhoon imager (GE Healthcare) and SameSpots software (NonLinear Dynamics), respectively. A total of 967 spots were detected and, after volume normalization, 6.3% of spots presented variation of its expression levels. Differentially expressed spots across the groups in study were selected for protein identification by MALDI tandem Mass Spectrometry (MS). Identified proteins were annotated according to molecular function and associated pathways to disclose altered mechanisms induced by SHS on exposed workers.

This study represents a global assessment of IAQ in Lisbon smoking entertainment places and health effects on exposed workers. Most importantly, it may contribute to the better understanding of pathogenesis mechanisms due to SHS exposure. In addition it may lead to the discovery of specific biomarkers for occupational SHS-exposure that might precede respiratory diseases on their employees and promote more effective therapies.

*Ethics Committee approval for this study was secured from Instituto Ricardo Jorge's Ethics Committee, Lisbon.*

*This work supported by Fundação Calouste Gulbenkian, Administração Central do Sistema de Saúde, FCT/PolyAnnual Funding Program and FEDER/SaudeXXI Program, Portugal. VDM, SP, and TS, are recipients of FCT fellowships.*

*The authors would like to thank the establishments 'owners/managers and their workers for cooperating in this study.*