Assessment of Potential Health Risks of Portuguese Wildland Firefighters' Occupational Exposure: Biomonitoring Approach

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Abstract

Introduction: Worldwide, forest fires are among the most common forms of natural disasters. In the closing years of the last century there was an increase of the burned area in some parts of the globe, including in Europe. Portugal has been particularly affected by large forest fires and megafires, which have been occurred mainly in the central and northern regions. The proximity of firefighters to fire exposes them to high levels of toxic compounds making this occupation one of the most dangerous and leading International Agency for Research on Cancer to classified occupational firefighting activity as possibly carcinogenic to humans. Up to date, the existing studies are mainly focused on environmental monitoring, existing limited information regarding biomonitoring assessments during real scenarios of wildland fires combat. This study aims to evaluate the impact of firefighting occupational exposure at molecular and cellular levels, considering personal exposure levels. Early-effect biomarkers (e.g., micronucleus, DNA strand breaks and oxidative DNA damage) will be analyzed in order to understand the mechanisms of action through which woodsmoke may impact firefighters' health, including the risk of cancer. Methodology: This ongoing prospective longitudinal study will comprise three different stages, specifically pre-exposure, exposure, and post-exposure to fire season. Around 200 wildland northern Portuguese firefighters will be involved in this study. Characterization of the study population will be conducted via questionnaires. Firefighters' personal exposure levels will be assessed by means of metabolites in exhaled breath, using an artificial olfactory system (e-nose technology). Buccal and urine samples will be used to measure genomic instability through micronucleus test in buccal epithelial cells and urothelial cells. DNA damage and oxidative DNA damage will be evaluated in peripheral blood lymphocytes using the comet assay. Statistical analysis will be performed to determine the relationship between personal exposure levels to toxic compounds and the early-effect biomarkers over the three different phases of the study. Expected results: The obtained results will support a more accurate and comprehensive assessment of occupational risks among wildland firefighters, crucial to prevent/reduce the associated health impacts. This work will contribute to the establishment of recommendations/good practices to improve firefighters' working conditions, allowing better definitions of policies and prevention strategies highly needed in this sector.

Keywords: Biomonitoring, Occupational, Air pollution, Biomarkers, Wildland firefighter.

INTRODUCTION

In the closing years of the 20th century, it was observed an increase of forest burned area, including in European region (Mouillot & Field, 2005). Through the last 3 decades, Portugal has been severely affected by forest fires which have been occurring mostly in the central and northern areas (San-Miguel-Ayanz et al., 2018). Higher drought periods associated with global warming have been increasing the number, duration and severity of fires among forests (Littell et al., 2016).

Firefighters are required to respond to emergency situations, including participation in wildfire combat. During these events, firefighters face continuous exposure to high temperatures and smoke, a complex mixture of gas-, liquid- and solid phase chemicals (Ward, 2001). Many smoke compounds, such as particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and toxic metals (e.g., lead, mercury, and cadmium), have well-known adverse health effects on humans (Cascio, 2018; IARC, 2010). For that reason, firefighting is considered one of the most dangerous occupations (IARC, 2010). Exposure to smoke pollutants may occur through different routes, namely inhalation, dermal absorption, and ingestion (Ruby et al., 2002). Growing evidence has suggested an association between wildfire smoke exposure and respiratory diseases (Cascio, 2018; Reinhardt, 2000), cardiovascular problems (Navarro et al.,2019) and all-cause mortality (Reid et al., 2016). In fact, firefighters are exposed to a complex mixture of compounds, and many of them are classified by International Agency for Research on Cancer (IARC) as probable (e.g., ethylbenzene, isoprene, styrene) or known human carcinogens (e.g., benzene, benzo[a]pyrene, formaldehyde, PM) (IARC, 2010). Increased rates of mortality and cancer incidence among firefighters (when compared to the general population) were found by Daniels et al. (2014). Consistently, a recent meta-analysis reported an increased incidence risk of some cancers among firefighters (e.g., colon, testicular, rectum, bladder, and cutaneous melanoma) (Jalilian et al., 2019).

Wildland firefighters face significant exposure to a variety of smoke pollutants through inhalation and dermal absorption, ranging from carbon monoxide, volatile organic compounds (VOCs), and particles. To protect themselves, firefighters are required to wear proper personal protective equipment (PPE), namely: structural helmet, mask, gloves, boots, and fire-resistant clothing. However, there is some reluctance to wear protection since it adds heat stress and physiological demands upon wildland firefighter. In addition, firefighters' skin may be exposed to combustion products through or around PPE or from the cross-transfer of contaminants on PPE to the skin. Moreover, it is important to highlight that firefighting occupational exposure does not end in the field but accompanies professionals in their return to fire stations through different manners (e.g., contaminated equipment and materials). Although firefighting is classified as potentially carcinogenic to humans by IARC, firefighters are still among the least studied occupations in terms of exposure and relationship to occupational diseases, mostly due to the difficulty involved in the collection of data on personal exposure during firefighting events.

Up to date, the majority of the previous studies has been focused on environmental monitoring. Biomonitoring complements the data on environmental firefighter exposure and provides insight into the biological processes related to that exposure. A major advantage of this approach is that biological monitoring can be performed retrospectively what is particularly useful due the difficulty of sampling in the field. Blood and urine have been the most frequently used biological matrices to determine firefighters' smoke exposure. However, other alternatives have been used, such as the measurement of chemical metabolites in exhaled air. Electronic nose (e-nose) system, for example, is a simple, inexpensive and non-invasive tool that allow the analyse gas samples and may be used for monitoring diseases according to specific breathprints (breath profile) (Scarlata et al., 2015). The use of biomarkers of exposure in the assessment of firefighters' occupational exposure should be accompanied of effect biomarkers to better characterize their exposure and the potential health related outcomes. Comet assay is a versatile method to detect DNA damage at the single cell level (Olive & Banáth, 2006); this technique have been commonly used in biomonitoring studies (Azqueta et al., 2020), inclusively in some that involved wildland firefighters (Abreu et al., 2017). Micronuclei assay, other cytogenetic endpoint, is from a great importance to detect early effects of environmental genotoxic carcinogens (Costa et al., 2013). The application of this assay using epithelial cells such urothelial and buccal cells are of particular relevance because most of the cancers are of epithelial origin (Hinck & Näthke, 2014). The use of both micronucleus assay and comet assay provides information about past and current exposures, respectively.

Biomonitoring of firefighters' occupational exposure during real scenarios of wildland fires combat have been subject of few studies. Thus, the aim of this study is to evaluate the impact of firefighting occupational exposure at molecular and cellular levels, considering personal exposure levels.

METHODOLOGY

This ongoing prospective longitudinal study will comprise three different stages, specifically pre-exposure, exposure, and post-exposure to a whole fire season. Around 200 wildland northern Portuguese firefighters, from different fire stations, will be involved in this study. In a previous phase, subjects will be fully informed on the nature of the study (objectives, risks and benefits); it will be stressed that their participation in this study is totally voluntary and that may be stopped at any point. In case of acceptance, participants will be asked to sign an informed consent already approved by an Ethical Committee. A characterization of study population will be conducted via questionnaires. These will be distributed to participants, in the three phases of the study, to collect data on socio-demographic factors, diet, physical activity, medical and occupational history. Relevant occupational data will be collected at those individuals that are involved in fire activities (e.g., occupational exposure time, usage of PPE, acute health symptoms, etc.). Data on relevant confounding variables as tobacco smoking and grilled or smoked food ingestion will be also collected. This task will run in parallel with the measurement of personal firefighters' occupational exposure and biomonitoring. Firefighters' personal exposure-levels will be assessed though the metabolites in exhaled breath, using an artificial olfactory system (e-nose technology). Exhaled air samples will be collected using Tedlar bags; briefly, subjects will be asked to inhale, hold breath, and consequently exhale into the Tedlar bag. E-nose system incorporates an on-board principal component analysis algorithm that will allow the automatic discrimination of VOCs profiles in each individual breath sample. Early effects may indicate early sub-clinical alterations that may be associated with pathological events. Thus, in the present study early effects will be evaluated at molecular and cellular levels in systemic (peripheral blood) and local target issues (buccal and urothelial cells). Data on genotoxicity and genomic instability associated with wildland firefighters' occupational exposure will be assessed through different techniques, namely comet assay and micronucleus assay. DNA damage will be evaluated in peripheral blood lymphocytes using the comet assay as described in previous works (Esteves et al., 2020; Costa et al., 2008).Oxidative DNA damage will be determined through the enzyme modified version of comet assay where an enzyme incubation step (OGG1) will allow the specific detection of 8-oxoguanine adducts, a biomarker of oxidative stress. Comets will be analyzed through fluorescence microscopy with the Comet Assay V analysis software (Perceptive Instruments). A total of 150 cells will be analyzed per sample, whereas DNA damage and oxidative DNA damage will be expressed as the percentage of DNA in the tail.

Genomic instability will be assessed through the micronucleus assay using buccal and urothelial cells. The analysis of effect biomarkers using epithelial cells (buccal and urothelial) presents several advantages (comparatively with the use of blood lymphocytes) since they are the first-contact tissue of inhaled contaminants that may promote early genotoxic events induced by carcinogenic agents entering the body and because its collection is obtained via minimally invasive methods. For the collection of buccal cells, subjects will be asked to rinse the mouth with tap water a few times in order to remove unwanted debris. Buccal cells from each subject cheek (left and right), will be collected using a cytobrush and suspended in a proper buffer to be further analyzed. In order to collect urothelial cells, a midstream clean catch collection of urine from the second or third void of the day will be requested to the subjects. All biological samples will be transported in a cooler (4°C) to be further processed. Micronucleus assay will be performed according to the procedure described by previous authors (Thomas et al., 2009; Bolognesi et al., 2013). Two thousand epithelial cells per individual will be registered and scored for micronuclei, nuclear buds and nucleoplasmic bridges. Slides will be stained using a DNA specific staining in order to guarantee a good contrast between nucleus and cytoplasm, avoiding the false positive readings. Cells in apoptotic or necrotic will also be scored; the death parameters evaluated will be condensed chromatin, karyorrhectic, pyknotic, and karyolitic cells. Descriptive statistics will be performed in order to describe the characteristics of the study group. Moreover, additional statistical approaches will be performed to determine the relationship between personal exposure levels to toxic compounds and the early-effect biomarkers over the three different phases of the study. Regression modelling approaches will be performed in order to include possible confounding factors.

EXPECTED RESULTS

The increasing incidence of wildfires episodes and long fire seasons highlight the need for occupational studies among wildland firefighters. Scientific evidence has been contributing to the establishment of some measures related to firefighters' occupational health and safety such as the use of PPE, the quick removal of protective equipment after fire suppression activities followed by showering to reduce the exposure to potentially toxic compounds. However, there are still knowledge gaps in the scientific literature regarding the health effects of woodsmoke exposure among wildland firefighters in real scenarios of exposure, particularly in the evaluation of the effects of a wildfire season at the molecular level (e.g., DNA strand breaks, oxidative DNA damage and micronucleus). This study will explore the biological mechanisms of action of woodsmoke exposure among these professionals to establish possible associations between short- and long-term firefighting occupational exposures with acute and chronic health impacts. Results here obtained will take into account factors that may affect the relationship between environmental exposures and the uptake of toxic compounds, including the different absorption rates within the subjects, the use of PPE and other personal behaviors. The characterization of Portuguese firefighters' occupational exposure and potential health risks are particularly relevant because Portugal is one of the European countries more highly affected by wildfires every year.

Finally, we expect to provide important data from biomarkers of exposure and early-effect biomarkers to a better characterization of firefighters' occupational exposure. This work will contribute to the establishment of recommendations/good practices to improve firefighters' working conditions, as well as allow better definitions of policies and prevention strategies highly needed in this sector.

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