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Tracing biomarker of PAH-exposure and susceptibility factor (GSTM-polymorphism) among cancer patients in Pakistan

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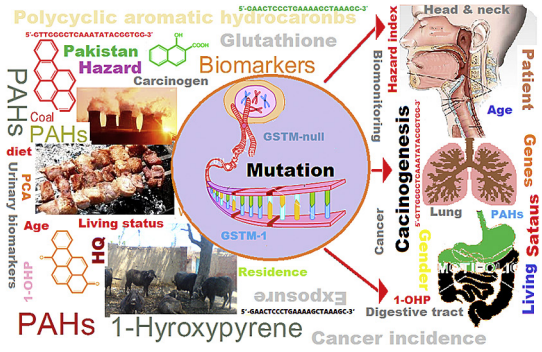
HIGHLIGHTS

- Urinary 1-OHP and genetic susceptibility factor in cancer patients.
- A probe into the association between GSTM-polymorphism and urinary 1-OHP concentrations.
- High levels of urinary 1-OHP were observed in certain cancer patients.

GRAPHICAL ABSTRACT



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ABSTRACT

We studied cancer patients for possible PAH exposure, using urinary concentration of 1-hydroxypyrene (1-OHP) as a biomarker of internal dose of polycyclic aromatic hydrocarbons (PAHs). The subjects included in this study belonged to various socio-demographic backgrounds, and were diagnosed with cancer (i.e. lung, head and neck or digestive tract cancer). In general, we observed high concentration of urinary 1-OHP among digestive tract cancer patients, compared with the controls (CN) (mean 1.06, median 1.03 and mean 0.62, median 0.63 μmol/mol-Cr in digestive tract cancer patients and controls respectively). The concentrations of urinary 1-OHP were higher than the background level of PAHs; therefore, these groups could have been exposed to PAHs. Highest urinary 1-OHP concentration was observed in digestive tract cancer patients (median 1.25 μmol/mol-Cr) with GSTM-1 genotype. The results of PCA were consistent with qualitative and quantitative data analysis. The contribution of urinary

Abbreviations: GSTM-1, glutathione-*s*-transferase M-1; PAHs, polycyclic aromatic hydrocarbons; 1-OHP, 1-Hydroxypyrene; HPLC, High performance liquid chromatography; PCA, Principal components analysis; PCR, Polymerase chain reaction; CYP-450, Cytochrome-P450.

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PAH-tracer
Cancer
GSTM
Pakistan

1-OHP eigenvector revealed a relatively high PAH-exposure among cancer patients compared with CN, while diet and age were influential parameters among cancer patients, which could have a strong link in cancer etiology in the selected exposure groups.

1. Introduction

The unplanned industrialization and the lack of government policies to control/reduce environmental pollution is continuously deteriorating urban ecosystem In Pakistan, which is a cause of numerous eco-health problems. Pakistan is one of the socio-economically disadvantaged countries, where the presence of xenobiotics is barely understood and its link with the public health and related issues is not entirely established (Kamal et al., 2012, 2014a). Recently, few studies have been conducted in Pakistan that highlighted the significance of commonly occurring persistent organic pollutants (POPs) especially the Polycyclic Aromatic Hydrocarbons (PAHs). The exposure to PAHs is very important in the context of public health as they are ubiquitous and gained much attention of scientists all over the world because of their toxicity, carcinogenicity, widespread occurrence and several human health effects (Shen et al., 2013; Kamal et al., 2014b,c).

Human cancer is a major cause of mortality and is second to heart diseases in developed countries (WHO, 2013). Since there is a wide gap in the information on the cancer causation in some countries. Recently, a large number of chemicals have been linked to the cancer etiology, still the link between two is being sought in many parts around the world. Unlike some occupational carcinogens, ubiquity of PAHs has potential implication for the health among the general population as well. Several congeners of PAHs are potential carcinogens, to which, people are exposed via all the major routes of exposure; however, a higher percentage of exposure is accounted for by the intake of contaminated food. Many authors have reported such health implications associated with PAHs exposure from several countries (Goldman et al., 2001; Birkett and Lester, 2003; Cioroiu et al., 2011; Kamal et al., 2014a,c). Furthermore, the genetic polymorphism in xenobiotic metabolizing genes has been associated with individual susceptibility to cancers in various occupational cohorts (Porru et al., 2014). In this context, the glutathione-*s*-transferase (GSTM) is an important phase-II enzyme, and has been reported to play a significant role in the activation and detoxification of PAHs respectively (Ciarrocca et al., 2014). Null-genotype (GSTM-null) is associated with an increased risk of various cancers among different ethnic groups (Ramesh et al., 2004; Zhang et al., 2014). Some recent studies (Kamal et al., 2011, 2014c, 2015a,b,c,d; 2016a,b) in Pakistan highlight the role of PAHs in hemotoxicity among several chronically exposed segments of population, and also presumed lifetime lung cancer risk in various exposure scenarios. However, there is still no biomonitoring study involving cancer patients to probe the cancer incidences in Pakistan. The present study, therefore, aimed to explore environmental and genetic factors, which could be important in the cancer epidemiology in Pakistan. In order to probe gene-environment-interaction, association between GSTM-polymorphism and urinary 1-OHP was co-investigated.

2. Materials and methods

2.1. Study-population and design

A case-control study design was adopted; and the samples were collected under the approved protocol from the ethical review committee of the Quaid-I-Azam University Islamabad (Pakistan). The non-smokers and working class of patients (n = 64) and the controls (n = 20) subjects were recruited with informed written consent. The cases, included patients with lung (n = 20), head and neck (H&N) (n = 26) and digestive tract (n = 18) cancer visiting the Institute of Nuclear Medicine and Oncology (INMOL) hospital Lahore. The inclusion criteria for workers were that the subject should be the non-smoker, and work or be exposed 6 h/d and 6 d/week in same residential areas for ≥5 years. Additionally, we included only those subjects in this study, who were not permanently admitted in the hospital, and were diagnosed with cancer. The participation rate of the patients was 65%, while that of controls was 72%. We collected samples of non-hospitalized subjects in order to assess the PAHs exposure from ambient residential environment. The subjects were interviewed, and their consents were documented the same day they visited their respective consultant in OPD. Samples were taken in the evening, to ensure post shift-representation of urine samples. Participants for an age-matched non-occupationally exposed (and currently working) group of healthy male and female subjects were recruited from Lahore district. Only those subjects were contacted, whom a complete record of living status and the personal characteristic could be accessed. Since, the data from many subjects were not completely available, therefore, only a small number of subjects, meeting the selection criteria were included for further investigation. Thus, the small number of sample represents the difficulty in accessing participants (patients, or their guardians); selection criteria and the confidentiality of their family. A qualified professional collected blood and spot urine samples from each subject, and also documented their demographic information. Participation of each subject was voluntary, and all the participants were informed about the nature of research before taking the samples. All safety measures were taken during the sampling procedure and all healthcare accessories, including disposable gloves and sterilized containers were used to avoid erroneous results. All participants filled a short questionnaire to provide confidential information on their occupation. The information included the work/exposure hour/day (h/d), work experience. Moreover, their health/living status, age, height, body mass index (BMI), education and the histories of known medical problems were also documented.

2.2. Blood sampling and genotyping

From each subject, 3–4 ml blood (in the EDTA vacutanors tubes) and 100 ml urine samples (in sterile PP bottles) were collected on the same day during sampling; the specimens were immediately transferred to the analytical laboratory (Environmental Biology and Ecotoxicology laboratory, Quaid-I-Azam University Islamabad), and after proper labeling/tagging kept refrigerated at 4 °C until analysis. Genomic DNA was extracted from peripheral lymphocytes using extraction kit (cell bios). GSTM-polymorphism was determined by using PCR according to a previously described method by Soya et al. (2007) using β-globin as an internal control (Singh et al., 2009). A 215-bp GSTM-1 fragment was amplified using the following primers: