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Study of trace metal imbalances in the blood, scalp hair and nails of oral cancer patients from Pakistan

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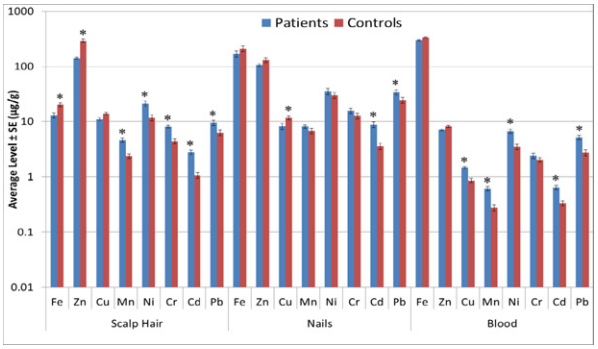
HIGHLIGHTS

- The study investigates the disproportion of trace metals in oral cancer patients.
- Concentrations of Cd, Ni and Pb were found significantly higher in the patients.
- Correlations among trace metals were significantly diverse in the patients/control.
- CA showed interferences by trace metals in blood, hair and nails of the patients.
- Trace metals showed considerable variations for various cancer stages and types.

GRAPHICAL ABSTRACT



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ABSTRACT

Oral cancer is an important cause of cancer morbidity and mortality globally and exposure to trace metals alongside tobacco, alcohol and HPV are the important etiological factors in its development. Selected essential and toxic trace metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were measured in the blood, scalp hair and nails of oral cancer patients and counterpart controls by atomic absorption spectrometry. Mean concentrations of Cd, Ni and Pb were found to be significantly higher ($p < 0.05$) and those of Cu, Fe and Zn were considerably lower in the blood, scalp hair and nails of the patients than the controls. Most of the metal concentrations exhibited higher dispersion and asymmetry in the blood, scalp hair and nails of the patients compared with the controls. The correlation study revealed significantly diverse relationships among the metals in blood, scalp hair and nails of both donor groups. Variations in the metal levels were also noted for various stages (I, II, III & IV) as well as the types (adenocarcinoma and squamous cell carcinoma) of oral cancer. Multivariate cluster analysis of the metal levels in the patients were also significantly dissimilar than the controls. The study evidenced considerably divergent variations in the metal levels in oral cancer patients in comparison with the controls.

1. Introduction

Worldwide, the general population is exposed to elevated concentrations of trace metals either voluntary through supplementation or involuntarily through intake of the contaminated food and water or contact with contaminated soil, dust and air. Therefore, metal poisoning is considered as a silent epidemic and associated with many maladies including cancer. Oral cancer is the sixth leading cause of cancer-related mortality on the global scale and may arise as a primary lesion originating in any of the oral tissues, by metastasis from a distant site of origin, or by extension from a neighbouring anatomic structure (Hosthor et al.,

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2014; Aiken, 2013). Globally, it accounts for approximately 4% of all cancers and 2% of all cancer deaths (Hosthor et al., 2014). In spite of recent advances in oncology and surgery, the mortality and morbidity rates in oral cancer patients remain unchanged (Hosthor et al., 2014).

The main histological types of oral cancer are squamous cell carcinoma, malignant melanoma, acinar cell carcinoma and adenocarcinoma (Zini et al., 2010). Squamous cell carcinoma originating in the mucosal linings contains >90% of oral cancers and highest incidence rates arise in India, Brazil, France and Pakistan (da Silva et al., 2011; Warnakulasuriya, 2009). Staging of oral cancer are used to develop a treatment plan and to define patient's prognosis (Aiken, 2013; AJCC, 1997). Consumption of tobacco is the predominant risk factor due to elevated concentration of carcinogenic exposure and failure to clean the carcinogens from the mucosal surface (Khlifi and Hamza-Chaffai, 2010). Alcohol, betel quid chewing, viruses (HPV), candida, genetic mechanisms, chronic irritation, exposure to metals and diets low in vegetables/fruits are also implicated in the aetiology of oral cancer (Kumar et al., 2016; Khlifi et al., 2013; Gangane et al., 2007; Khanna and Karjodkar, 2006).

Determination of trace metal concentrations in body tissues and fluids has proven useful in evaluating nutritional status, diagnosis of diseases, indications of systemic intoxication and to assess the environmental exposure. Among various matrices, blood is the best indicator for assessing recent past exposures (days to weeks) to the metals because of the capability of erythrocytes to bind these metals and retain them for some time (Schultze et al., 2013). Furthermore, it provides quick and reliable information regarding the trace metal metabolism in human body (Qayyum and Shah, 2016). Hair and nails analysis as a non-destructive monitoring tool for metal exposure assessment has a long tradition in human toxicology (Mikulewicz et al., 2013). There are many advantages for nails and hair as indicators; they are non-invasive, inexpensive, provides easy sampling, preparation, transportation, storage and reflect exposure from a long period (weeks to years depending on the length) (Priya and Geetha, 2011). Because of slow growth rate, generally nails appear to be more suitable in chronic exposure studies, while hair is useful in monitoring of exposure during discrete periods (Palmeri et al., 2000). Nevertheless, exogenous material adhering to nails and hair may influence both intra- and inter-individual variability in metal levels (Slotnick et al., 2005).

Many epidemiological studies have demonstrated the carcinogenic effects of trace metals exposure including oral cancer (Yuan et al., 2011; Jayadeep et al., 1997; Su et al., 2010). Most of the trace metals undergo redox cycling reactions and have the ability to generate reactive radicals in the biological systems (Mulware, 2013). It is generally accepted that ROS via oxidative stress produced by the metals eventually causes DNA damage, strand breaks and alterations in the cellular redox balance, whereby insufficient cellular repair mechanisms may involve wide variety of diseases including oral cancer (Das et al., 2007). Smoking may enhance the oxidative stress through the production of ROS which is an important event in the development of oral cancer (Shetty et al., 2015; Burlakova et al., 2010). Numerous studies indicated that oxidative stress plays a significant role in oral carcinogenesis (Choudhari et al., 2014; Kryston et al., 2011). However, very limited data related to the trace metals and oral cancers are available in Pakistan (Cancela et al., 2010). Thus, there is a dire need to study the interrelationship of trace metals, which could have clinical and diagnostic significance. So the present study is intended; 1) to measure the concentrations of various essential and toxic trace metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the blood, scalp hair and nails of oral cancer patients in comparison with the controls, 2) to find out the mutual associations among the metal levels by the correlation study, 3) to explore the multivariate apportionment of the metals in blood, scalp hair and nails of the patients and controls by cluster analysis. Viable variations in the metal levels with respect to histopathological types/stages of oral cancer are also evaluated, thereby investigating whether these metals have any presumptive benefits in the diagnosis and/or prognosis of oral cancer. Further, this study will help to regulate the possible association between the elevated and/or lower concentrations of selected metals in different tissues and the onset/progress of the oral cancer in Pakistan.

2. Materials and methods

2.1. Study population

In the present study, the patients were selected among the newly diagnosed oral cancer patients admitted in Nuclear Oncology & Radiotherapy Institute (NORI), Islamabad, Pakistan. The protocol of study was approved by the ethical review committee of the institute prior to the sample collection. Subjects were selected on volunteer basis and an informed signed consent was obtained before sampling. The blood, scalp hair and nails samples of oral cancer patients were collected from the freshly diagnosed patients prior to any treatment (i.e., surgery, chemotherapy or radiotherapy) and they were not taking any mineral supplement for the last three months. Oral cancer was confirmed by biopsy test and types and stages were identified by histopathological and radiological examinations. The controls or healthy subjects were also selected on volunteer basis from the same localities with matched age groups, similar socioeconomic status, food habits and who were not suffering from any cancerous lesions. The healthy subjects were initially briefed about the purpose and objectives of the study and then a written consent was obtained. A proforma was filled by all the participants to record the information, such as, gender, age, weight, height, habitat, smoking habits, dietary habits, cancer type/duration, medicine, occupation, hobbies and tumour grade etc., at the time of sample collection. None of the patients or controls was consuming alcohol on continuous basis. Physical examinations were performed in the institute to measure the participant's biochemical data (blood pressure, sugar, urine examination, renal function test, liver function test, etc.) and body mass index (BMI).

2.2. Collection and processing of the blood samples

The blood sample (about 3 mL) was drawn from an antecubital vein and transferred to a vacutainer tube at room temperature (BD Vacutainer Ref. 366,430); it was stored in a refrigerator until further processing. Exactly known amount of blood sample was transferred from storage tube to the digestion flask. A mixture of HNO_3–$HClO_4$ (10:1 v/v) was added to each flask, which was subsequently heated (80 °C) to a soft boil until dense white fumes evolved. After digestion, the flasks were left to cool at room temperature and then diluted to 25 mL with doubly distilled water (Qayyum and Shah, 2016). Blanks containing all the reagents in the same sequence (without blood sample) were also prepared with each batch of the samples.

2.3. Collection and processing of the hair samples

About 3 g of hair sample was collected from the sub-occipital zone of the head at 1 cm from the scalp, as strands 3–5 cm long with a pair of plastic scissors. Each sample was placed individually in a zip-mouthed polyethene bag and stored in the desiccator until further processing. Each scalp hair sample was thoroughly washed to remove the exogenous impurities (Sen and Das Chaudhary, 2001; Pereira et al., 2004). Hair samples were cut in pieces of 2–3 cm in length and mixed with 50 mL of detergent solution (Colgate Palmolive Cleaner, 5% w/v) in a conical flask and shaken on an auto-shaker for 30 min at 320 vibrations per minute. The samples were left for 2 h undisturbed then washed with plentiful water until all detergent was leached out. Afterward, each sample was washed with 30 mL Triton X-100 (0.5% v/v) and again shaken on the auto-shaker for 20 min. Later the samples were rinsed with excess of doubly distilled water and ultimately dried in an electric oven for overnight at 70 °C and cooled to room temperature in a desiccator (Qayyum and Shah, 2014).