Journal of Trace Elements in Medicine and Biology 27 (2013) 307–311

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

CLINICAL STUDIES

Trace elements (copper, zinc, selenium and molybdenum) as markers in oral sub mucous fibrosis and oral squamous cell carcinoma

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ARTICLE INFO

Article history:
Received 18 December 2012
Accepted 3 April 2013

Keywords:
Copper
Zinc
Selenium
Molybdenum
Oral squamous cell carcinoma

ABSTRACT

Oral cancer is a major cause of cancer morbidity and mortality worldwide and is prevalent in most areas where tobacco related practices are observed. Essential elements play a role in many biochemical reactions as a micro-source and there is growing evidence that their concentrations are altered on the onset and progress of malignant disease. In this study the levels of copper (Cu), zinc (Zn), selenium (Se) and molybdenum (Mo) in serum of patients with oral sub mucous fibrosis (OSMF) ($n = 30$) and oral squamous cell carcinoma (OSCC) ($n = 30$); were determined and the alterations of these critical parameters were analyzed in comparison with controls ($n = 30$) to identify predictors amongst these parameters for disease occurrence and progression. The serum Cu and Zn were established using Flame Atomic Absorption Spectrometry. Serum estimation of Se and Mo was done by graphite furnace atomic absorption spectrometry (GFAAS). Data analysis revealed a marked, progressive and significant increase in Cu levels in precancer (OSMF) and cancer (OSCC) groups as compared to the normal group. The level of Zn in serum was slightly elevated in OSMF and OSCC though not statistically significant. Cu/Zn ratio was slightly but not significantly elevated. Serum levels of Se and Mo were significantly decreased in the precancer and cancer groups as compared to the normals.

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Introduction

In most regions of the world, about 40% of head and neck cancers are known to be OSCC originating in the oral cavity. OSMF is a chronic pre malignant condition largely affecting the youth, particularly in the Indian sub-continent [1].

Trace elements Cu, Zn, Se and Mo are essential for proper functioning of life supporting processes as they have an important role in many biochemical reactions. They are required in small concentrations and are essential components of biological systems or of structural portions of biologically active constituents [2,3]. Cu is primarily found in plasma (90–95%) as part of the oxidative enzyme ceruloplasmin [4]. It is also part of various enzymes like tyrosinase, uricase, and cytochrome oxidase which participate in oxidative processes in the cell metabolism. Zn stimulates gene transcription and cell multiplication and is critical for activation of RNA and DNA polymerase activity and is important in multiplication of tumour cell. Decreased Zn levels are associated with increased oxidative stress at the cellular level [5].

The role of Se as a chemo preventive agent is attributed to its antioxidant effects [6]. Antioxidant enzymes glutathione peroxidase (GSH-Px) and thioredoxin reductase contain Se that catalyze the reduction of hydrogen peroxide and organic hydroperoxide thus preventing the cell membrane from oxidative damage. Similarly, the main function of Mo is as a co-factor in xanthine oxidase, aldehyde oxidase and sulphite oxidase [7]. It plays an important role in purine metabolism making it an essential trace element in human nutrition. Cu, Zn, Se and Mo are cofactors of anti oxidant enzymes and are involved in the destruction of free radicals through the enzyme systems. They help maintain the integrity of the membrane, reducing the risk of cancer and also slowing the ageing process [8].

Literature reports indicate that a number of studies have examined the relation of Cu and Zn levels in serum, blood, saliva, etc. with various carcinomas in general and OSCC in particular.

Abbreviations: Cu, copper; Zn, zinc; Se, selenium; Mo, molybdenum; OSMF, oral sub mucous fibrosis; OSCC, oral squamous cell carcinoma; GFAAS, graphite furnace atomic absorption spectrometry; Cu/Zn ratio, copper/zinc ratio; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; GSH-Px, glutathione peroxidase; LOD, limit of detection; NIST, National Institute of Standards and Techniques; SPSS, Statistical Package for Social Science; HEPA, High Efficiency Particulate Air; AAS, atomic absorption spectrometry; ICPMS, Inductively Coupled Mass Spectrometry; EDXF, Energy Dispersive X-ray Fluorescence; PIXES, Proton induced X-ray Emission Spectrometry.

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http://dx.doi.org/10.1016/j.jtemb.2013.04.003

The normal serum levels of Cu and Zn are 0.6–1.6 μg/ml and 0.6–1.5 μg/ml respectively [15–23]. Reports of Se are few and those of Mo are scarce. However normal serum levels of Se and Mo are 60–150 ng/ml and 0.0–1.7 ng/ml respectively [24–32]. In the present study Cu, Zn, Se and Mo levels in serum were estimated in patients diagnosed with OSCC, OSMF and compared with normal control group. Several studies indicate an increased Cu/Zn ratio in malignancy and the same has been employed for a prognosis of the disease [33,34].

The initiative was undertaken to study the change in the profile of these elements in the three study groups and evaluate their usefulness so that they may be considered as biomarkers in the process and progress of carcinogenesis.

Materials and methods

This study was carried out in Nair Hospital Dental College, Mumbai in association with Bhabha Atomic Research Centre, Mumbai. 30 patients with OSMF and 30 patients with OSCC with histopathologically proven lesions were included in this study. For comparison thirty normal subjects were also selected. The age group of these patients ranged from 20 to 79 years. The symptoms and signs of the patients were evaluated, after through history taking [1,16,22].

Laboratory analysis

The glass tubes used for collection of the blood for trace elements estimation were cleaned twice with Suprapur® (Merck) Nitric acid in 1:1 dilution and then rinsed with double distilled water twice before storing them in acid cleaned polypropylene bags.

A 5 ml sample of venous blood was collected with help of a Teflon catheter was collected directly into the clean test tubes as mentioned previously. The blood collected was allowed to clot for 30 min at room temperature and then centrifuged at 3000 rpm for 10 min. The obtained serum was stored in the trace element free glass tubes cleaned as mentioned previously. The same was further stored at −20 °C until estimation. Cold chain was maintained during the transportation of the blood samples to the Ultra Trace Analytical Facility. The entire collection procedure was checked meticulously for Cu, Zn, Se and Mo estimation and found to be essentially free from contamination.

Analytical procedure

Cu and Zn

Serum was evaluated by Flame Atomic Absorption Spectrometry employing a GBC 906AA AAS unit with deuterium-arc background correction.

Analytical and instrumental parameters

Hollow cathode lamp for Cu and Zn, wavelength 324.7 nm and 213.8 nm respectively, air acetylene flame and nanopure water (18.3 Ω) were used. All measurements were performed using integrated absorbance.

The refrigerated samples were thawed to room temperature and suitably diluted with nanopure water to bring the mentioned element in the optimum analytical range. The samples were aspirated into the air acetylene flame.

The results of Cu and Zn were expressed as μg/ml. The coefficient of variation was from 2 to 5% and the average accuracy was >95% when analyzing the reference material.

Se and Mo were determined by GFAAS employing GBC Savanta Ultra Z AAS with longitudinal Zeeman Correction.

Analytical and instrumental parameters

Ω pyrolytically coated partition graphite tubes, EDL Se λ 196 nm and Hydrochloric acid Mo λ 313.2 (GBC, Australia) were used.

Se

1 ml of serum sample was taken in micro centrifuge tubes and 100 μL of Suprapur (Merck). Nitric acid was added to it. The micro centrifuge tubes were warmed in water bath at 70 °C for 5 min and centrifuged at 9000 rpm for 10 min. Supernatant was decanted immediately and Se was analyzed in supernatant using GFAAS. Palladium nitrate was used as matrix modifier.

Mo

1 ml of serum sample was transferred to a pre cleaned platinum dish. Sample was incinerated in a clean room to remove organic matter. The residue was made up to 0.5 ml with 2% nitric acid. Mo was estimated by GFAAS in this solution.

The results were expressed as $\mu g\,L^{-1}$. The detection limit or LOD (limit of detection), is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. The detection limit (3σ blank) for Se was <5 $\mu g\,L^{-1}$ and for Mo was <0.15 $\mu g\,L^{-1}$. The average accuracy was >95% when analyzing the reference material.

Quality assurance

In the present study elemental standards (Cu, Zn, Se and Mo 1 mg/ml) traceable to NIST and Seronorm™ Trace Elements Serum-reference material Level 2 (Nycomed Pharma AS, Oslo) were employed. The calibration of the AAS and validation of the analytical results were carried out using the same. (The t test at 95% confidence limit gave a value less than critical value $t_{0.05}$ is 2.78 $n = 5$.) Spiked sample recoveries and split specimen analysis was also carried out.

All the samples were processed in Laminar Air Flow benches which are equipped with HEPA filters working at an efficiency of 99.7% for 0.5 particles to minimize sample contamination.

Statistical analysis

The data was subjected to statistical analysis using both Microsoft Office Excel (Microsoft Corporation, Redmond, WA, USA) and Statistical Package for Social Science (SPSS) software. The chosen confidence level was 95%. Univariate statistical methods were used to calculate descriptive statistical parameters (mean, median and standard deviation) for each parameter in the three groups. The Anova test was used for data normal distribution. The Pearsons Correlation was used to compare variance of the samples. Tukey test, Kruskal Wallis one way analysis of variance, Mann–Whitney (for results with a non-normal distribution) and Linear Regression analysis were applied for analysis.

Results

In this randomized controlled clinical trial 48.5% of cases were in the age range of 20–39 years. The age (in years) range of patients with OSMF was 35.67 as compared to 50.15 in the OSCC group and 37.30 in the normal group. The mean age in precancer and cancer group was higher than normal and the difference was statistically significant (p 1.89E−05). It is observed that patients report to the hospital at late stage of disease and there is a trend of more males presenting with disease than females in the outpatient department. Majority of patients in this study were males as they are usually earning members of the family and seek medical attention.

Majority of our study group consisted of males (include statistics of both male and female patients) who had tobacco, areca

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