RESEARCH ARTICLE

Association between Trace Element and Heavy Metal Levels in Hair and Nail with Prostate Cancer

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Abstract

   While associations between trace elements and heavy metals with prostate cancer are still debatable, they have been considered as risk factors for prostate cancer. Thus, this study aimed to detect any links between selected minerals and heavy metals including Se, Zn, Cu, Mn and Fe with prostate cancer. A case control study was carried out among 100 subjects (case n=50, control n=50), matched for age and ethnicity. Trace elements and heavy metals level in hair and nail samples were determined by ICP-MS. Mean selenium levels in hair and nail of the cases were significantly lower as compared to controls. A similar trend was noted for zinc in both hair and nail samples, whereas the mean level of copper was significantly higher in cases than controls. Similar elevation was noted for iron and manganese (p<0.05 for all parameters). Low levels of selenium and zinc and high levels of copper, iron and manganese appear to be associated with the risk of prostate cancer. Further studies to elucidate the causal mechanisms and appropriate chemopreventive measures are needed.

Keywords: Prostate cancer - heavy metals - trace elements - hair and nail samples - case-control study

Introduction

   Prostate cancer is the sixth most common type of cancer worldwide (Sapota et al., 2009). Both genetic and environmental risk factors including diet (fat, vegetables and fruits, dairy products and certain micronutrients and vitamins intake), smoking, alcohol consumption, sexual and physical activity, hormones and body size are associated with increased risk of prostate cancer among Asian population. These factors play an important role in development and progression of tumour cells either by acting directly in a causative pathway or indirectly, by acting on genes related disease susceptibility. These suggest indirect mechanisms include regulation of hormone level or regulation of carcinogenic agent's metabolism (Jarup, 2003).

   Several epidemiological studies have demonstrated that exposure to metals has so much toxic and carcinogenic affects on humans and animals (Vinceti et al., 2007). According to epidemiological data (Harris et al., 2003; Mahata et al., 2003), heavy metals are confirmed human carcinogens; lead, cobalt, and iron are observed as potential carcinogens. Excess occupational and environmental exposure to metals is considered to be a major cause of metal-related cancer and also associated with increased cancer risk. Prostate cancer mortality was found to be strongly contributed by cadmium (Cd), followed by zinc (Zn) and chromium (Cr) (Killilea et al., 2007).

   Changes in the balances in the optimum levels of trace elements such as copper (Cu), zinc (Zn) and selenium (Se) may affect biological pathways and have been associated with many diseases including cancer and chronic disorders (Jarup, 2003). Previous study among Malaysian men reported that there was an association between low fat diet, high intake of fruits, vegetables, lycopene rich foods and being physical active at middle age with reduced risk of prostate cancer (Shahar et al., 2011). However the role of trace elements and heavy metals has not been fully investigated among Asian men, therefore this study aimed to determine the association between trace elements and heavy metals with prostate cancer risk among men in Klang Valley of Malaysia. This study also aimed to specifically evaluate associations between prostate cancer with anthropometric and socio-demographic factors.

Materials and Methods

Subjects

   This was a case control study on 50 prostate cancer patients (cases) and 50 healthy men (controls) conducted from August 2010 until May 2011 at Kuala Lumpur. Cases were newly diagnosed prostate cancer patients (using biopsy) recruited from Universiti Kebangsaan Malaysia Medical Centre. Those who were at the fourth stage of prostate cancer, undergoing chemotherapy or radiation, severely stressed as determined using Hospital Anxiety

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and Depression Score test (HADS) (Montazeri et al., 2003) and had history of metastatic and prostatectomy were excluded. A minimum age of 50 years was required for both case and control group (ranges 50-80 years). Controls were healthy men around Klang Valley, who had not been diagnosed with chronic diseases including benign prostatic hyperplasia, kidney stone, hypertension, diabetes mellitus, cardiovascular disease, epilepsy and arthritis. Individuals who were taking trace elements and heavy metals supplements for the past three months were excluded from the study. Cases and controls were matched for both age and ethnicity. Sample size was calculated with 95% confidence interval and 80% study power following the method of Sample Size Determination in Health Studies. Subjects were interviewed for their socio-demographic profile, smoking habits, medical history, educational level and marital status.

The study was approved by the Universiti Kebangsaan Malaysia Ethics Committee and informed consent was obtained from each subject.

Anthropometric Measurements

Anthropometric measurements including weight, height, waist and hip circumferences were carried out on subjects. The weight and height of subjects were measured by a calibrated TANITA digital scale and a portable stadiometer (SECA 214) to the nearest 0.1 kg and 0.1 cm, respectively (MOH, 2008). We calculated body mass index (BMI) as weight (kg) divided by height (m²). Measuring tape (Lufkin W606PM) was used to measure waist at the narrowest levels over light clothing and hip circumferences to the nearest decimal.

Trace Elements and Metals Analysis

A total of 5 mg of nails and 5 mg of hair for each subjects were collected for this analysis. Samples were washed thorough stirring with different solvents in sequence: acetone, deionized water and 0.5% Triton X-100 solution (Chatt and Katz, 1988; Gammelgaard et al., 1991). The samples were then subjected to acid digestion procedure using nitric acid, hydrogen peroxide and deionized water. Sample solutions were diluted to exactly 10 mL and were ready for the measurement process (Mehra and Juneja, 2005). An inductively coupled plasma mass spectrometer (ELAN 6000, Perkin- Elmer Norwalk, USA) was used for the detection of trace elements and metals (Se, Zn, Cu, Fe, Mn).

Statistical analysis

Descriptive statistics was used for socio-demographic data of the subjects. Trace elements and metals concentrations in hair and nail were expressed as arithmetic mean and median value in μg/g with standard deviation. The statistical significance of mean values between case and control group was determined by applying independent sample t-test and Mann-Whitney test. The level of significance was set at $p<0.05$. The association between trace elements and heavy metals level in hair and nail of the subjects with prostate cancer was determined using Odds Ratio from the binary logistic regression at $p<0.05$.

Results

One hundred men aged 50 to 86 years, consisted of 50 prostate cancer patients and 50 controls participated in this study. The ethnic compositions of both cases and controls were 47% Malays, 33% Chinese and 20% Indians, as shown in Table 1.

Table 1. shows that the mean age of cases was comparable with controls (72.4±6.74 vs 71.7±7.86). Cases had lower mean BMI than controls (23.16 ± 4.62kg/m² vs 25.37 ± 3.51 kg/m², p<0.05). In addition, BMI ≥ 25 kg/m² (overweight) was found in 30% of cases and 60% of controls.

The mean level of selenium and zinc in both hair and nail of cases were significantly lower as compared to controls (p<0.05), as shown in Table 2. In contrast, cases had a higher concentration of copper, iron and also manganese in their hair and nail as compared to controls (p<0.05).

Table 3. shows odds ratio (OR) for prostate cancer across medians concentrations of trace elements and heavy metals in hair and nails of study participants. OR are shown are adjusted for ethnicity, family history, smoking and alcohol consumption. Median concentration of trace elements and heavy metals of control group was considered as a reference due to unavailability of the cut-off point for these elements in hair and nail samples in previous studies. According to this table, there was a significant association between selenium in hair (OR 5.48, p<0.002) and nail (OR 5.91, p<0.001), manganese in hair (OR 3.56, p<0.02) and nail (OR 3.78, p<0.007) and iron level in nail (OR 5.47, p<0.001) with the occurrence of prostate cancer.

Table 1. General Characteristics of Study Participants

Variable	Case	Control	P
Age (y)	72.4±6.74[b]	71.7±7.86	0.4[a]
Weight	64.7±13.67	73.5±11.63	0.001
Height	1.66±0.08	1.7±0.7	0.07
BMI	23.16±4.62	25.37±3.51	0.007
waist	89.51±11.85	91.45±10.11	0.4
Hip	94.95±11.35	99.95±7.62	0.014
Waist/hip	0.94±0.13	0.91±0.6	0.1
Ethnicity (%)			0.182[c]
Chinese	20 (40%)	13 (26%)	
Indian	11 (22%)	9 (18%)	
Malay	19 (38%)	28 (56%)	
Education (%)			0.087
Primary or less	23 (46%)	11 (22%)	
Secondary	18 (36%)	22 (44%)	
University graduates	9 (18%)	17 (34%)	
Monthly income			0.001
>RM1500	24 (48%)	42 (84%)	
Occupation			0.001
Retirement	38 (76%)	9 (18%)	
Smoking			0.229
Yes	30 (60%)	24 (48%)	
Alcohol consumption			0.688
Yes	24 (48%)	22 (44%)	
Family history			0.106
Yes	16 (32%)	9 (18%)	

[a]Values are resulted from independent sample t-test, [b]Valuses are Mean±SD, [c]Values are resulted from chi-square test

Table 2. Concentrations of Trace Elements and Heavy Metals in Hair and Nails of Study Participants

Parameters	Case Mean±SD	Median (25%-75%)	Control Mean±SD	Median (25%-75%)	P[a]
Concentrations in hair					
Selenium	7.15±3.5	6.0 (4.48-9.74)	10.4±4.52	11.35 (6.88-14.00)	0.001
Zinc	3.29±2.22	3.10 (1.48-3.99)	4.29±2.53	3.75 (2.92-4.85)	0.018
Cupper	0.09±0.03	0.08 (0.06-0.1)	0.07 ±0.02	0.07 (0.05-0.08)	0.029
Ferrus	1.23±0.968	0.97 (0.65-1.5)	1.21±1.472	0.72 (0.41-1.09)	0.25
Manganese	0.07±0.04	0.07 (0.05-0.09)	0.055±0.05	0.02 (0.01-0.10)	0.001
Concentrations in nails					
Selenium	7.23±3.11	6.92 (5.26-8.98)	9.03±3.69	10.17 (5.90-11.69)	0.001
Zinc	2.70±1.49	2.83 (1.31-4.07)	3.97±4.06	3.32 (2.73-4.36)	0.01
Cupper	0.07±0.03	0.06 (0.05-0.09)	0.06±0.02	0.05 (0.04-0.08)	0.08[b]
Ferrus	1.58±0.88	1.50 (0.89-2.04)	0.92±0.74	0.65 (0.41-1.44)	0.001
Manganese	0.10±0.06	0.1 (0.06-0.13)	0.05±0.04	0.05 (0.01-0.08)	0.001

[a]Values are resulted from Mann-Whitney U-test, [b]Values are resulted from independent-sample t-test

Table 3. Odds Ratio for Prostate Cancer Across Medians Concentrations of Trace Elements and Heavy Metals in Hair and Nails of Study Participants

Parameters	N	Case N (%)	Control N (%)	Crude Odd Ratio (95% CI OR)	Adjusted Odd Ratio (95% CI OR)[b]	P value[a]
Concentrations in hair;						
Selenium: <Median (abnormal)	57	33 (84.6)	24 (50)	5.5 (1.94-15.52)	5.48 (1.86-16.16)	0.002
>Median (normal)	30	6 (15.40%)	24 (50%)	1	1	
Zinc: <Median (abnormal)	56	32 (64%)	24 (49%)	1.85 (0.82-4.14)	2.32 (0.95-5.63)	0.06
>Median(normal)	43	18 (36%)	25 (51%)	1	1	
Copper: <Median (normal)	48	28 (70%)	20 (48.80%)	1	1	
>Median (abnormal)	33	12 (30%)	21 (51.20%)	0.6 (0.26-1.46)	0.56 (0.22-1.38)	0.2
Manganese:				3.71 (1.29-10.69)	3.56 (1.19-10.6)	0.02
<Median (normal)	20	7 (14.30%)	13 (38.20%)	1	1	
>Median (abnormal)	63	42 (85.70%)	21 (61.80%)			
Ferrous: <Median (normal)	44	19 (38%)	25 (50%)	1	1	
>Median (abnormal)	56	31 (62%)	25 (50%)	1.63 (0.73-3.61)	1.55 (0.66-3.63)	0.3
Concentrations in nai;						
Selenium: <Median (abnormal)	32	34 (81%)	24 (50%)	4.2 (1.63-11.05)	5.91 (1.99-17.53)	0.001
>Median (normal)	58	8 (19%)	24 (50%)	1	1	
Zinc: <Median (abnormal)	59	34 (68%)	25 (50%)	2.12 (0.94-4.78)	2.4 (0.99-5.8)	0.053
>Median (normal)	41	16 (32%)	25 (50%)	1	1	
Copper: <Median (normal)	43	23 (55.30%)	20 (41.50%)	1	1	
>Median (abnormal)	45	24 (44.70%)	21 (58.50%)	0.57 (0.24-1.33)	0.43 (0.17-1.12)	0.08
Manganese: <Median (normal)	31	10 (20%)	21 (51.20%)	1	1	
>Median (abnormal)	60	40 (80%)	20 (48.80%)	4.2 (1.66-10.50)	3.78 (1.43-9.98)	0.007
Ferrous: <Median (normal)	33	8 (16%)	25 (50%)	1	1	
>Median (abnormal)	67	42 (84%)	25 (50%)	5.2 (2.05-13.4)	5.47 (2.03-14.72)	0.001

[a]Values are resulted from binary logistic regression, [b]OR are adjusted for ethnic, family history, smoking and alcohol consumption

Discussion

Unlike previous studies which suggested that development of prostate cancer may be affected by environmental factors such as alcohol consumption and smoking, our data did not find any significant association between these factors with incidence of prostate cancer (Gsur et al., 2004), probably due to a small number of the samples who smoke and consumed alcohol.

The results of the present study showed that prostate cancer patients had lower concentration of selenium and zinc in their hair and nails than control group. These results showed that low levels of selenium and zinc and high levels of ferrous, copper and manganese, increased the risk of prostate cancer. Most of the previous studies determined the association between risk of prostate cancer and trace elements concentration in the plasma (Brooks et al., 2001; Shahar et al., 2009; Adaramoye et al., 2010). According to our knowledge, present study is

the first study which determined such an association in hair and nail samples of Asian people. Selenium is a well documented cofactor to antioxidant enzymes (Dunna et al., 2011). The best-known example of selenium function is the reduction of hydrogen peroxide and damaging lipid and phospholipid hydroperoxides to harmless products (water and alcohols) by the family of selenium-dependent glutathione peroxidises. This function helps to maintain membrane integrity, protects prostacyclin production and reduces the likelihood of propagation of further oxidative damage to bio-molecules such as DNA, lipoproteins and lipids with the associated increased risk of conditions such as cancer and atherosclerosis (Reyes et al., 2002).

In the present study, we observed statistically significant differences in the concentration of the zinc in hair and nails of the cases compared to the controls. Additionally, zinc deficiency increased the risk of prostate cancer which is consistent with previous studies (Costello et al., 2011; Epstein et al., 2011). Costello (2011) reported

that zinc was markedly decreased in prostate cancer tissue samples versus normal and hyperplasic tissue samples. The possible explanation for this relation is that, the peripheral zone glandular epithelial cells of the prostate which is responsible for production and secretion of prostatic fluid, evolved as highly specialized and unique citrate-producing cells. The normal peripheral zone accumulates uniquely high zinc levels, being approximately ten fold greater than those of most other soft tissues (Costello et al., 2011).

In this case-control study high level of iron in hair and nail of the subjects increased risk of prostate cancer by 1.5 and 5.4. Similar observations were noted in other case-control studies (Siah et al., 2005; Choi et al., 2008). Iron is the most abundant transition metal in the human body. However, as a transition metal, iron's loosely bound electrons in its outer shell facilitates the production of reactive oxygen species (ROS), resulting in DNA single breaks and oxidative stress (Bae et al., 2009). The role of ROS in prostate cancer with human variation in response to ROS damage and repair exacerbating ROS-related DNA damage in the prostate was supported in recent experimental studies (Muzandu et al., 2005). Although epidemiological studies have shown inconsistent results but excessive iron intake from either foods or dietary supplements can be a source of ROS (Chan et al., 2005; Lee and Jacobs, 2005; Zhou et al., 2005).

Furthermore, a significantly higher percentage of cases had abnormal copper and manganese status in their hair and nails as compared to controls which increased risk of prostate cancer. Copper plays an important role in the carcinogenic process that may be also linked with its ability to bind to some proteins and thus to acquire the angiogenic activity. On the other hand, copper mediates the involvement of cellular proliferation via the activation of angiogenic growth factors (Banas et al., 2010). Additionally, because copper is expected to initiate angiogenesis, elevated concentration of this element is likely to promote prostate cancer by increasing the blood supply for tumour growth. This explains an increase in Cu levels in cancer-affected tissues in malignancies (Uauy et al., 1998; Majumder et al., 2009).

Recently, some evidence suggests that Fe and Mn concentrations were higher in the serum of patients with malignant lymphoma and lung cancer (Wu et al., 1988; Zhao and Han, 1998). Pasha et al. (2008) reported higher concentration of manganese, magnesium and plumbum in plasma of prostate cancer patients as compared to controls (Pasha et al., 2008). Manganese is an essential trace element in the body which has several biochemical and chemical properties similar to iron. Some previous studies revealed a metabolic interaction between the Fe and Mn, especially at the level of intestinal absorption (Chua et al., 1996). In our data, the increased level of Mn in hair and nails of the patients with prostate cancer may increase the occurrence of prostate cancer by 3.5 and 3.7, which should be clarified in further studies.

This study has shown the element distribution patterns of prostate cancer patients and the excess levels of trace elements and heavy metals observed in the hair and nail among prostate cancer patients could be either a cause or a consequence of the pathological stages. Concerning the role of minerals and heavy metals in the origination or advancement of prostate cancer, emerges one possible surmise that the raised levels of trace elements and heavy metals could have led to the formation of free radicals or other reactive oxygen species that adversely affect DNA, thereby causing prostate cancer. It is a suspected human prostatic carcinogen and has been shown to induce prostatic tumours and proliferative lesions in rats (Reyes et al., 2002).

There are some strengths in the present study that should be taken into account. This is the first study which assessed the association between prostate cancer with trace elements and heavy metals in hair and nail sample of Malaysian people. Until recently, all of studies are limited to evaluate such an association in serum. However, we could not to identify the dietary, occupational and environmental hazards. Indeed, we need more long-term studies to determine the relation between trace elements and heavy metals status with risk of prostate cancer.

In conclusion, low level of selected trace elements (Se, Zn) and high level of minerals (Cu) and heavy metals (Fe, Mn) were associated with increased risk of prostate cancer. There is a need to identify the causes of the scenario and also conduct further nutrition, lifestyle and occupational hazards intervention in order to reduce the risk of prostate cancer among Malaysian men.

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