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Determination of heavy metals concentration in cancer patients of faridkot region of punjab (India) using hair as biomarker

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Abstract

The study was carried out in Faridkot, which is located in south west of Punjab (India). Hair samples of cancer patients were used determine the concentration of heavy metals, as hair samples indicates long exposure of heavy metals in a person's body. Cotton belt of Punjab suffered from an increase in number of cancer patients in last decade. Principal component analysis, a data reduction technique is used to see the correlation of metals in terms of chemical interrelations.

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Introduction

Metallic elements play important biological roles and are found in all organisms. Exposure to heavy metals and environmental contamination is a serious concern throughout the world (Orisakwe, 2014). Economic development, excess use of fertilizers, industrial revolution and rapid population growth are main concerns regarding contaminated environment. Metal pollution can be triggered from release of toxic metals in drinking water. In certain natural activities like weathering and decomposition of ores and metal rocks may transfer heavy metals in groundwater which further led to contamination of groundwater and human exposure to the same. It is also noted that polluted crops and water cause contamination. Drinking water and food is also a route. As the breakdown of metals is not possible, they stay in body until excreted. Heavy metals contamination in human body is primarily done via three routes i.e. inhalation, absorption and ingestion. Inhalation is most important occupational exposure. The metals accumulate in bones, hair, nails and tissues (Michalak *et al.*, 2012).

Punjab is located in the northwest region of India. It is one of the leading food grains producing state of India. Cancer prevalence is higher in Punjab as compared to national average i.e. 800/million/year. In Punjab, cancer rate in Malwa region is higher as compared to Majha and Doaba region which is 1089/million/year compared to 647/million/year and 881/million/year (DHFW, 2013). Faridkot is located at the South-Western part of Punjab. It is located in Malwa region of Punjab, which shares its boundaries with Ferozepur, Moga, Bathinda and Muktsar. It is the smallest district of Punjab which comprises of two blocks; Faridkot and Kotkapura. Heavy metal contamination is a serious concern in this area. This study focuses on local people who have lived there for a long period without migrating. Farming is the occupation of people selected. Fertilizers, herbicides and pesticides are used in high amount during the season. The excess use of fertilizers and pesticides is considered as main reason for high number of several diseases in Malwa belt. Cancer mortality rate is also directly correlated with farming (Singh, 2008)

The use of hair as biomarkers is widely used during past three decades for the assessment of different exposures, diagnosis of disease and evaluating nutritional and forensic status. (Chatt *et al.*, 1980; Katz and Chatt, 1988; Valkovic, 1988; Batzevich, 1995; Bencjo, 1995). Hair is used for toxicological analysis as it differs from urine and blood samples because it shows wide range of detection from few months to several years which can help in investigating past consumption. Hair samples are solid and durable in nature. With developed analytical technologies, new methods enables hair samples to be analyzed for organic substances as they have high sensitivity. Moreover simplicity of collecting, handling and transporting of sample also makes hair as suitable biomarker for detection. Although before hair was dismissed as unreliable test for detection of metals, but now it is proved that hair is a reliable material for testing the occupational and environmental exposure.

In the previous studies conducted in this region, a considerable high amount of heavy metals have been reported in soil and water. Malwa area of Punjab was previously known as 'Food Bowl of India' now known as 'cancer capital' of India. Health issues such as early aging, reproductive abnormality and behavioural disorders, premature greying of hairs have been reported in this region. (Halder 2007; Mittal *et al.*, 2014).

The purpose of this study was to see the amount of heavy metals in cancer patients in the faridkot area of Malwa region of Punjab. Although hair was considered as unreliable source for detection of heavy metals but it was demonstrated that concentration of metals in hair shows the concentration as that of bone. In liver, heart and brain tissues, lesser concentrations than hair were found.

Materials and methods

Study Area

The study area is from the rural area of Faridkot District located in South West of Punjab, India. Geographically the area lies in 30° 40' N latitude and 74° 75' E longitude with an area of 1458 square kilometres. Neighbouring districts include Ferozepur,

Muktsar, Bathinda and Moga. For livelihood people are dependent on agriculture. Water sources for irrigation are canal and tubewells.

Collection of Data

The cancer patients' data was collected from Guru Gobind Singh Medical College and Hospital, Faridkot. After this Information regarding type of cancer, food habits, etc was directly obtained from patients in person. A questionnaire bearing patient number should be prepared for the same.

Collection of Samples

Hair samples were collected with the help of stainless scissors. A tuft of hair can be cut directly from centre of head. From the scalp there are less chances of contamination. The collected samples must be transferred immediately to zip lock polybag. The zip lock polybag and the questionnaire should have same number. It should be advised to candidates that no hair colour/bleach is applied to hair before one week.

Pre-Digestion

External impurities such as sweat and dust present in hair may interrupt the analytical results. To remove the external impurities the hair samples must be washed, dried and weighted properly before digestion.

Digestion

Digestion of hair samples was done in closed vessel microwave digestion system (Milestone Ethos Easy) using HCl: HNO_3 in the ration of 1:3. Ultrapure nitric acid was used for digesting the sample.

Fig. 1. Map of India showing Faridkot region of Punjab.

Elemental Analysis

The elemental analysis (for Al, B, Ba, Cd, Cr, Cu, Fe, Mg, Mn, Pb, Sr, Ni and Zn) in hair samples is done with the help of ICPMS. PCA was applied to see the correlation between the metal for a combined effect on the body.

Results and discussion

Statistical analysis

Multivariate analysis has been performed to understand the geochemical characteristics and chemical interrelations. The Kaiser Meyer Olkin test for measuring sample adequacy was 0.624 which was higher than the threshold value 0.5, signifies the reliability and suitability of data for PCA. The significance value of Bartlett's test of sphericity was zero which must be lesser than 0.05.

Four principal components were extracted in the hair samples. In PC 1, high correlation between Barium, Copper, Magnesium and strontium was seen. It has shown a variance of 22.2% and significant correlation (r = 0.649 to r = 0.909). Barium is not considered as carcinogenic due to ingestion, but barium chromate is considered as carcinogenic. In a study conducted by Blaurock Busch *et al.* It was reported that double concentration of barium was found in breast cancer patients to that of healthy persons. Barium is used in many industries (Dallas *et al.*, 2001). In Hair dyes, organic dye cleaning solvents and petroleum fuels are few in which barium is used. In sulphate form it is used in pesticides and fertilizers. Exposure to Barium may occur from drinking water and air during manufacturing operations. Gastrointestinal disturbances and muscle weakness may be caused due to short exposure of barium as drinking water. Long time exposure may result in kidney damage. Just like strontium it belongs to alkaline earth metal. A high level of copper is may be due to industrial and farming exposure. High levels of strontium are found in the hair samples of cancer patients. As source of Sr is rocks, soil, dust and coal, High Sr level is reported in water and soil. In Ca deficiency, Sr may have negative effects on bone metabolism. The drinking water level for strontium is 4mg/L as per EPA recommendation.

Table 1. Total Variance Explained (Extraction Method: Principal Component Analysis).

Component	Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative%
1	2.886	22.201	22.201
2	2.608	20.059	42.260
3	2.107	16.210	58.470
4	1.885	14.502	72.972

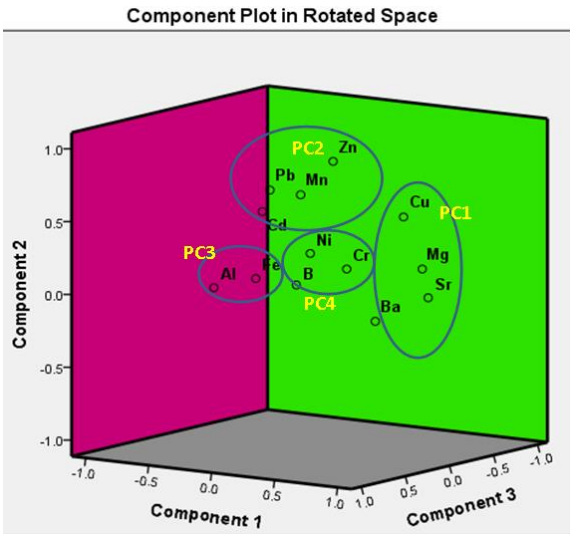

Fig. 2. Component Plot in Rotated Space.

The PC2 accounted for 20.05% of the variance, with high loadings of Cd, Mn, Pb and Zn. A significant correlation ranges from r = 0.535 to r = 0.882 have been seen. The strong correlation among the metals is may be due to the common source. Cadmium is used in various industries, ranges from production of alloys, pigments and batteries. Skin absorption is rare in case of cadmium but exposure from smoking, metal industries and from eating contaminated food may take place. Trace amounts of cadmium is found in leafy vegetables, potatoes and grains (Tchounwou *et al.*, 2012). Acute absorption of cadmium may result in nausea, vomiting, abdominal pain and loss of consciousness (Baselt *et al.*, 1995). Chronic exposure of cadmium is carcinogenic (Waalkes *et al.*, 1995; Waalkes *et al.*, 1992). High manganese content in soils is reported due to excess use of fertilizers. As to tackle the problem of Manganese deficiency in plants, it is added in fertilizers which further results in high Mn content in soil. (Schulte *et al*, 2004). These high Mn content further leaches down to groundwater. A high Mn level of groundwater is directly related to increase number of colon cancer and lung cancer

death rates (Spangler *et al.*, 2010). Lead is present in environment in small amounts. High concentration of lead is in environment due to manmade activities like mining, industrial & domestic applications. Lead is used in paints and ceramic products (Centers for Disease Control, Atlanta; 1991). Deteriorating lead paint on interior surfaces contribute to elevated level of lead in blood of children (Lanphear *et al.*, 1998). The nervous system is one of the main targets of lead poisoning. Early symptoms include headache, loss of memory, poor attention and irritability. Through drinking water, the highest percentage is absorbed by kidney followed by liver and soft tissues like heart and brain (Flora *et al.*, 2006). High zinc and cadmium is reported in soil due to addition of fertilizers for increasing the fertility of soil.

The PC3 accounted for 16.21% of the variance with a significant correlation (r = 0.878 to r = 0.919) between Aluminium and Iron. In previous studies high permissible limits of aluminium and iron in water have been reported. Apart from fertilizers, steel industry waste is may be a reason for high iron and aluminium content in the region. It is a result of dumping waste without treatment.

The PC4 accounted for 14.5% of the variance with a significant correlation ranges from r = 0.528 to r = 0.813. B, Cr and Ni are highly correlated in PC4. Waste of chemical and metallurgical industries, is directly linked with the high chromium in environment. As chromium is used as anticorrosive agent in cooking systems and boilers, it may be a reason of high chromium in human body. Chromium is released in air from various industrial applications. (ATSDR, US; Toxicological Profile for chromium) Environmental and occupational exposure to chromium may result in renal damage, allergy and asthma and in extreme conditions it may result in cancer of respiratory tract. (WHO: Chromium). Ingestion of Chromium (VI) may results in cardiovascular, gastrointestinal and neurological effects. Nickel is found in batteries, magnets, coins and plumbing fixtures. As this is an essential element for plants, it is found in fertilizers also. Excess use of fertilizers results in contamination of soil by nickel.

Conclusion

High usage of heavy metals play important role in causing dreaded diseases, out of which cancer is one. Man-made activities, which may include industrial and agricultural activities is one main reason of heavy metal pollution. For different metals there may be one or more common sources. Some metals may lie in close proximity. Out of industrial activities, release of waste in water or on land results in causing water and soil pollution. The deposition of excess heavy metals in water and soil causes extreme health disorders in people surrounding the area. Presence of high concentration of heavy metals in Faridkot region is a reason of high cancer rate in that area.

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Declaration of Interest

The article is approved by all authors and no conflict of Interest is there.

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