Biomonitoring of 33 Trace Elements in Blood Samples from Inhabitants of Southern India by ICP-MS

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Abstract: Trace elemental levels have a profound effect on human health. However, only limited information is available on biomonitoring of trace elements in India. Due to rapid industrial development and urbanization, pollution levels have increased and therefore there is a need to monitor trace elemental concentrations in human beings. In this work 33 trace elements (Li, Be, Rb, Sr, Cs, Ba, V, Mn, Co, Ni, Cu, Zn, Se, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Yb, Lu, Th, Cd, Tl, Pb, U, As and Ag) in 98 human blood samples, collected from occupationally non-exposed volunteers, living in Greater Visakhapatnam in southern part of India, were monitored. The samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS). The samples for analysis were prepared by treating with supra pure nitric acid and digesting in a microwave digester. The method of validation is described for all 33 elements and results about internal and external quality assurance are discussed. The information about the sample donors was collected by questionnaire-based interviews. Statistical data has been presented (mean values, geometric mean values, ranges and selected percentiles) for all elemental concentrations in blood.

Keywords: Biomonitoring, Whole blood, Trace elements and ICP-MS

1. Introduction

Trace elements play an important role in biological processes and have profound effect on human health. Deficiency of essential trace elements or accumulation of potentially toxic elements in the human body causes various diseases (Fraga 2005, Heitland and Koster 2006; and Ivanenko et al. 2013). But very little information is available on the concentration of the ranges of the trace elements, which can vary with age, sex, habits, geographical conditions, pollution levels and various other factors (Alessandro 2005). Elements like Pb, Cd, Se, Zn, Hg and few others are measured frequently and reference values are well established, but elements like Be, Li, V, Rb, Sr, Co etc., along with others have to be studied and there is a need to establish the reference levels. Several studies on trace elements in India such as Fe, Co, Ni, Zn and Se were carried out by different researchers (Sreelatha et al. 2004; Savita et al. 2011) in serum samples. Fourteen minor and trace elements (Na, K, P, Fe, Br, Co, Cr, Cs, Hg, Rb, Sb, Se, Sr and Zn) have been determined in pre and postoperative blood samples of breast cancer affected subjects (Vivek and Garg 1998). But none of them studied the other trace elements such as Li, Rb, Ce, V, etc.

Biomonitoring of trace elements in human body fluids is used as a diagnostic and toxicological indicator in human beings. According to Vivek Singh and Garg (1998), blood is the most reproducibly accessible body fluid analyzed for monitoring the trace elemental status in humans. However few of the studies have been carried out using blood as a medium. Rodushkin et al. (1999) have determined 50 elements in blood samples of 31 human subjects from Sweden. White and Sabbioni (1998) have determined seven elements in blood of unexposed British subjects. Minoia et al. (1998) have determined 35 elements in blood of the Italian population. Trace element reference values for blood were presented by Cornelis et al. (1994) for the Belgian population, by Poulsen et al. (1994) for the Danish population, by Kucevra et al. (1995) for the Czech and Slovak population and by Wilhelm et al. (2004) for the German population, by Ivanenko et al. (2013) for the Russian population, by Xiaobing Liu et al. (2014) for the Chinese population. The above studies concluded that reference values of the trace elements differ among different population groups.

According to Alessandro et al. the concentration ranges of the trace elements, can vary with age, sex, habits, geographical conditions, pollution levels and various other factors (Alessandro 2005). The main aim of the study is to assess the trace element levels in inhabitants of Greater Visakhapatnam area. The present study area has been surrounded by many industries such as steel plant, casting industries, thermal power plant and many others, further Visakhapatnam is a coastal city and the inhabitants consume lot of sea food. Advancement in the multielemental analysis instrumentisation like ICPMS and ICP-OES it is possible to monitor trace elements simultaneously. Introduction of the reaction/ collision gas cell technique removes polyatomic interferences more efficiently, and determination of trace elements simultaneously with good precision and accuracy in whole blood samples becomes possible. In the present study 33 trace elements in 98 whole blood samples collected from different subjects living in Visakhapatnam city in Southern India were monitored. The samples were digested using microwave accelerare reaction system. The digested blood samples were analysed using ICPMS with collision/reaction gas cell and the complete validation of the method along with internal and external quality assurance has been described. Statistical data about 33 trace elements are provided, which will be useful for the researchers working in the fields of clinical and toxicological studies.
2. Procedure for Paper Submission

2.1 Review Stage

Materials and methods

Instrumentation

The blood samples were analyzed with an Agilent 7700s Inductively Coupled Plasma Mass Spectrometer with a 27.12MHz solid state generator. The instrument has an octapole - based collision / reaction cell with helium as collision and hydrogen as reaction cell gases. A flow of 4.4 mL min⁻¹ helium (Indian gases, India) with a purity of 99.999% (V/V) was introduced into the cell. 9Be, 51V, 55Mn, 59Co, 60Ni, 63Cu, 66Zn, 75As, 82Se, 85Rb and 88Sr were analyzed in He gas mode and the remaining isotopes were analyzed in no gas mode. Here Pt sampler and skimmer cones were used with an orifice diameter of 1.0 and 0.7mm, respectively and the sample introduction system was equipped with PFA micro flow concentric nebulizer combined with a Scott double pass spray chamber. The spray chamber was Peltier cooled at 2°C to ensure temperature stability and to reduce the water vapor present in the nebulizer gas flow. The ICP torch consists of an injector tube with a large inner diameter of 2.5mm to reduce the risk of particle deposition or clogging. The instrument was optimized to get highest signal to background ratio with a solution containing 7Li, 59Co, 89Y and 205Tl along with the ratios of 140Ce16O+/140Ce+ 1.5% and 140Ce2+/140Ce+<5% for a solution with 1µg L⁻¹. ICPMS instrument operating conditions were as follows RF power 1500w, outer gas flow 15 L min⁻¹, carrier gas flow 0.75 L min⁻¹, nebulizer gas flow 1.0 L min⁻¹. Nebulizer pump 0.1 rps, integration time 0.1 sec.

Sample collection and sample preparation:

Blood samples of 98 occupationally non-exposed human subjects were collected. The subjects were living in and around greater Visakhapatnam, a coastal city in southern India. This area has a population density of 384 inhabitant’s km⁻² and is close to many industrial establishments such as steel plant, casting industries, fertilizer plant, thermal power plant, rare earth extraction industry, pharmaceutical and drugs, and food industry. All the volunteers gave their consent for the use of their blood samples for the present survey. Information on exposure conditions were collected by questionnaire-based interviews and the following data about age, gender, place of residence, occupation, smoking habits, and fish consumption prior to sample collection are available. The age group of the subjects ranges from 18 to 70 years. Sixty three subjects of this group are female and thirty five are male. Twenty subjects are smokers and 78 subjects are non-smokers. Nineteen subjects are alcohol consumers and eighty one subjects never consumed alcohol. Seventeen subjects have consumed seafood within 48 h prior to sample collection. The bar diagram depicted below in Fig. 1 represent the characteristics of the population under study.

A sample of 5mL blood was drawn with stainless steel needles into BD vacutainer tubes containing K3EDTA as the anti-coagulant. Samples were collected at Andhra university medical lab and Visakha diabetic center, Visakhapatnam. Samples were stored at -20°C and digested within 48 hours using microwave digester (CEM MARS). The Microwave digestion system was equipped with 100mL Teflon PFA vessels and a turntable. In each vessel about 0.5mL of blood sample was taken and 1mL of supra-pure nitric acid was added and then made up to 10mL with deionized water. Routinely, 12 vessels were subjected to the following digestion procedure. Samples were allowed to predigest for a minimum of 15 min and then the vessel is closed by using a Capping station (CEM Corp). The sample digestion process was done in the following steps - in the first step temperature was gradually increased from room temperature to 150°C with a ramp time of 20 min. The temperature was maintained for 5 min and then the vessels allowed to cool down to room temperature (Table 2). After cooling, the digested samples were diluted to a final volume of 25mL with deionized water and analyzed. For each set of 10 samples two blanks were prepared in the similar way (without samples) to check for possible contamination. The samples were stored in the refrigerator at 4°C until the analysis was carried out. During this study ultrapure water (18.2 mV) obtained from the water purification system (Millipore synergy) and suprapure nitric acid were used. Each sample was spiked with internal standard containing Bi, Ge, In, 6Li, Sc, Tb, Y (SPEX CertiPrep). All the plastic ware and pipette tips used for blood, acid, water, standards and internal standard solution were rinsed with dilute nitric acid and then deionized water in that sequence.

Standard solutions and control samples:

The multi-element standard solution containing Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Ho, La, Lu, K, Li, Mg, Mn, Na, Nd, Ni, Pb, Pr, Rb, Sc, Sm, Se, Sr, Tb, Th, Ti, Tm, U, V, Y, Yb and Zn each at a concentration of 100 µg L⁻¹ (SPEX CertiPrep) was used. 33 elements including Li, Be, Rb, Sr, Cs, Ba, V, Mn, Co, Ni, Cu, Zn, Se, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Yb, Lu, Th, Cd, Tl, Pb, U, As and Ag were detected. The standard curve was prepared with 0, 0.25, 0.5, 1, 5, 10, 25, 50, 100 µg L⁻¹ concentrations. Accuracy and validity of the method has been assessed with the human whole blood (Seronorm level 3) certified for the elements As, Cd, Co, Cu, Mn, Ni, Se, Ti, Pb and Zn. For the uncertified elements the method was validated with spiked pooled blood. Detailed submission guidelines can be found on the author resources Web pages. Author resource guidelines are specific to each journal, so please be sure to refer to the correct journal when seeking information. All authors are responsible for understanding these guidelines before submitting their manuscript. For further information on both submission guidelines, authors are strongly encouraged to refer to http://www.ijser.org.

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\[
\int_{0}^{\infty} \exp(-\lambda |z_i - z_j|) J_i(\lambda r) J_0(\lambda r) d\lambda.
\]

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Table 1: Units for Magnetic Properties

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Quantity</th>
<th>Conversion from Gaussian and CGS EMU to SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>magnetic flux</td>
<td>(1 \text{ Mx} = 10^4 \text{ Wb} = 10^6 \text{ Vs} )</td>
</tr>
<tr>
<td>(B)</td>
<td>magnetic flux density, magnetic induction</td>
<td>(1 \text{ G} = 10^4 \text{ T} = 10^{-8} \text{ Wb/m}^2 )</td>
</tr>
<tr>
<td>H</td>
<td>magnetic field strength</td>
<td>(1 \text{ Oe} = 10^{-4}(\text{A/m}) \times 10^{-1} \text{ T} )</td>
</tr>
<tr>
<td>m</td>
<td>magnetic moment</td>
<td>(1 \text{ erg/Cm} = 1 \text{ emu/cm}^2 = 10^{-7} \text{ J/T} )</td>
</tr>
<tr>
<td>M</td>
<td>magnetization</td>
<td>(1 \text{ erg/(G-cm)} = 1 \text{ emu/cm}^2 )</td>
</tr>
<tr>
<td>(4\pi M)</td>
<td>magnetization</td>
<td>(1 \text{ G} = 10^{-4}(\text{A/m}) \times 10^{-1} \text{ T} )</td>
</tr>
<tr>
<td>(\sigma)</td>
<td>specific magnetization</td>
<td>(1 \text{ erg(G-cm)} = 1 \text{ emu/g} = 1 \text{ A/m/kg} )</td>
</tr>
<tr>
<td>J</td>
<td>magnetic dipole moment</td>
<td>(1 \text{ erg(G-cm)} = 1 \text{ emu/cm}^2 )</td>
</tr>
<tr>
<td>(\mu)</td>
<td>magnetic polarization</td>
<td>(1 \text{ erg(G-cm)} = 1 \text{ emu/cm}^2 )</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>susceptibility</td>
<td>(1 = 4\pi )</td>
</tr>
<tr>
<td>(d)</td>
<td>mass susceptibility</td>
<td>(1 \text{ cm}^3/g = 4\pi \times 10^{-3} \text{ m}^3/\text{kg} )</td>
</tr>
<tr>
<td>(\mu)</td>
<td>permeability</td>
<td>(1 = 4\pi \times 10^{-7} \text{ H/m} )</td>
</tr>
<tr>
<td>(\mu_0)</td>
<td>relative permeability</td>
<td>(\mu = \mu_0 )</td>
</tr>
<tr>
<td>(W)</td>
<td>energy density</td>
<td>(1 \text{ erg/cm}^3 = 10^{-6} \text{ J/cm}^3 )</td>
</tr>
</tbody>
</table>

Statements that serve as captions for the entire table do not need footnote letters.

*Gaussian units are the same as cgs emu for magnetostatics;\nMx = maxwell, G = gauss, Oe = oersted; Wb = weber, V = volt, s = second, T = tesla, m = meter, A = ampere, J = joule, kg = kilogram, H = henry.

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Theorems and related structures, such as axioms corollaries, and lemmas, are formatted using a hanging indent paragraph. They begin with a title and are followed by the text, in italics.

Theorem 1. Theorems, corollaries, lemmas, and related structures follow this format. They do not need to be numbered, but are generally numbered sequentially.

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Proof. The same format should be used for structures such as remarks, examples, and solutions (though these would not have a Q.E.D. box at the end as a proof does).

7. End Sections

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9. Acknowledgment

The authors wish to thank A, B, C. This work was supported in part by a grant from XYZ.

References


**Author Profile**

Author and coauthors are pursuing Masters Degree Program in Electric Power Engineering in University, Country.