The Determination of Mercury and Selenium in Maternal and Neonatal Scalp Hair by Inductively Coupled Plasma-Mass Spectrometry

Ibrahim B.-A. Razagui and Stephen J. Haswell
1University of Hull Postgraduate Medical School, Academic Department of Obstetrics and Gynaecology, Hull Maternity Hospital, Hedon Road, Hull, HU9 5LX, United Kingdom and 2School of Chemistry, University of Hull, Cottingham Road, Hull, HU6 7RX, United Kingdom

Abstract

As part of a prospective study to evaluate maternal and neonatal scalp hair mineral profiles in normal and pathological pregnancy cases, the suitability of inductively coupled plasma-mass spectrometry (ICP-MS) was evaluated for the quantitation of mercury and selenium as part of a multi-element determination procedure. Treatment of hair samples included a closed-system microwave-assisted digestion in screw-capped PTFE vials of 10-mL capacity with concentrated nitric acid (2 mL) as the matrix solubilization medium. A digestion time of 10 min at 40% microwave power yielded limpid digestates, which, after appropriate dilution with deionized water to give a final acid concentration of 20% (v/v), were used for direct ICP-MS measurements within a rectilinear calibration range from 0 to 10 pg/L. Analytical recovery results for mercury ranged from 97 to 102%, whereas those for selenium ranged from 96 to 101%. Results from concurrent analyses of a human hair reference material showed a high degree of concordance with certified values. The results obtained suggest that the closed-system microwave-assisted digestion procedure described was effective in minimizing the risk of volatility-associated losses that can be encountered in the assays of both metals. The results also indicated that the ICP-MS system used in this study was suitable for including both metals as part of a multi-element quantitation procedure. Using this method, analytical results were obtained for mercury and selenium levels from 100 pairs of maternal and neonatal scalp hair samples collected at the postnatal ward of Hull Maternity Hospital, Hull, United Kingdom.

Introduction

The toxicity of mercury to humans and animals is well-recognized, and the adverse health effects associated with exposure to the elemental and various compound forms of this metal have been the subject of various publications (1-3). Biochemical features associated with exposure to mercury include altered catalytic efficiency and specificity of enzyme systems and permeability of membranes involved in nutrient transport (4).

Exposure to mercury during pregnancy has raised particular concerns regarding feto-toxicity (5,6) because the metal in its elemental and organic forms readily crosses the placental barrier (7).

Apart from a diet based on fish and other seafood, inadvertent exposure to mercury during pregnancy may result from mercury-based dental restorations (8).

The uptake of this form of mercury by the systemic circulation via the alveolar space is quite rapid and has been observed to cross the placental barrier preferentially to other forms of the metal (7).

Furthermore, pregnancy has been associated with decreased maternal mercury burden, which suggests the possibility of increased maternal mercury elimination via the placenta and uptake by the fetus (7).

In the United Kingdom, dental treatment is available gratis to expectant mothers, and pregnant women of low socioeconomic status are likely to take advantage of this service. Thus, this population group is probably at a higher risk of exposure to elemental mercury vapor released during insertion of or replacement of mercury-based dental amalgam or both (9-11).

This risk can be compounded by the fact that the use of protective rubber dams to reduce exposure to mercury vapor during dental amalgam replacement is a discretionary rather than mandatory dental practice in the United Kingdom.

The potential adverse effects of chronic low-level exposure to elemental mercury vapor emanating from dental amalgam restorations on fetal and postnatal development remain a subject of active research.

In contrast to mercury, selenium is an essential nutrient, and its most recognized role is that of a component of the enzyme glutathione peroxidase, which forms part of the organism's antioxidant defense mechanisms against free radical damage (12,13).

It has been suggested that selenium may impart some protective effect against mercury toxicity (14). This suggestion appears to be based on the strong affinity that both metals have for the cysteinyl and histidyl side chains of amino acid residues for which they compete for binding (15). These amino acids are common components of globular proteins, which
are involved in a range of important biological functions, including those affecting the immune and endocrine systems and nutrient transport. However, there appear to be no systematic studies evaluating mercury burden in relation to selenium status in humans.

In view of the important implications of both elements to human health, establishing reliable biochemical indices for assessing their systemic status continues to be a domain of active research.

Scalp hair has long been recognized as a valuable biopsy material for assessing mercury burden (16,17). In recent years, its use has been extended to the assessment of chronic selenium status following reported evidence of a close relationship between hair and whole blood selenium concentrations (18).

Chronic selenium status in humans has been observed to vary geographically according to the metal’s concentrations in the soil and, hence, the food supply (19,20). Thus, assessing selenium status on the basis of dietary surveys would be of limited value, particularly in industrialized countries where the food supply is often of multiple geographical origins and the diet is heterogeneous in composition.

The basis for considering hair as a substrate for assessing chronic selenium status is similar to that for mercury. Both elements readily bind to the sulfhydryl groups of thioamino acids, which are abundant components of the hair matrix proteins. Their levels in scalp hair can therefore serve as a useful adjunct to other biochemical indices by providing a presumptive indication of their systemic levels as affected by a variety of factors including environmental exposure and diet.

Neonatal scalp hair would be a particularly valuable substrate as the developing fetus would have been exclusively exposed to the intrauterine environment. Mineral levels in neonatal scalp hair may, therefore, provide a unique insight into fetal systemic mineral status as affected only by the maternal circulation. The determination of mercury in biological materials including hair has largely been based on cold vapor atomic absorption spectrophotometry (CVAAS) (21).

The procedure is based on treating the sample with stannous chloride to effect the release of elemental mercury vapor which is then measured optically. However, this procedure can result in analytical bias emanating from volatility-associated losses, adventitious contamination during preparation, and matrix-effect interference.

Spectrofluorometry has been widely used for the determination of selenium in hair and other biological substrates (22,23). Quantitation is based on reacting selenium with an organic ligand such as 3,3'-diaminobenzidine (DAB), or 2,3-diaminonaphthalene (DAN) to form a stable fluorescing complex, the fluorescence intensity of which is proportionally related to the metal's concentration. However, interference from coextractants can often cause reduced sensitivity. Although CVAAS and spectrofluorometry have gained wide recognition for their respective applications to the determination of mercury and selenium, there remains a need for more sensitive and rapid and less laborious methods for their assays.

In recent years, inductively coupled plasma-mass spectrometry (ICP-MS) has generated much interest as a more viable instrumental method for the determination of a range of elements (24). Apart from its ability to provide detection limits at trace levels, it offers the considerable advantage of a one-time simultaneous and rapid multi-element determination procedure.

However, there appears to be a paucity of analytical data on mercury and selenium scalp hair levels obtained from the use of ICP-MS. Thus, it was felt appropriate to examine whether this advantage can be extended to include metals that are commonly assayed by separate and different methods.

As part of a prospective study to evaluate the concentrations of a range of nutritionally and toxicologically significant elements in maternal and neonatal scalp hair of normal and pathological pregnancy cases, this paper describes an analytical procedure using ICP-MS for the quantitation of mercury and selenium in term pregnancy mothers and their neonates.

The method is based on the solubilization of the sample matrix in a closed-system microwave acid digestion to minimize risk of volatility-associated losses followed by direct measurements using ICP-MS with one set of instrumental parameters, which allows the simultaneous quantitation of several elements including mercury and selenium.

Materials and Methods

All glassware used was soaked in nitric acid and rinsed with deionized water.

PTFE digestion vessels were soaked overnight in a composite solution of nonionic detergent, EDTA, and citric acid (1%, w/v) and rinsed with a liberal amount of deionized water.

Reagents

All reagents used for sample digestion and the preparation of standard elemental solutions were Aristar grade (BDH, Poole, Dorset, U.K.).

Deionized water was of high purity (18 MΩ cm resistivity; Elgastat UHQ PS, Elga, High Wycombe, Bucks, U.K.).

Multi-elemental standard solutions of a concentration range between 0 and 10 μg/L in 20% (v/v) nitric acid were prepared from a series of dilutions of individual stock solutions (1000 μg/mL) for mercury and selenium. Other elements included magnesium, chromium, iron, zinc, copper, cadmium, and lead.

Equipment

The ICP-MS instrument used was a Fisons Instruments (Winsford, Cheshire, U.K.) PlasmaQuad 2 Plus.

Optimization of the instrument’s operating parameters was effected by means of a tuning solution containing the elements beryllium, yttrium, indium, lanthanum, bismuth, and uranium at 10 μg/L in a matrix of 20% nitric acid (v/v).

The transient analyte peaks were monitored using the time-resolved analysis mode. The instrument’s operating parameters were as follows: forward power, 1350W; reflected power, 0W; aerosol gas flow rate, 0.94 L/min; intermediate gas flow rate, 1.1 L/min; outer gas flow rate, 13.0 L/min; nebulizer (De Galan type), 2.0 × 10⁻⁶ mbar; spray chamber, glass, water-cooled,
10°C; sample flow rate, 1.0 mL/min (peristaltic pump). Time-
resolved analysis peak-jumping parameters were as follows:
time per slice, 1.00 s; points per peak, 3; detector mode, pulse
counting; selected isotopes, $^{200}\text{Hg}$, $^{202}\text{Hg}$, $^{78}\text{Se}$, $^{80}\text{Se}$, and $^{82}\text{Se}$. Data processing and acquisition were accomplished by
means of a computer program.

The microwave oven used for sample digestion was a CEM
(Matthews, NC) MDS-81D, which had the following features: a
maximum available power of 650 W in 1% increments, pro-
grammable separately timed stages, eight-position carousel, a
capping station, a cooling tank, and a pressure control monitor.

Sample collection

Maternal and neonatal samples were collected 24–48 h post-
partum from the suboccipital region of the scalp using sur-
gical-grade scissors. Each sample collected was stored in an
autosealable polythene bag. Distal ends of maternal sample
were discarded to leave a length of approximately 5 cm of the
proximal ends. Neonatal samples, because of their limited
length and quantities, were left uncut.

Washing procedure

As a preparatory step to maximize surface area contact for the
washing and digestion procedures, each sample was minced
with a stainless steel surgical scalpel until individual hair seg-
ments were approximately 1 cm in length. This was followed by
mixing each cut sample to make it homogeneous.

Each hair sample was washed sequentially with acetone,
deonized water, and acetone, using three successive portions.
The samples were then dried in an oven at 105°C.

Accurate weights of each sample prepared were obtained
using a Mettler Toledo (Beaumont Leys, Leicester, U.K.) MT5
microbalance of 5.1-g capacity and 0.001-mg graduation.

Digestion procedure

Each hair sample weighing approximately 25–30 mg was
transferred into a PTFE digestion vial of 10-mL capacity, and
concentrated nitric acid (2 mL) was pipetted into the vial. The
vial was loosely capped and the sample left to digest at room
temperature for about 20 min in order to reduce any foaming
that might ensue.

Each vial was then tightly screw-capped and placed inside a
microwave PTFE digestion tube of 120-mL capacity containing
10 mL of deionized water as the conductive heat medium.

PTFE digestion tubes, each containing two PTFE sample
vials, were tightly closed and placed in the rotating carousel.

Two sample blanks were treated in a similar manner with
each run. The system was then connected to a pressure mon-
itor, and digestion was set for 10 min at 40% power.

After the vials were allowed to cool to room temperature,
each digestate was transferred into a volumetric flask and
dilution made with deionized water to give a final acid concen-
tration of 20% (v/v).

Dilution factors of 5 and 12.5 were found appropriate for
neonatal and maternal samples, respectively, to bring sample
mineral concentrations within the instrument’s working cali-
bration range.

Results and Discussion

Sample washing

Although neonatal hair samples are unlikely to be associated
with the same risk of exogenous metal contamination as adult
samples, they were subjected to the same washing procedure as
maternal samples. This was a precautionary measure to remove
any residual contamination that may have emanated from con-
tact with the amniotic fluid.

Furthermore, hair samples obtained from neonates delivered
by caesarian sections are likely to have come into contact with
maternal blood, which could have caused exogenous contam-
nination. However, whereas perceptibly higher levels of zinc
and iron were observed in unwashed hair samples obtained
from neonates delivered by caesarian section (unpublished
data), no evidence of such effect was observed for mercury or
selenium levels.

Digestion conditions

The use of a closed system microwave-assisted digestion
provided a number of advantages including affecting sample

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<th>Table I. Concentration of Mercury and Selenium Obtained from the Analysis of a Hair Reference Material (NIEH–CRM5)*</th>
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<td><strong>Element</strong></td>
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</tr>
<tr>
<td>Mercury</td>
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<td>Selenium</td>
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* Results are the mean of triplicate analyses plus or minus one standard deviation.
† Range of values.

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<th>Table II. Analytical Recovery Values Obtained for Mercury and Selenium using the Described Procedure*</th>
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<tr>
<td><strong>Element</strong></td>
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* Results are the mean of triplicate analyses.
matrix solubilization in a short time with a minimal risk of volatility-associated loss of analytes.

The risk of adventitious contamination, which can occur in open-vessel digestion procedures, is also minimized, and the containment of acid digestion fumes within a closed vessel reduces the risk of corrosive damage to the oven and fume hood.

The relatively low microwave power selected (40%) for a digestion time of 10 min was found to be sufficient to yield digestates that were invariably limpid and free from particulate matter. Initial trials with lower microwave power (20-35%) and shorter digestion times (5-8 min) resulted in incomplete solubilization and opacity of the digestate. Conversely, longer digestion times (12-20 min) at 40% power yielded no perceptible differences in the quality of the digestate but occasionally resulted in excessive buildup of pressure.

The inclusion of codigestants such as hydrogen peroxide or perchloric acid was not considered necessary or appropriate. Both enhance digestion by helping maintain solubilization and preventing formation of tarry residues. However, apart from being potentially explosive, increased effervescence by hydrogen peroxide can increase the risk of volatility-associated losses. Moreover, perchloric acid may not always be suitable for ICP-MS instruments equipped with Ryton nebulizers, which can be susceptible to perchloric acid attack, leading to reduced nebulizer performance (25).

Nitric acid provided an effective sample solubilization medium without resorting to a multi-acid digestion. This is probably due to the relative simplicity of the sample matrix, which, compared with other biological materials, has a lower content of lipids, complex carbohydrates, and alcohols. During preliminary trials with adult and neonatal samples, it was observed that upon addition of nitric acid, excessive effervescence gradually ensued from adult samples. No effervescence was observed with neonatal samples. Excessive effervescence may be attributed to the relatively more complex chemical structure of adult hair. In addition to higher concentrations of matrix proteins and some minerals, sebacious secretion products are likely to be more prevalent in adult hair, thus increasing the content of lipids and other organic constituents.

As a precautionary measure to reduce the risk of uncontrollable oxidation, maternal samples were allowed to digest overnight at room temperature in loosely capped digestion vessels before microwave-assisted digestion.

Calibration and precision

Measurements from a series of standard elemental solutions gave an optimum instrument calibration range for concentrations between 0 and 10 µg/L.

The detection limits for both metals (three times the standard deviation of the mean of the blank) were based on the measurements of six blanks. Detection limits were 0.39 µg/L for mercury and 0.25 µg/L for selenium.

Reproducibility and precision of the procedure were evaluated by running concurrent analyses of triplicate samples of a human hair reference material (NIEH-CRM 5).

The results showed a high degree of concordance with certified values (Table I).

Analytical recovery was evaluated by the addition of four measured quantities of mercury and selenium to a composite sample made from comparable proportions of three maternal subsamples. The levels of recovery obtained ranged from 97 to 102% for mercury and from 96 to 101% for selenium (Table II).

The within-sample variation coefficient for the analyses of maternal samples was less than 3%.

The method would appear to be appropriate for hair analysis, including that of neonatal hair, which is often obtainable in limited quantities compared with adult hair. However, the relatively high concentrations of a wide range of elements in hair and the low detection range afforded by ICP-MS reduce the need for laborious and time-consuming preconcentration.
treatments and offer the possibility of subsampling. Furthermore, the ease of sample introduction by nebulization and the high degree of ionization provided by a stable ionization source such as plasma obviates the need for protracted digestion and sample volume reduction often associated with other analytical methods.

The instrumental method also offers the potential advantage of differential quantitation of stable isotopes. This can be a useful means of helping the identification of sources of metal exposure and uptake by relating the relative concentrations of stable isotopes in the diet or environment to their relative distributions in biological tissues including scalp hair. This also offers the possibility of investigating the biokinetics of elements in humans without resorting to radiotracer methods, which may be unavailable and ethically objectionable.

Using the described procedure, a total of 100 pairs of samples from term pregnancy mothers and their neonates from the Hull area were analyzed for a range of nutritionally and toxicologically significant elements, including mercury and selenium. Table III gives a summary of the results for mercury and selenium levels, which are also illustrated in Figure 1 and show their respective distributions in the maternal and neonatal samples analyzed.

A detailed profiling of the results and assessment of their possible significance in relation to a range of clinical parameters will be included in a separate publication that is currently in preparation.

Acknowledgment

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References


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