Assessment of selenium and mercury in biological samples of normal and night blindness children of age groups (3–7) and (8–12) years

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Abstract The causes of night blindness in children are multifactorial and particular consideration has been given to childhood nutritional deficiency, which is the most common problem found in underdeveloped countries. Such deficiency can result in physiological and pathological processes that in turn influence biological sample composition. This study was designed to compare the levels of selenium (Se) and mercury (Hg) in scalp hair, blood, and urine of night blindness children age ranged (3–7) and (8–12) years of both genders, comparing them to sex- and age-matched controls. A microwave-assisted wet acid digestion procedure was developed as a sample pretreatment for the determination of Se and Hg in biological samples of night blindness children. The proposed method was validated by using conventional wet digestion and certified reference samples of hair, blood, and urine. The Se and Hg in biological samples were measured by electrothermal atomic absorption spectrometry and cold vapor atomic absorption spectrometry, prior to microwave acid digestion, respectively. The concentration of Se was decreased in scalp hair and blood samples of male and female night blindness children while Hg was higher in all biological samples as compared to referent subjects. The Se concentration was inversely associated with the risk of night blindness in both genders. These results add to an increasing body of evidence that Se is a protecting element for night blindness. These data present guidance to clinicians and other professional investigating deficiency of essential micronutrients in biological samples (scalp hair and blood) of night blindness children.

Keywords Night blindness · Selenium · Mercury · Biological samples · Wet acid digestion methods · Children · Age groups (3–12) · Atomic absorption spectrophotometer

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Introduction

Night blindness is considered as an early sign of vitamin A deficiency. Low intake of fruit and vegetables containing beta carotene, which the body converts into vitamin A, may also contribute to a vitamin A deficiency (Christian et al. 2001). The relationship of micronutrient deficiency with the risk of developing certain major visual disorders and the therapeutic role of these micronutrients are now being recognized

(VandenLangenberg, 1998). Childhood nutritional deficiency is the most common problem found in underdeveloped countries. Such deficiency can result in physiological and pathological processes that, in turn, affect normal physiology of human body (Weatherall and Clegg 2001). But it is important to recall that night blindness is a concomitant of a wide range of retinal diseases, many of which are hereditary in nature.

Minerals may function as regulators of antioxidant enzymes; thus, their deficiency may negatively affect the cellular antioxidant defense system (Kathleen 1999). Epidemiological evidence points to the potential of antioxidant vitamins E and C, carotenoids, zinc, and selenium in the prevention and possible treatment of macular degeneration and night blindness (Kathleen 1999). Selenium (Se) is an activator of glutathione peroxidase (Gpx) (Singh et al. 1984). Currently undergoing clinical trial SELECT examining the protective effect of Se in macular degeneration in men should clarify whether this element plays a role in the pathogenesis of macular degeneration. Regardless of this trial, Se inhibited vascular endothelial growth factor production in the epithelial cancer cells in vitro (Jiang et al. 2000). Thus, it is possible that Se could also participate in the regulation of angiogenesis in the eye impeding the development of macular degeneration and night blindness.

Mercury (Hg) has no positive role in the human body (Bergan and Rodhe 2001); in fact, a safe level of Hg exposure is very difficult to determine. The Hg and its compounds affect the central nervous system, kidneys, and liver and can disturb immune processes and cause tremors, impaired vision and hearing, paralysis, insomnia, and emotional instability (Cernichiari et al. 1995; Adimado and Baah 2002). During pregnancy, Hg compounds cross the placental barrier and can interfere with the development of the fetus, and cause attention deficit and developmental delays during childhood (Batista et al. 1996). The Hg increases free-radical production (Clarkson 1997); inactivates antioxidant defenses (Insug et al. 1997); binds to thiol-containing molecules (Wiggers et al. 2008); binds to Se forming selenomercury complexes, reducing Se availability for glutathione peroxidase (GPx) activity (Kobal et al. 2004); inactivates glutathione, catalase, and superoxide dismutase (Park et al. 1996); increases lipid peroxidation (Miller et al. 1991); and increases oxidation of low density lipoprotein (LDL) (Miller et al. 1991).

The Se antagonizes some of the adverse effects of Hg by forming a seleno-mercury complex in tissue that is less toxic (Ganther et al. 1972; Sumino et al. 1977). The Se is the selenocysteine part of the enzyme glutathione peroxidase, which detoxifies hydrogen peroxide and lipoperoxides (Dlouha et al. 2008; Patrick 2004). The relationship between Hg and Se has been subject to numerous studies (Lourdes et al. 1991; Parkman and Hultberg 2002; Chen et al. 2001; Jin et al. 2006; Yang et al. 2008). The majority of the papers point to a negative relationship between the two elements (Chen et al. 2001; Jin et al. 2006), showing mitigating effects of Se on Hg toxicity. Studies have shown that intake of Se can repress the toxic effects of high Hg levels (Paulsson and Lundbergh 1991; Chen et al. 2001).

In view of the above facts, it is important to determine the essential and toxic trace elemental concentrations in biological samples of humans having physiological disorders, such as myocardial infarction, hypertension, diabetic mellitus, etc. Various biopsy materials such as serum, scalp hair, urine, and other body fluids may be used as bio-indicators for these purposes (Afridi et al. 2006). Blood elemental analysis provides information about what the body has recently (hours to days and in some cases weeks) absorbed. Blood levels are largely independent of tissue deposition (Polkowska et al. 2004).

Atomic absorption spectrometric methods are frequently used for the specific determination of very low elemental concentrations in environmental (Tuzen and Soylak 2005) and biological samples (Kazi et al. 2009; Tuzen & Soylak, 1995; Soylak et al. 1995, 1998). At present, the mineralization methods frequently employed for the analysis of biological samples are wet digestion with concentrated acids using either convective systems or microwave ovens (Zhu et al. 1992). The main advantage of microwave-assisted pretreatment of samples is the small amount of mineral acids required and reduced production of nitrous vapors.

The aim and objective of our present study was to assess the concentrations of Se and Hg in the biological samples (scalp hair, blood, and urine) of night blindness and normal children of both genders and correlated with control subjects of age matched groups (3–7) and (8–12) years. The variation in levels of these trace elements in night blindness patients as compared to referent subjects was evaluated. The biological samples were prepared by microwave-assisted acid digestion method, and the validity of analytical procedure was checked by corresponding conventional wet acid digestion of certified reference materials.

Material and methods

Apparatus

The analysis of Se was carried out by means of a double beam PerkinElmer atomic absorption spectrometer model A. Analyst 700 (Norwalk, CT, USA) equipped with a graphite furnace HGA-400 (PerkinElmer), a pyrocoated graphite tube with an integrated platform and an autosampler AS-800 (Perkin Elmer). The Hg in samples and standard solutions was determined by atomic absorption spectrometer, A. Analyst 700 (Perkin-Elmer) using MHS-15 chemical vapor generation system (PerkinElmer). The instrumental parameters are shown in Table 1. A Pel (PMO23, Osaka, Japan) domestic microwave oven (maximum heating power of 900 W) was used for digestion of the biological samples. Acid washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

Reagents and glass wares

Ultrapure water obtained form ELGA labwater system (ELGA, Bucks, UK) was used throughout the work. All reagents were of analytical grade obtained from Merck (Darmstadt, Germany) and checked for possible trace metal contamination. Working standard solutions of Hg and Se were prepared immediately prior to their use, by stepwise dilution of certified standard solutions

Table 1 Measurement conditions for electrothermal and cold vapor AAS 700

Parameters	Se	Hg
Lamp current (mA)	12.5	6.0
Wave length (nm)	196.0	253.7
Slit width (nm)	0.2	0.2
Drying temp °C/ramp/hold(s)	140/15/5	140/15/15
Ashing temp °C/ramp/hold(s)	1100/10/20	110/10/20
Atomization temp (°C)/ramp/hold) (s)	2100/0/5.0	2200/0/5.0
Cleaning temp (°C)/ramp/hold) (s)	2600/1/3	2600/1/3
Chemical modifier	$Mg(NO_3)_2$	$Mg(NO_3)_2$
Sample volume (10 μl), cuvette=cup, carrier gas=(200 ml/min)		

Background correlation (D_2 lamp) used for all elements

D_2 deuterium lamp, *mA* milli ampere, *nm* nanometer, *°C* degrees centigrade, *s* second, *mm* millimeter, *L/min* liter/minute, *ml/min* milliliter/minute, *μl* microlitre

(1000 ppm) Fluka Kamica (Buchs, Switzerland), with 0.2 M HNO_3. The stock standard solution of modifiers, $Mg(NO_3)_2$ (5.00 g/L) were prepared from $Mg(NO_3)_2$ (Merck). All solutions were stored in polyethylene bottles at 4 °C. For the accuracy of methodology, the Clincheck control-lyophilized human urine, human whole blood (Recipe, Munich, Germany), and human hair (BCR 397) were used. Solution of sodium tetrahydroborate was prepared by dissolving $NaBH_4$ powder (Acros Organics New Jersey, USA) in 0.05 M KOH. All glassware and plastic materials used were previously soaked for 24 hours in 5 M nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

Sample collection and pretreatment

The study was conducted on 150 children aged ranged between 3 and 12 years of both genders, registered in the hospital as patients with ocular problems. A total of 82 children reported poor vision in the day and in the night, and 68 children have good visions in the day but poor vision in dim light or in the night. The healthy children of same city and residential area of Hyderabad City were not registered in hospital but checked by the ophthalmologist for eyesight. A control group of 210 healthy children with the same age and with normal eyesight was chosen; detail was reported in Table 2. The samples from both groups were collected for three times, in a year, to evaluate any variation in the concentration of trace metals in patients and normal subjects during 1 year. The parents of night blindness and normal subjects were interviewed and asked to complete a questionnaire in order to collect details concerning physical data, ethnic origin, health, medical reports, and dietary habits (questionnaire is given). The biochemical tests were described in Table 3. Both vitamin A and carotene were analyzed by the method of Kimble (1939) (Kimble 1939). The factors used to convert the L values obtained with the Evelyn colorimeter to International Units of vitamin A and pg of carotene. All patients and control subjects underwent a routine eye examination including visual acuity, slit lamp examination, and ophthalmoscopic test; all tests were performed by well-trained and standardized specialists in eye hospital. A file of complete information, and all the demographical data was compiled. The consent of each contributor to use data was asked. This

Table 2 The number of subjects as control and night blindness patients

Age groups	Control groups		Night blindness patients	
	Male	Female	Male	Female
3–7	46	49	39	31
8–12	58	57	43	37
Total	104	106	82	68

research project was evaluated and approved by the Higher Education Commission, Pakistan.

Collection of biological samples

Venous blood samples (3–5 ml) were collected using metal-free Safety Vacutainer blood collecting tubes (Becton Dickinson, Rutherford, NJ, USA) containing >1.5 µg ethylenediaminetetraacetic potassium salt (K_2EDTA/mL) and were stored at −20 °C until analysis. All identified cases and controls were asked for 10–100-ml urine spot sample in a wide-mouth jar previously washed with 10% nitric acid and rinsed with deionized water, then urine was transfer into acid-washed 50 ml polyethylene tubes (Kartell, Milan, Italy) with

Table 3 Clinical and biochemical characteristics of normal and night blindness subjects

Parameters	Male		Female		Normal range
	Normal	Night blindness	Normal	Night blindness	
3–7 years					
Height (cm)	106.4±6.42	95.2±5.61	105.7±5.39	92.9±6.33	
Weight (kg)	19.3±1.56	15.8±2.33	18.6±1.12	13.9±1.24	
Serum ferritin (µg/L)	31.9±1.4	19.7±2.32	33.7±2.7	17.8±1.8	<30
Hemoglobin (mg/dL)	11.5±0.6	9.9±1.4	11.8±0.6	9.6±0.5	11.5–16.5
Hematocrit (%)	43.7±3.7	34.4±1.7	40.2±2.7	33.9±2.3	35–55
Vitamin A (IU)	136±5.45	52.4±4.98	137±5.68	53.8±4.67	>136–149
Carotene (µg/100 ml plasma)	148.7±13.8	59.8±9.56	149.3±11.3	56.4±8.25	>140
Red blood count RBC (mm^3)	4.4±0.6	3.21±0.6	4.5±0.4	3.4±0.8	3.5–5.5
MCV (µm)	83.4±3.24	74.3±4.5	86.2±1.9	73.6±3.4	75–100
MCH (pg)	31.4±1.5	24.5±1.3	33.6±1.1	26.3±0.7	25–35
MCHC (g/dL)	34.3±3.1	29.9±2.3	36.8±2.5	31.2±2.3	31–38
WBC (mm^3)	6.9±0.82	7.4±0.5	6.67±2.28	7.51±0.42	3.5–10
Platelets (mm^3)	165.5±16.5	179.2±19.4	172±8.9	193±14.8	100–400
8–12 years					
Height (cm)	137.7±1.32	125.4±3.28	136.9±1.68	124.7±2.07	
Weight (kg)	31.9±1.54	24.3±1.87	32.2±1.64	24.9±1.53	
Serum ferritin (µg/ L)	36.5±2.7	28.9±2.34	37.3±4.4	29.4±3.52	<30
Hemoglobin (mg/ dL)	12.2±1.3	11.3±1.4	12.5±1.23	11.8±2.65	11.5–16.5
Hematocrit (%)	45.3±3.21	36.9±2.42	46.5±1.76	34.5±4.6	35–55
Vitamin A (IU)	145±3.66	56.9±3.65	142±3.97	58.9±5.36	>136–149
Carotene (µg/100 ml plasma)	159±14.9	57.2±5.43	153±9.87	54.3±5.62	>140
Red blood count RBC (mm^3)	4.62±0.35	3.79±0.32	4.76±0.62	3.89±0.54	3.5–5.5
MCV (µm)	85.4±3.4	72.2±5.1	88.2±4.32	75.8±3.5	75–100
MCH (pg)	32.9±2.6	27.8±2.15	35.5±2.67	29.8±2.56	25–35
MCHC (g/ dL)	37.2±2.46	29.8±3.6	37.9±1.8	29.9±3.62	31–38
WBC (mm^3)	7.33±1.52	7.13±1.23	7.24±0.36	7.02±0.48	3.5–10
Platelets (mm^3)	236±19.7	247±15.6	229±20.2	245±22.6	100–400

cm centimeter, *kg/m²* kilogram/square meter, *µg/L* microgram/liter, *mmHg* millimeter of mercury, *mmol/L* millimoles/liter, *mg/dL* milligram/deciliter, *%* percent, *IU* international unit, *mm³* cubic millimeter, *µm* micrometer, *pg* picogram, *g/dL* gram/deciliter

polypropylene lids which were decontaminated before handling. Urine samples were stored at −15 °C until they were analyzed. Prior to sub-sampling of urine, the samples were shaken vigorously for 1 min to ensure a homogeneous suspension. The samples of hair were obtained using stainless-steel scissors from the nape of the neck. The scalp hair samples were washed as reported in our previous studies. After washing, hair samples were dried at 80 °C for 6 h. Hair samples were put into separate plastic envelopes with an identification number for each participant.

Microwave-assisted acid digestion (MWD)

Duplicate samples of scalp hair (200 mg) and 0.5 mL of blood and urine samples of each night blindness children and control individuals were directly placed into Teflon PFA flasks, then added 2 ml of a freshly prepared mixture of concentrated HNO_3-H_2O_2 (2:1, v/v) to each flask. The flasks were kept for 10 min at room temperature and then placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80 % of total power (900 W). Complete digestion of blood and urine samples required 2–4 min, while 5–8 min was necessary for scalp hair samples. After the digestion, the flasks were left to cool and the resulting solution was evaporated to semidried mass to remove excess acid. About 5 ml of 0.1 M nitric acid were added to the residue and filtered through a Whatman No. 42 filter paper and diluted with deionized water up to 10.0 ml in volumetric flasks. Blank extractions were carried through the complete procedure.

The Hg determination was carried out by CVAAS, using sodium tetrahydroborate as reducing agent and hydrochloric acid as carrier solution. About 500 μL of the each stock sample solution of CRM and real samples were transferred to PTFE flasks of the MHS-15 system and 10 mL of a 0.15 mol/L HCl solution was added, along with 40 μL of the antifoam agent. The system was sealed and 3 % (m/v) $NaBH_4$ was added for 5 s to the PTFE flask. A stream of high purity argon gas at a flow rate of 200 mL/min carried the generated Hg vapor to the quartz cell, and the absorbances of the generated Hg atoms were measured. Calibration was performed using aqueous standards of Hg (1–5 ppb) and subjected to CV AAS procedure as described above.

The validity and efficiency of microwave-assisted acid digestion method was checked with certified values of CRMs of all three biological samples and with those obtained from conventional wet acid digestion method (CDM), reported elsewhere (Kazi et al. 2008; Afridi et al. 2009) (Table 4). The % recoveries varied in the range of 96.4–99.0 for two analytes understudy. Non-significant differences were observed ($p > 0.05$) when comparing the values obtained by (MDM and CDM), (paired t test) as reported in our previous works (Kazi et al. 2008; Afridi et al. 2009).

Statistical evaluations

The statistical analyses were performed with Excel X State computer program (Microsoft Corp., Redmond, WA, USA) and Minitab 13.2 (Minitab Inc., State College, PA, USA). Student t test was used to assess the significance of the differences between concentrations of trace elements in the biological samples of night blindness patients and referents, calculated by unpaired (two sample) t test.

Analytical figure of merit

The linear range of the calibration curve for Hg and Se reached from the quantification limit (LOQ) up to 5.0 and 50 μg/L, respectively, and correlation coefficient (r^2) of 0.999 and 0.999 for Hg and Se, respectively. The limit of detection (LOD) and quantitation (LOQ) were calculated by $LOD = 3 \times {}^s/_m$ and $LOQ = 10 \times {}^s/_m$, respectively, where s is the standard deviation of 10 measurements of reagent blanks and m is the slope of the calibration graph. The LOD of Hg and Se was 0.133 and 0.052 μg/L, respectively. The method was assured by the analysis of triplicate samples, reagent blank, procedural blanks, CRMs sample, and standard addition method.

Result

The results reported in Table 5 shows the concentrations of Hg and Se in three biological samples of night blindness children as related to controls. Trace mineral patterns in biological samples are providing fruitful data not only as diagnostic procedure but also in providing answers pertaining to treatment.

Table 4 Determination of Se and Hg in certified samples by CDM and MWD ($N=10$)

Elements	CDM	MWD	T value[a]	% recovery[b]	Certified values
Certified sample of whole blood (µg/L)					
Se	114.6±2.47 (2.15)	113.5±3.68 (3.24)	0.247	99.0	116±23.5
Hg	3.31±0.31 (9.06)	3.27±0.25 (7.64)	0.225	98.8	3.4±1.0
Certified sample of urine (µg/L)					
Se	69.2±1.20 (1.73)	68.5±1.96 (2.86)	0.388	98.9	70±14.8
Hg	3.72±0.19 (5.10)	3.63±0.23 (6.34)	0.519	97.6	3.9±1.5
Certified sample of human hair (µg/g)					
Se	1.92±0.15 (6.25)	1.85±0.12 (8.10)	0.0203	96.4	2.00±0.08
Hg	12.2±0.21 (1.72)	11.9±0.23 (1.93)	0.146	97.5	12.3±0.874

[a] Paired t test between CDM and MWD DF=9 T (critical) at 95 % CI=2.262, $p=0.05$

[b] % recovery was calculated according to $\left[\frac{MDM}{CDM}\right] \times 100$

Values in parentheses are RSD

The concentrations of Se in the scalp hair samples of male control children of both age groups, 3–7 and 8– 12 years, at 95 % confidence limit (95 % Cl 1.47–1.69 and 1.58–1.70 µg/g, respectively), were found to be

Table 5 Selenium and mercury concentrations in biological samples (scalp hair, blood, and urine samples) of normal and Night blindness subjects

Age groups	Male $n=186$			Female $n=174$		
	Normal	Night blindness	p value	Normal	Night blindness	p value
Selenium						
Scalp hair (µg/g)						
3–7 range[a]	1.58±0.23 1.47–1.72	0.69±0.18 0.58–0.80	0.002	1.63±0.26 1.49–1.75	0.66±0.13 0.58–0.73	0.001
8–12 range[a]	1.65±0.14 1.58–1.71	0.71±0.12 0.66–0.78	0.001	1.72±0.15 1.64–1.80	0.69±0.09 0.65–0.73	0.001
Blood (µg/L)						
3–7 range[a]	198±7.24 194–202	82.3±5.28 79.8–85.2	0.001	202±9.23 197–206	86.5±7.92 82.6–90.2	0.001
8–12 range[a]	204±8.90 199–208	76.8±7.45 73.2–79.9	0.001	206±8.4 202–209	78.5±4.82 76.4–81.0	0.001
Urine (µg/L)						
3–7 range[a]	17.6±3.48 15.8–19.2	39.8±7.20 36.3–42.4	0.002	17.9±6.28 14.8–21.0	38.6±5.50 36.4–41.2	0.001
8–12 range[a]	20.6±3.9 18.7–22.5	46.8±5.4 44.3–49.2	0.001	20.5±4.42 18.3–22.8	47.4±6.28 44.2–50.5	0.001
Mercury						
Scalp hair (µg/g)						
3–7 range[a]	0.79±0.04 0.77–0.81	1.69±0.12 1.63–1.75	0.001	0.75±0.03 0.73–0.77	1.59±0.15 1.52–1.67	0.001
8–12 range[a]	0.85±0.03 0.82–0.87	1.76±0.16 1.68–1.80	0.001	0.83±0.04 0.81–0.85	1.73±0.17 1.65–1.80	0.001
Blood (µg/L)						
3–7 range[a]	0.74±0.06 0.71–0.77	1.64±0.08 1.60–1.68	0.001	0.71±0.04 0.69–0.73	1.58±0.06 1.55–1.61	0.001
8–12 range[a]	0.80±0.09 0.77–0.84	1.72±0.05 1.69–1.74	0.001	0.79±0.07 0.76–0.83	1.67±0.04 1.65–1.69	0.001
Urine (µg/L)						
3–7 range[a]	0.46±0.05 0.43–0.48	0.74±0.06 0.71–0.77	0.001	0.43±0.04 0.41–0.45	0.70±0.08 0.66–0.74	0.001
8–12 range[a]	0.49±0.05 0.47–0.52	0.77±0.05 0.75–0.80	0.006	0.48±0.08 0.44–0.52	0.76±0.07 0.72–0.79	0.001

[a] Minimum–maximum values

higher as compare to night blindness children of both age groups (95 % CI 0.60–0.78 and 0.65–0.77 µg/g, respectively). The level of Se in blood samples of male and female control children (95 % CI 195–208 and 196–210 µg/L, respectively) was found to be significantly higher ($p<0.001$) than male and female night blindness subjects (Table 5). The excretion of Se was higher in night blindness subjects of both genders.

The elevated level of Hg was observed in scalp hair of night blindness children of both genders (Table 5). The level of Hg in blood samples of night blindness children is higher (95 % CI 1.59–1.75 µg/l and 95 % CI 1.56–1.70 µg/l) as compared to values of Hg found in blood samples of control children (95 % CI 0.76–0.83 µg/l and 95 % CI 0.68–0.84 µg/l) of male and females, respectively, of both age groups. The excretion of Hg was high in night blindness children of both genders.

The unpaired student t test at different degree of freedom between psoriasis and referents of both genders were calculated at different probabilities. Our calculated t value exceeds that of t critical value at 95 % confidence intervals, which indicated that the difference between mean values of all four metals in referents and psoriasis persons showed significant differences ($p<0.001$).

Discussion

The present study provides data on Se and Hg concentrations in scalp hair, blood, and urine obtained from the night blindness and normal children of both genders living in Hyderabad, Sindh, Pakistan.

The biological (scalp hair and blood) samples of children with ocular problem had the lower average selenium concentration, while controls had higher concentrations with an average of 8.5 % higher than patients (Tables 5). The result shows that the level of Se in blood and scalp hair samples of night blindness patients was lower than referent children. The minerals, particularly zinc and Se, have a role in retinal metabolism and as antioxidants. Selenium concentration was higher in female children as compare to night blindness children. Gender is also a factor to consider due to the differences in the intake of energy, iron status, or hormonal influences, which can affect the bioavailability of trace elements (Kazi et al. 2008b).

The Se is an essential trace element that is needed to be consumed in the diet. It has antioxidant and neuroprotective features and is an important component of several antioxidant enzymes like glutathione peroxidase, thioredoxin reductases, and selenoproteins (Ognjanovic et al., 2012). The Se is incorporated into protein to make selenoproteins, which are important antioxidant enzymes called glutathione peroxidase (National Institute of Health 2004). According to Newswire (2001), antioxidants are a group of substances that protect tissues, cells and important compounds like protein, and DNA against the destructive power of oxygen and its relatives. Antioxidants encompasses vitamins C, E, beta carotene, and some minerals, and they are essential for good health and can help fight off heart diseases, cancer, age-related eye problems, and aging itself (Newswire 2001). The Se containing proteins, (selenoproteins) are important components of several antioxidant systems (e.g., glutathione peroxidase) that actively protect body organs against the damage created by free radicals and reactive oxygen species (Li and Shah 2004; Huang et al. 2002).

Participants with a history of ocular problems had higher mercury concentrations in hair and blood than those without such a history, with $p<0.001$. The average concentration of mercury was 50–58 % higher in the patient of ocular problem than controls. The Hg is present in coal and oil, and when these are burned, it vaporizes, eventually falling with precipitation into water and farmland (Shanker et al. 1998). In water, Hg is picked up by lower forms of life where it is methylated (its most toxic form) and works its way up the food chain to larger fish and humans. The Hg is extremely toxic with an affinity for neural tissue. It combines with the sulfide bonds in mitochondria, robbing the cell of energy and causing cell death (FDA Consumer Advisory 2001). Although adults can experience neurological effects when exposed to high concentrations of methyl mercury, advisories have mainly arisen because of the increasing concerns regarding methyl mercury's effects in the developing nervous systems of unborn and growing children. Alarmingly, while the placental barrier can stop many toxic elements, methyl mercury is an exception in that it not only crosses the placenta, but it also accumulates at higher concentrations on the fetal side than on the maternal (Kerper et al. 1992). Worsening the situation for the developing fetus, Hg also crosses the blood-brain barrier and exhibits long-term retention once it gets across (Kerper et al. 1992).

The Se can act as a growth factor; has powerful antioxidant and anticancer properties; and supports

normal thyroid hormone homeostasis, immunity, and fertility (Kuklinski et al. 1992). The study of Se physiology has become one of the fastest growing areas in biomedical research, and its role in protection against Hg toxicity is also gaining increased attention (Kuklinski et al. 1992). It is reasonable then to assume that not only does Se have an effect on Hg's bioavailability, but Hg may also have an effect on Se bioavailability. Therefore, the understanding of the "protective effect" of Se against Hg exposure may actually be backwards. The Hg's propensity for Se sequestration in brain and endocrine tissues may inhibit formation of essential Se-dependent proteins (selenoproteins). Hence, Se's "protective effect" against Hg toxicity may simply reflect the importance of maintaining sufficient free Se to support normal Se-dependent enzyme synthesis and activity (Behne et al. 2000; Hill et al. 2003).

The Se-deficient rodents are more susceptible to the prenatal toxicity of methyl mercury, and it is noteworthy that exposure to Hg reduced the activity of the selenoprotein glutathione peroxidase in the fetal/neonatal brain (El-Demerdash 2001). Additionally, when rodents are depleted of Se perinatally, the thyroid hormone economy of the fetus is disturbed (El-Demerdash 2001). Thyroid hormones are essential for normal neurological development. When disruption of thyroid hormone regulation occurs at vulnerable periods of development, irreversible neurological damage can result. Iodothyronine deiodinases are selenoproteins which regulate the tissue levels of thyroid hormones (Watanabem 2001). Therefore, severe Se deficiency may be detrimental to the developing brain. Considering Hg's extremely high binding affinity for Se and its ability to cross the blood-brain barrier, it is reasonable to suspect the Hg-Se interaction may have a role in developmental pathophysiology (Watanabem 2001).

The concentrations of selenium in blood samples of control children, found in present study, are higher than the findings of Hungary (Cser et al. 1996), Belgium

Table 6 Comparison of different elemental contents (μg/g) in biological samples of children from various parts of the world

Countries/authors	Specimens	Elements	Age (years)	$x \pm s$
Hungary				
Cser et al. 1996	Whole blood (μgl/l)	Selenium	1–5	58.43±2.5
			6–10	65.54±5.5
			11–15	64.75±3.95
Belgium				
Van Caille-Bertrand et al. 1986	Whole blood (μg/l)	Selenium	2–15	77.4±7.69
England				
Lombeck et al. 1980	Whole blood (μg/l)	Selenium	4–9	105±7.05
			3–15	116±5.93
			6–7	101±7.25
Germany				
Heinzow et al. 1989	Whole blood (μg/l)	Selenium	2–15	77.4±6.38
Poland				
Wasowicz et al. 1994	Whole blood (μg/l)	Selenium	1–3	75.8±6.08
			3–7	82.9±7.25
Spain				
Montuori et al. 2006	Scalp hair (μg/g)	Mercury	4	0.938±0.908 (0.189–5.627)
Japan				
Murata et al. 2004	Scalp hair (μg/g)	Mercury	7	1.64 (0.45–6.32)
USA				
McDowell et al. 2004	Scalp hair (μg/g)	Mercury	1–5	0.22 (0.18–0.25)
Germany				
Pesch et al. 2002	Scalp hair (μg/g)	Mercury	8–10	0.23±0.20 (<0.06–1.7)
	Urine (μg/l)			0.45±0.71

(Van Caillle-Bertrand et al. 1986), England (Lombeck et al. 1980), Germany (Heinzow et al. 1989), and Poland (Wasowicz et al. 1994) studies (Table 6). The concentrations of mercury in scalp hair samples of our referent children were lower than those reported from Spanish (Montuori et al. 2006) and Japanese (Murata et al. 2004) studies, while higher than USA (McDowell et al. 2004) and German (Pesch et al. 2002) studies (Table 6).

Conclusion

The results of the present study show that night blindness children tend to exhibit lower hair- and blood levels of Se and higher Hg concentration, and these are associated with higher urinary levels of these elements. It was also concluded that levels of mercury were higher in children having ocular problems. Among other things, regular monitoring of metals in biological/clinical specimens therefore plays an important part in, among other things, identifying possible sources of contamination/intoxication, as well as in preventing, through early detection, the onset of metal-related illness in individuals. Children are particularly susceptible to essential elemental deficiency due to their increased mineral needs for rapid growth and the relatively low selenium and zinc content of their diets due to poverty. It is necessary to add these minerals via food supplements. The results of this study provided guidance to clinicians and other professionals investigating deficiency of essential trace metals (Se) and excessive levels of toxic metals (Hg) in biological samples of healthy and anemic children.

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