Evaluation of Essential Trace and Toxic Elements in Biological Samples of Normal and Night Blindness Children of Age Groups 3–7 and 8–12 Years

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Abstract The most common cause of blindness in developing countries is vitamin A deficiency. The World Health Organization estimates 13.8 million children to have some degree of visual loss related to vitamin A deficiency. 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 samples composition. Vitamin and mineral deficiency prevents more than two billion people from achieving their full intellectual and physical potential. This study was designed to compare the levels of Zn, Mg, Ca, K, Na, As, Cd, and Pb in

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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 As, Ca, Cd, K, Pb, Mg, Na, and Zn 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 concentrations of trace and toxic elements were measured by atomic absorption spectrophotometer prior to microwave-assisted acid digestion. The results of this study showed that the mean values of As, Cd, Na, and Pb were significantly higher in scalp hair, blood, and urine samples of male and female night blindness children than in referents ($p < 0.001$), whereas the concentrations of Zn, Ca, K, and Mg were lower in the scalp hair and blood but higher in the urine samples of night blindness children. These data present guidance to clinicians and other professional investigating deficiency of essential mineral elements in biological samples (scalp hair and blood) of night blindness children.

Introduction

Night blindness (NB) 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 [1]. Such a deficiency may result from a diet low in animal foods (the main source of vitamin A, Zn, Fe, and Cu) such as eggs, dairy products, organ meats, and fish [1]. Christian et al. [1] showed that vitamin A deficiency is widespread in developing countries. Zn is necessary for the enzyme that activates vitamin A in the vision, and it is necessary for the normal metabolism of vitamin A and its deficiency is thought to interfere with vitamin A metabolism in several ways. Brody [2] mentioned that without Zn, vitamin A cannot be converted to its active form, and it is required in hepatic synthesis and secretion of the retinol binding protein (RBP), a primary plasma transport protein for retinal (vitamin A). Semba [3] study has been showed that Zn deficiency may be related with the etiology of night blindness because it interacts with vitamin A deficiency. Zn deficiency results in a decreased activity of the enzymes that release retinol from its storage form, retinyl palmitate, in the liver. Thus, even in the presence of vitamin A adequacy, night blindness could occur when Zn deficiency exists [4].

The relationship of micronutrient deficiency with the risk of developing certain major visual disorders and the therapeutic role of these essential mineral elements is now being recognized [5]. 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. Even when little was known of the cellular basis of these disorders, it was assumed that a deficiency in vitamin A was at the root of the problem—probably because of similarities in the dark-adaptation curve—and heroic doses of vitamin A were routinely administered in an attempt to cure the condition. Unfortunately, there are relatively few instances in which this form of therapy has proven effective [3].

The relationship of micronutrient deficiency with the risk of developing certain major visual disorders and the therapeutic role of these essential mineral elements are now being recognized [3]. 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 [6]. Ca is needed for the human beings in the formation of strong bones and teeth, for controlling blood-clotting mechanisms and to regulate the excitability of nerves and muscles. The majority of absorbed Ca is stored in the skeleton. Excess absorbed Ca is excreted in urine, feces, and sweat. The Ca content of the human body is 25 to 30 g at birth (0.8% of the body weight) and between 900 and 1,300 g in adult men (up to 1.7% of body weight) as reported by Weaver et al. [7]. Over 99% of the total Ca of the body is located in the bones, where it accounts for 39% of the total body bone mineral content as stated by Weaver [8]. Most available data indicate that calcium intake levels of about 800 mg/day are associated with adequate bone mineral accumulation in prepubertal children. The benefits of greater levels of intake in this age group have been studied inadequately [9, 10]. One study found a benefit of calcium supplements to children as young as 6 years of age [11].

Calcium enters into the retina through voltage-gated Ca channels (VGCCs), which regulates the release of neurotransmitter from the ribbon synapses of photoreceptor and bipolar neurons [12]. Neuronal VGCCs are critical to numerous cellular functions including synaptogenesis and neurotransmitter release [13]. Many enzymatic reactions required Mg as cofactor. The efficiency or deficiency of Mg may create different physiological disorders including cardiovascular disease [14]. The study of Goto et al. [15] showed that severe visual loss and legal blindness, which may be caused by the induced hyperexcitability and toxicity of the N-methyl-D-aspartate receptors, have been observed in Mg-deficient patients. Saris et al. and Laurant and Touyz [16, 17] studied that Mg is involved in several processes, including hormone receptor binding and getting of Ca channels, transmembrane ion flux, regulation of adenylate cyclase, muscle contraction and neuronal activity, control of vascular tone, cardiac excitability, and neurotransmitter release. Kirschmann [18] reported that Mg increases the body's ability to utilize Ca, P, Na, K, and vitamins C, E, and B complex. Quamme [19] investigated that normal intake of Mg is approximately 300 mg/day and about one third of the intake is absorbed by the gastrointestinal tract. The renal Mg handling is essentially a filtration–reabsorption process even though Mg secretion has been suggested. Primary prevention of osteoporosis is an important public health goal for Americans. This requires adequate intake and retention during childhood and adolescence of calcium and possibly magnesium. We found that standard dietary approaches, aimed at increasing dairy product consumption to increase calcium intake, led to greater total calcium absorption in pubertal but not in prepubertal children over the range of intakes from 750 to 1,350 mg/day. We found further that a magnesium intake consistent with the 1989 RDA was only able to maintain slightly more than one half of the children aged 9–14 years in positive magnesium balance [20]. Magnesium is necessary for Na/K ATPase which is responsible for active transport of K intracellularly during the action potential duration [21]. Most available data indicate that potassium intake levels in the children of different age groups are associated with age group that is 500 mg or 13 mEq for infants' birth–6 months age groups, 700 mg or 18 mEq for the age groups 7–12 months, 1,000 mg or 26 mEq for 1-year children, 1,400 mg or 36 mEq for children 2–5 years, 1,600 mg or 41 mEq for the children of 6–9 years, and 2,000 mg or 51 mEq for children of age group over 10 years [22, 23].

Potassium is a very significant body mineral, important to both cellular and electrical functions. It is one of the main blood minerals called "electrolytes" (the others are Na and Cl), which means it carries a tiny electrical charge (potential). K is the primary positive ion (cation) found within the cells, where 98% of the 120 g of K contained in the body is found (Wielopolski et al.) [24]. Rosanoff [25] investigated that Mg helps to maintain the K in the cells, but the Na and K balance is as finely tuned as those of Ca and P or Ca and Mg. Grant

et al. [26] investigated that Na is beneficial to the human body but only when combined with K, Ca, Mg, and other elements. In addition, some people are sensitive to high concentrations of Na. Rocchini [27] study showed that many people consume much more Na than their body needs. The human body is capable of handling up to 3,000 mg of Na. The study of Weinberger et al. [28] showed that the adequate intake or daily amount of Na sufficient to meet the needs of most healthy people is 1,500 mg/day (3,800 mg of salt) for 19- to 50-year-olds with normal blood pressure. Older adults and the elderly require somewhat less Na based on lower energy intakes [29]. The arrhythmogenic effect of Mg deficiency may be related to its effect in maintaining intracellular K. Magnesium is also involved in regulating K influx through different K channels. A deficiency in night blindness Mg can lead to a decrease in intracellular K due to a less efficient Na/K ATPase system and also by the loss of inward rectification [29].

Human cells employ metals, such as copper, zinc, and iron, to control significant metabolic and signaling functions making them essential for life. Other metals can be potentially toxic such as the heavy metals: lead, nickel, cadmium, mercury, and thallium. Lead, in particular, is a neurotoxin that has been linked to visual deterioration [30], central and peripheral nervous system disorders [31], renal dysfunction [32], and hypertensive cardiovascular disease [33]. Recently, Eichenbaum and Zheng [34] showed that the retina and choroid can accumulate the heavy metal, lead.

Lead-induced toxicities continue to be a major public health concern. In addition to its toxic effect on the central nervous system, Pb toxicity has been linked to renal dysfunction, hypertensive cardiovascular disease, and auditory disturbances and visual disability [35]. Recent studies from a rat retinal model indicate that low-level exposure to Pb produces scotopic visual deterioration, characterized by diminished rod-mediated electroretinogram, rod and bipolar apoptotic cell death, and inhibited cyclic guanine monophosphate phosphodiesterase [30]. While Pb toxicity has been linked to visual dysfunction in mammals, few studies have been performed to quantitate Pb distribution in human eyes. Even less has been learned about molecular and cellular mechanisms underlying Pb-induced visual disturbance. The retinal pigment epithelium (RPE) serves as a defensive barrier to the retina in the same way as the choroid plexus (CP) functions to the brain [36]. The previous studies have shown that the CP sequesters Pb, resulting in reduced secretion of transthyretin (TTR) to the cerebrospinal fluid [37]. TTR is a 55,000-Da protein and functions as a carrier protein for thyroid hormones, retinal, and retinol-binding protein. In the eyes, TTR plays a critical role in transporting retinol to the photoreceptor for the phototransduction process [36]. However, little quantitative information is available concerning the distribution of TTR in human eyes. Cadmium toxicity has been associated with renal disease, hypertension, and an increased prevalence of cardiovascular disease [38]. Heavy metals like arsenic, nickel, and cadmium are ubiquitous pollutants that have permanently contaminated air, water, and soil [39, 40]. The toxic effect of heavy metals usually involves an interaction between the heavy metal ion and the specific target protein, resulting in a change in protein structure and function [39]. Cells involved in the transport of trace metals are particularly susceptible to toxicity. The retinal pigment epithelium is a metal-chelating tissue that is capable of binding essential and toxic heavy metals because of the high affinity of metals to melanin in retinal pigment epithelium melanosomes [40].

In view of the above facts, it is important to determine the essential trace and TEs concentrations in biological samples of humans having physiological disorders such as HT [41]. Among the various biopsy materials, serum, scalp hair, urine, and other body fluids may be used as bio-indicators for these purposes [42]. Atomic absorption spectrometric methods are frequently used for the specific determination of very low elemental

concentrations in biological samples [43]. At present, the mineralization method frequently used for the analysis of biological samples is wet digestion with concentrated acids, using either convective systems or microwave ovens [43]. The main advantage of microwave-assisted samples pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors.

The main objectives of our study were (a) to assess the concentrations of essential trace and toxic elements in the biological samples (scalp hair, blood, and urine) of night blindness and normal children of both genders and with ages ranging between 1–5 and 6–10 years old, (b) to evaluate that the variation of these elements in night blindness children was related with age and sex factors, and (c) to investigate about the etiologies of the detected night blindness and to evaluate the role of nutrition, based on dietary history to screen high-risk children for night blindness in Pakistan.

Materials and Methods

Apparatus

A Perkin-Elmer Model 700 (Norwalk, CT, USA) atomic absorption spectrometer, equipped with a flame burner and graphite furnace HGA-400, a pyrocoated graphite tube with integrated platform, and an autosampler AS-800 were used for the analysis of understudied elements. The instrumental parameters are shown in Table 1. The Ca, Mg, Na, and Zn were measured under optimized operating conditions by flame atomic absorption spectrometry with air–acetylene flame, while As, Cd, and Pb were determined by electrothermal atomic absorption spectrometry. The signals were measured as the absorbance peaks in the flame absorption mode, while integrated absorbance values (peak area) were used in graphite furnace. A Pel (PMO23) 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 Glasswares

Ultrapure water obtained from ELGA labwater system (Bucks, UK) was used throughout the study. Concentrated nitric acid 65% and hydrogen peroxide 30% were purchased from Merck (Darmstadt, Germany) and were checked for possible trace metal contamination. Standard solutions of As, Ca, Cd, K, Mg, Na, Pb, and Zn were prepared by dilution of certified standard solutions (1,000 ppm) Fluka Kamica (Bush, Switzerland). Dilute working standard solutions were prepared immediately prior to their use by stepwise dilution of the stock standard solution with 0.2 M HNO_3. The stock standard solution of modifiers $Mg(NO_3)_2$ (5.00 g l^{-1}) was prepared from $Mg(NO_3)_2$ (Merck), while Pd stock standard solution, 3.00 g l^{-1}, was prepared from Pd 99.999% Sigma Aldrich (Milwaukee, WI, USA). All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, the certified reference materials (CRMs), human hair BCR 397 (Brussels, Belgium), Clincheck control-lyophilized human urine, and human whole blood (Recipe, Munich, Germany) were used.

All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, certified samples of human hair BCR 397 (Brussels, Belgium), NIST 2670 urine reference material (Bureau Drive, Gaithersburg, MD, USA) for Ca, K, Mg, and Na while Clincheck control-lyophilized human urine used for As, Cd, Pb, and Zn, and Sero-

Table 1 Measurement conditions for electrothermal atomization AAS 700 and for Ca, Mg, K, and Na

Parameters	As	Zn	Cd	Ca	Pb	K	Mg	Na
For electrothermal atomization AAS 700								
Lamp current (mA)	10.0		6.0		8.0			
Wavelength (nm)	193.7		228.8		283.3			
Slit width (nm)	0.7		0.7		0.7			
Drying temperature (°C)/ramp/hold (s)	140/15/15		140/15/5		140/15/5			
Ashing temperature (°C)/ramp/hold (s)	1,300/10/20		850/10/20		700/10/20			
Atomization temperature (°C)/ramp/hold (s)	2,300/0/5.0		1,650/0/5.0		1,800/0/5.0			
Cleaning temperature (°C)/ramp/hold (s)	2,600/1/3		2,600/1/3		2,600/1/3			
Chemical modifier	$Mg(NO_3)_2 + Pd(NO_3)_2$		5 µg Pd as $Pd(NO_3)_2$		15 µg $Mg(NO_3)_2$			
For Ca, Mg, K, and Na								
Wave length (nm)		213.9		422.7		766.5	285.2	589
Slit width (nm)		0.7		0.7		0.7	0.7	0.2
Lamp current (mA)		7.5		7.5		10	7.5	10
Burner height (mm)		7.5		12.5		7.5	7.5	7.5
Oxidant (air; l/min)		17.0		17.0		17.0	17.0	17.0
Fuel (acetylene; l/min)		2.0		2.0		2.0	2.0	2.0

Sample volume (10 µl), cuvette = cup, carrier gas = (200 ml/min). Background correction (D_2 lamp) used for all elements

201705 human whole blood, level-3-0337 (Billingstad, Norway) for Ca, K, Mg, and Na while Clincheck control-lyophilized human blood used for As, Cd, Pb, and Zn, were used as CRMs. All glassware and plastic material used were first soaked for 24 h in 2 M nitric acid, washed with distilled water, and finally rinsed with deionized water, dried, and stored in a class 100 laminar flow hood.

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, and the 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. The biochemical tests were described in Table 3. Both vitamin A and carotene were analyzed by the method of Kimble [44]. The factors used to convert the L values obtained with the Evelyn colorimeter to international units of vitamin A and picograms 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 the data was asked. This research project was evaluated and approved by the Higher Education Commission, Pakistan.

Venous blood samples (3–5 ml) were collected using metal-free Safety Vacutainer blood collecting tubes (Becton Dickinson, Rutherford ®, USA) containing >1.5 μg K_2EDTA/ml and were stored at −20°C until analysis. Urine was voided directly into an acid-washed 100-ml polyethylene tubes (Kartell1, Milan, Italy) which had been decontaminated before handling. Between sampling sessions, the container was wrapped in a clean polyethylene bag. Urine samples were acidified with 1% ultrapure HNO_3. Prior to sub-sampling for analysis, the sample was shaken vigorously for 1 min to ensure a homogeneous suspension. Hair samples of children were collected from the children in an area of the cranium 2 to 3 cm above the nape of the neck. The scalp hair samples were washed as in our previous

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

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

studies [45, 46]. 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.

Conventional Digestion Method

Duplicate 0.5 ml of each certified urine and blood samples and 0.2 g of human hair samples BCR 397 were placed individually into 50-ml Pyrex flasks. Five milliliters of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) was added to each flask, which was heated on an electric hot plate at 80°C, for 2 to 3 h, till clear transparent digests were obtained. Final solutions were made up to 10 ml with 2 M HNO_3. The final solutions were collected in polyethylene flask and stored at 4°C till analysis. Duplicate biological samples (scalp hair, blood, and urine) of each night blindness and control human subjects were treated as described above for reference samples.

Microwave-Assisted Acid Digestion Method

A microwave-assisted digestion procedure was carried out, in order to achieve a shorter digestion time. Duplicate samples of scalp hair (200 mg) and 0.5 ml of blood and urine samples of each night blindness and control individuals were directly placed into Teflon PFA flasks. Two milliliters of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) was added to each flask then left for 10 min. After this period, the flasks were placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80% of total power (900 W), during 2 to 3 min for blood and urine and 4 to 5 min for hair samples. After the digestion, the flasks were left to cool and the resulting solution was evaporated almost to dryness to remove excess acid and then diluted to 10.0 ml in volumetric flasks with 0.1 M nitric acid. Blank extractions (without sample) were carried out performing the complete procedure of both methods.

To establish the validity described for our methodology, five replicas samples of each certified sample (human hair, blood, and urine) were digested as reported above. All experiments were conducted at room temperature (30°C) following the well-established laboratory protocols. All digests obtained from both methods were analyzed for Zn, Cu, and Fe by flame atomic absorption spectrophotometer. The concentrations were obtained directly from calibration graphs after correction of the absorbance for the signal from an appropriate reagent blank. The validity and efficiency of the microwave-assisted digestion method was checked with those obtained from conventional wet acid digestion method [47].

Analytical results of the certified reference materials, obtained by both digestion methods, were closed to that of the certified values, which confirmed the reliability of the methods. The percentage recovery of all elements in CRM samples obtained by conventional digestion method varied between 97.8% and 99.9%, but it was time-consuming. The microwave-assisted digestion method was efficient and taken less than 10 min to complete the digestion of the three certified biological samples. Mean values for all the elements differed less than 1% to 2% from the certified values. The coefficient of variation differed less than 2% for the different elements. Nonsignificant differences were observed ($p > 0.05$) when comparing the values obtained by both methods (paired t test; Table 4).

Results

The results reported in Table 5 shows that the concentrations of essential trace and toxic elements (As, Ca, Cd, K, Mg, Pb, Na, and Zn) were altered in all three biological samples of night blindness children as related to controls. The concentrations of Zn in the scalp hair samples of control children of both age groups, 3–7 and 8–12 years, were found in the range of 172–215 and 224–267 µg/g while the level of Zn in male night blindness children of both age groups has in the range of 120–149 and 164–200 µg/g; the same trend was observed in females (Table 5). The range of Zn levels in blood samples of referent subjects of both gender (13.7–18.4 and 8.1–13.9 mg/l) was significantly higher as compared to the range of Zn values observed in blood samples of night blindness subjects (4.52–6.02 and 3.47–5.76 mg/l) in male and females, respectively (Table 5). The excretion of Zn was high in diabetic patients of both genders (Table 5).

The concentrations of Ca in the scalp hair samples of male control children of both age groups, 3–7 and 8–12 years, were found in the range of 2,510–4,500 and 1,850–3,810 µg/g

Table 4 Determination of Ca, Mg, K, Na, Zn, Cd, Pb, and As in certified samples by conventional (CDM) and microwave digestion method (MWD) (N=10)

Elements	Microwave digestion method	Conventional digestion method	T value[b]	% recovery[c]	Certified values
Certified sample of whole blood (µg/l)					
Ca	14.60±1.1 (7.53)	14.58±1.3 (8.92)	0.898	99.9	14.7±0.3
Mg	16.88±1.3 (7.70)	16.86±1.4 (8.30)	0.978	99.9	17.0±0.3
Na	1,202.7±33.8 (2.81)	1,193.1±19.8 (1.66)	0.310	99.2	1,210±40
K	1,011±48.7 (4.82)	1,009±36.7 (3.64)	0.936	99.8	1,014±30
Zn[a]	2.25±0.09 (4.00)	2.20±0.08 (3.64)	0.0912	97.8	2.27±0.68
Cd	1.21±0.07 (5.78)	1.187±0.09 (7.58)	0.0912	98.10	1.2±0. 4
Pb	105.67±8.2 (7.76)	104.8±7.3 (6.96)	0.0012	99.18	105±24
As	–	–	–	–	–
Certified sample of urine (µg/l)					
Ca	104.5±7.9 (7.56)	104.3±6.8 (6.52)	0.938	99.8	105±5
Mg	62.68±5.6 (8.93)	62.56±4.5 (7.19)	0.937	99.8	63±3
Na	2,609.5±59.8 (2.29)	2,604.3±73.5 (2.82)	0.947	99.8	2,620±140
K	1,495.5±28.9 (1.93)	1,491.0±39.8 (2.67)	0.876	99.7	1,500
Zn	205±6.0 (2.93)	201±7.0 (3.48)	0.0647	98.05	210±50
Cd	11.83±0.91 (7.69)	11.72±0.85 (7.25)	0.44027	99.07	11.8±2.5
Pb	41.2±1.96 (4.75)	40.77±2.86 (7.01)	0.0038	98.9	41.0±9.9
As	33.8±1.6 (4.73)	33.6±1.2 (3.57)	0.00001	99.4	34.0±5.0
Certified sample of human hair (µg/g)					
Ca	1,550.6±55.3 (3.56)	1,546.0±45.6 (2.95)	0.889	99.7	1,560.0±40[d]
Mg	199.0±13.5 (6.79)	198.2±12.3 (6.2)	0.838	99.6	200±5[d]
Na	1.07±0.08 (7.48)	1.06±0.06 (5.66)	0.855	99.1	1.08±0.007
K	8.55±0.5 (5.85)	8.53±0.7 (8.21)	0.941	99.8	8.6±0.03
Zn	197±12.8 (6.2)	194±11.3 (5.7)	0.0345	98.6	199±5
Cd	0.53±0.025(4.72)	0.524±0.024 (4.58)	0.2256	98.87	0.52±0.024
Pb	33.29±1.21 (3.63)	32.56±1.18 (3.62)	0.096	97.8	33±1.2
As	0.32±0.02 (6.2)	0.31±0.023 (8.0)	0.00001	96.8	0.31±0.02

T (critical) at 95% CI=2.262, p<0.50. # indicative value. Values in parentheses are RSD

[a] Values indicates in mg/l

[b] Paired t test between CDM and MWD DF=9

[c] % recovery was calculated according to: $\frac{MDM}{CDM} \times 100$

while the level of Ca in male night blindness children of both age groups has in the range of 2,340–3,350 and 1,900–3,200 µg/g; the same trend was observed in females (Table 5). The range of Ca levels in blood samples of referent subjects of both gender (52.3–69.3 and 49.5–69.2 mg/l) was significantly higher as compared to the range of Ca values observed in blood samples of night blindness subjects (36.7–42.8 and 35.8–46.3 mg/l) in male and females, respectively (Table 5). The excretion of Ca was high in night blindness patients of both genders. The magnesium levels in hair revealed significant difference (p<0.005) between controls and patients; levels for healthy male and female of both age groups were found in the range of 789–946 and 782–635 µg/g while the range for patients was observed as 322–429 and 381–502 µg/g for both male and female, respectively (Table 5). In blood

Table 5 Trace element concentrations in biological samples (scalp hair, blood, and urine samples) of normal and night blindness subjects

Age groups	Male			Female		
	n=186			n=174		
	Normal	Night blindness	p value	Normal	Night blindness	p value
Zinc						
Scalp hair (µg/g)						
3–7	192±18.9	134±13.5	0.001	205±13.5	143±11.6	0.001
Range[a]	172–215	120–149		195–221	132–156	
8–12	245±17.8	182±17.5	0.001	253±12.7	173±12.2	0.001
Range[a]	224–267	164–200		243–262	161–195	
Blood (mg/l)						
3–7	15.3±2.06	5.16±0.61	0.001	16.3±2.2	5.47±0.57	0.001
Range[a]	13.7–17.2	4.52–5.79		14.1–18.4	4.91–6.02	
8–12	11.8±2.7	4.67±1.07	0.001	12.3±1.08	4.92±0.68	0.001
Range[a]	8.1–13.9	3.47–5.45		11.2–13.4	4.39–5.76	
Urine (mg/l)						
3–7	0.9±0.13	1.69±0.24	0.001	0.9±0.33	1.78±0.31	0.001
Range[a]	0.8–1.2	1.43–1.99		0.72–1.26	1.7–2.1	
8–12	1.13±0.24	1.73±0.28	0.002	0.8±0.26	1.63±0.28	0.001
Range[a]	0.7–1.42	1.38–2.1		0.5–1.12	1.44–1.92	
Calcium						
Scalp hair (µg/g)						
3–7	3,650±940	2,840±552	0.019	3,950±870	2,690±750	0.008
Range[a]	2,510–4,500	2,340–3,350		3,100–4,720	1,920–3,200	
8–12	2,760±982	2,530±590	0.021	3,140±1,100	2,220±641	0.013
Range[a]	1,850–3,810	1,900–3,200		1,985–4,230	1,650–2,900	
Blood (mg/l)						
3–7	62.4±6.5	39.8±2.9	0.001	59.5±7.4	37.5±3.54	0.001
Range[a]	55.3–69.3	36.7–42.8		52.3–66.8	36.9–41.9	
8–12	61.3±7.4	41.8±3.2	0.001	58.4±9.6	40.2±5.6	0.003
Range[a]	54.6–68.9	38.5–44.6		49.5–69.2	35.8–46.3	
Urine (mg/l)						
3–7	41.9±5.4	75.2±6.75	0.001	40.2±5.9	73.9±7.82	0.001
Range[a]	35.4–47.3	68.5–82.4		35.2–46.8	65.8–81.6	
8–12	50.7±10.9	79.5±8.7	0.001	44.4±8.6	76.8±5.22	0.001
Range[a]	40.3–61.2	70.2–88.3		36.8–54.2	71.3–82.5	
Magnesium						
Scalp hair (µg/g)						
3–7	885±74.6	384±52.8	0.001	852±60.9	365±42.6	0.001
Range[a]	807–946	324–429		789–920	322–409	
8–12	863±63.6	446±55.7	0.001	822±37.3	452±46.8	0.001
Range[a]	794–935	387–502		782–863	381–502	
Blood (mg/l)						
3–7	85.2±14.3	38.7±5.6	0.001	90.6±16.4	39.7±7.69	0.001
Range[a]	71.2–100	34.8–45.9		73.4–107	31.5–46.8	

Table 5 (continued)

Age groups	Male			Female		
	n=186			n=174		
	Normal	Night blindness	p value	Normal	Night blindness	p value
8–12	100±17.4	46.2±4.6	0.001	103±13.9	45.2±8.54	0.001
Range[a]	85.2–119	41.9–50.9		89.4–117	37.5–53.8	
Urine (mg/l)						
3–7	59.3±2.6	83.6±15.6	0.004	61.9±4.7	79.7±6.21	0.001
Range[a]	56.3–62.4	67.9–100		58.9–66.3	73.5–85.9	
8–12	66.6±5.3	89.5±18.5	0.006	66.9±5.5	92.8±7.97	0.001
Range[a]	60.5–71.9	69.4–112		60.4–72.3	84.7–101	
Potassium						
Scalp hair (µg/g)						
3–7	9.5±3.2	6.8±1.21	0.006	9.2±2.34	5.59±1.32	0.002
Range[a]	6.5–13.3	5.63–8.21		7.32–11.6	4.17–6.91	
8–12	16.9±5.7	13.9±1.9	0.019	15.9±2.63	12.3±2.42	0.009
Range[a]	11.2–21.2	11.7–16.0		13.4–18.5	9.93–14.8	
Blood (mg/l)						
3–7	1,330±55.2	1,030±44.9	0.002	1,290±49.8	1,000±36.9	0.001
Range[a]	1,280–1,402	969–1,080		1,235–1,350	960–1,050	
8–12	1,560±98.2	1,190±79.6	0.001	1,540±100	1,126±60.8	0.001
Range[a]	1,490–1,610	1,110–1,270		1,439–1,640	1,050–1,200	
Urine (mg/l)						
3–7	338±32.3	723±25.6	0.001	306±22.8	695±56.8	0.001
Range[a]	304–375	695–753		283–329	642–752	
8–12	502±45.3	799±39.8	0.001	470±59.8	785±65.4	0.001
Range[a]	452–549	746–845		406–532	723–860	
Sodium						
Scalp hair (µg/g)						
3–7	200±11.2	254±28.2	0.005	154±15.6	248±31.2	0.001
Range[a]	188–214	224–287		139–170	214–282	
8–12	218±27.7	260±36.8	0.01	213±17.3	268±26.2	0.007
Range[a]	185–246	225–300		193–235	235–293	
Blood (mg/l)						
3–7	338±32.3	468±36.9	0.005	306±22.8	448±26.3	0.001
Range[a]	304–374	423–516		280–329	415–470	
8–12	502±45.3	612±50.2	0.01	470±59.8	658±36.9	0.001
Range[a]	449–549	574–669		405–540	610–693	
Urine (mg/l)						
3–7	1,450±35.6	2,360±54.9	0.005	1,380±32	2,350±63.5	0.001
Range[a]	1,410–1,490	2,290–2,410		1,350–1,435	2,280–2,430	
8–12	2,590±52.5	3,310±72.6	0.01	2,240±70.2	3,350±86.7	0.001
Range[a]	2,430–2,645	3,240–3,390		2,180–2,360	3,270–3,440	
Cadmium						
Scalp hair (µg/g)						
3–7	1.14±0.37	2.78±0.54	0.001	1.03±0.25	2.56±0.41	0.001

Table 5 (continued)

Age groups	Male			Female		
	$n=186$			$n=174$		
	Normal	Night blindness	p value	Normal	Night blindness	p value
Range[a]	0.75–1.53	2.21–3.35		0.75–1.32	2.13–2.99	
8–12	1.83±0.24	3.4±0.6	0.001	1.63±0.31	3.25±0.46	0.001
Range[a]	1.52–2.11	2.75–4.05		1.30–1.97	2.87–3.72	
Blood (µg/l)						
3–7	2.56±0.43	4.48±0.32	0.001	2.47±0.36	4.18±0.25	0.001
Range[a]	2.11–3.04	4.04–4.84		2.09–2.86	3.81–4.47	
8–12	3.38±0.36	5.67±0.43	0.001	3.14±0.19	5.45±0.37	0.001
Range[a]	3.02–3.79	5.12–6.14		2.92–3.35	5.02–5.93	
Urine (µg/l)						
3–7	1.2±0.32	2.45±0.43	0.001	0.8±0.35	2.28±0.16	0.001
Range[a]	0.71–1.58	2.02–2.93		0.52–1.17	2.14–2.47	
8–12	1.42±0.28	2.9±0.37	0.001	1.3±0.39	2.8±0.31	0.001
Range[a]	1.02–1.83	2.24–3.45		0.82–1.79	2.37–3.25	
Arsenic						
Scalp hair (µg/g)						
3–7	0.52±0.03	1.03±0.13	0.001	0.43±0.02	1.01±0.15	0.001
Range[a]	0.47–0.56	0.89–1.18		0.40–0.46	0.84–1.17	
8–12	0.72±0.08	1.14±0.22	0.002	0.65±0.06	1.12±0.15	0.001
Range[a]	0.63–0.81	0.91–1.39		0.58–0.72	0.96–1.30	
Blood (µg/l)						
3–7	0.9±0.3	1.98±0.17	0.001	0.7±0.4	1.86±0.32	0.001
Range[a]	0.59–1.23	1.70–2.18		0.34–1.13	1.48–2.19	
8–12	1.2±0.4	2.49±0.25	0.001	0.9±0.2	2.35±0.31	0.001
Range[a]	0.77–1.63	2.20–2.76		0.67–1.15	2.02–2.68	
Urine (µg/l)						
3–7	2.2±0.6	3.56±0.42	0.006	1.9±0.5	3.39±0.38	0.001
Range[a]	1.56–2.83	3.14–3.99		1.39–2.49	3.00–3.79	
8–12	2.9±0.56	4.2±0.8	0.01	2.7±0.9	4.07±0.55	0.001
Range[a]	2.39–3.49	3.42–5.35		1.92–2.83	3.47–4.68	
Lead						
Scalp hair (µg/g)						
3–7	4.3±0.54	12.5±1.3	0.001	4.2±0.45	11.5±0.37	0.001
Range[a]	3.68–4.87	11.1–13.9		3.69–4.69	10.7–11.9	
8–12	6.9±1.5	16.4±2.6	0.001	6.43±1.36	15.6±1.26	0.001
Range[a]	5.33–8.32	14.2–19.2		4.93–7.82	14.3–16.9	
Blood (µg/l)						
3–7	144±20.2	276±23.6	0.002	137±20.8	265±27.4	0.001
Range[a]	121–167	247–302		115–160	240–286	
8–12	165±13.9	322±36.2	0.003	149±14.9	306±28.2	0.001
Range[a]	149–181	293–359		132–165	274–335	
Urine (µg/l)						
3–7	43.5±4.3	65.8±3.64	0.001	41.2±4.6	63.9±5.6	0.001

Table 5 (continued)

Age groups	Male			Female		
	n=186			n=174		
	Normal	Night blindness	p value	Normal	Night blindness	p value
Range[a]	38.6–49.2	61.5–69.2		36.8–46.5	57.9–69.8	
8–12	47.6±6.4	73.2±9.6	0.001	46.6±5.2	71.6±7.32	0.001
Range[a]	41.4–55.3	62.2–81.9		40.8–52.0	63.7–79.2	

[a] Minimum–maximum values

samples, the range of Mg values in healthy subjects of both age groups was found to be higher (71.2–107 and 85.2–119 mg/l) than those values obtained for Mg in blood samples of night blindness children (31.5–46.8 and 37.5–53.8 mg/l) for male and females, respectively (Table 5). The excretion of Mg was high in night blindness children of both genders. The concentrations of K in the scalp hair samples of male control children of both age groups, 3–7 and 8–12 years, were found in the range of 6.5–13.3 and 11.2–21.2 μg/g while the level of K in male night blindness children of both age groups has in the range of 5.63–8.21 and 11.7–16.0 μg/g; the same trend was observed in females. The range of K levels in blood samples of referent subjects of both gender (1,235–1,402 and 1,439–1,640 mg/l) was significantly higher as compared to the range of K values observed in blood samples of night blindness subjects (960–1,080 and 1,050–1,270 mg/l) in male and females, respectively (Table 5). The excretion of K was high in night blindness patients of both genders. The elevated level of Na was observed in scalp hair of night blindness children of both genders (Table 5). The level of Na in blood samples of night blindness children is higher (415–516 and 574–693 mg/l) as compared to values of Na found in blood samples of referents subjects (280–374 and 405–549 mg/l) of male and females, respectively, of both age groups. The excretion of Na was high in night blindness children of both genders.

The elevated level of As was observed in scalp hair of night blindness children of both genders (Table 5). The level of As in blood samples of night blindness children is higher (1.70–2.76 and 1.48–2.68 μg/l) as compared to values of As found in blood samples of referents subjects (0.47–0.81 and 0.40–0.72 μg/l) of male and females, respectively, of both age groups. The excretion of As was high in night blindness children of both genders (Table 5). The concentrations of Cd in the scalp hair samples of control children of both age groups, 3–7 and 8–12 years, were found in the range of 0.75–1.53 and 1.52–2.11 μg/g while the level of Zn in male night blindness children of both age groups has in the range of 2.21–3.35 and 2.75–4.05 μg/g; the same trend was observed in females. The range of Cd levels in blood samples of referent subjects of both gender (2.11–3.79 mg/l and 2.09–3.35 μg/l) was significantly lower as compared to the range of Cd values observed in blood samples of night blindness subjects (4.04–6.14 and 3.81–5.93 μg/l) in male and females, respectively (Table 5). The excretion of Cd was high in night blindness patients of both genders. The Pb levels in hair revealed significant difference ($p<0.001$) between controls and patients; levels for healthy male and female of both age groups were found in the range of 3.68–8.32 and 3.69–7.82 μg/g while the range for patients was observed as 11.1–19.2 and 10.7–16.9 μg/g for both male and female, respectively (Table 5). In blood samples, the range of Pb values in healthy subjects of

both age groups was found to be lower (121–181 mg/l and 115–165 μg/l) than those values obtained for Pb in blood samples of night blindness children (247–359 and 240–335 μg/l) for male and females, respectively (Table 5). The excretion of Ni 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 was calculated at different probabilities. Our calculated t value exceeds that of t critical value at 95% confidence intervals, which indicated that the difference between means values of all four metals in referents and psoriasis persons showed significant differences ($p<0.001$).

An increase in As, Cd, and Pb and decrease of Ca, K, Mg, and Zn concentrations were observed in night blindness children. Moreover, a decrease of Zn/As, Zn/Cd, and Zn/Pb ratios in biological samples of night blindness children as compared to controls was also observed (Table 6).

Discussion

Minerals play important role in the subtle biochemistry of the body as do vitamins. Virtually, all enzymatic reactions in the body require minerals as cofactors [48].

Table 6 Zn/As, Zn/Cd, and Zn/Pb mole ratio in biological samples (scalp hair, blood, and urine samples) of normal and night blindness children of both genders

Specimens	Age groups	Male		Female	
		Normal	Night blindness	Normal	Night blindness
Zn/As					
Scalp hair	3–7	423	149	546.1	162.2
	8–12	389.8	182.9	445.9	176.9
Blood	3–7	19.47	2.985	26.7	3.37
	8–12	11.26	2.149	15.7	2.40
Urine	3–7	0.469	0.544	0.543	0.602
	8–12	0.446	0.472	0.339	0.459
Zn/Cd					
Scalp hair	3–7	289.5	82.8	342.1	96.0
	8–12	230.1	92.0	266.8	91.5
Blood	3–7	10.3	1.98	11.3	2.25
	8–12	6.00	1.42	6.73	1.55
Urine	3–7	1.29	1.19	1.93	1.34
	8–12	1.37	1.03	1.06	1.00
Zn/Pb					
Scalp hair	3–7	4,921	1,181	5,379	1,370
	8–12	3,913	1,223	4,336	1,222
Blood	3–7	11.71	2.06	13.1	2.275
	8–12	7.881	1.598	9.1	1.772
Urine	3–7	45.1	52.3	52.2	57.9
	8–12	42.9	45.4	32.7	44.1

Micronutrients are integral functional components of many enzymes and transcriptional regulatory proteins [49]. The result shows that the level of Ca, K, Mg, and Zn in blood and scalp hair samples of night blindness patients was lower, while As, Cd, Na, and Pb levels were higher than referent children.

NB 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. 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. Night blindness may be an early sign of vitamin A deficiency. Such a deficiency may result from diets low in animal foods, the main source of vitamin A, zinc, iron, and copper [5]. The result shows that the level of Zn in blood and scalp hair samples of night blindness patients was lower than referent children. Zinc deficiency may result in a decreased synthesis of RBP, which transports retinol through the circulation, to tissues (e.g., the retina) [4]. Zinc deficiency results in a decreased activity of the enzymes that release retinol from its storage form, retinyl palmitate, in the liver. Thus, even in the presence of vitamin A adequacy, night blindness could occur when zinc deficiency exists. Zinc can be used in the treatment of ocular problems. Many physicians suggest that 15 to 30 mg of zinc per day is necessary for a healthy vision [5]. On the other hand, the long-term zinc supplementation may reduce copper levels (depending on the amount of zinc used) and is usually administered to people receiving a zinc supplement for more than a few weeks. Food rich in phytate significantly reduces the absorption of Zn, increasing the chance of Zn deficiency. Socioeconomic status of children was strongly associated with levels of Zn in hair samples, and the lowest concentration of Zn was found in children from the families with low incomes and in children who rarely consumed meat [50]. The supplementation with zinc and iron together has been found to improve vitamin A status among children with a high risk to suffer a deficiency of the three nutrients [51]. Our study results are consistent with previously reported study that Zn is known to be an etiological factor in NB and low serum levels can result from alcoholism and systemic conditions causing intestinal malabsorption. Patients with low Zn levels are often also deficient in vitamin A. Laboratory and clinical studies have shown that low serum levels of Zn and vitamin A are associated with NB and reduced electroretinograms [52].

Magnesium and potassium depletion also leads to impaired function of the Na/K cation pump embedded in the cell membrane [53]. This results in accumulation of intracellular Na and water, a decrease in cell K, and an increase in intracellular Ca accumulation. Importantly, if Mg and K depletion are not simultaneously corrected, then this may result in refractory K^+ repletion due to coexisting Mg depletion [54].

In the retina, Ca entry through voltage-gated Ca channels regulates the release of neurotransmitter from the ribbon synapses of photoreceptor and bipolar neurons. These channels translate the graded, electrical responses of photoreceptors and bipolar cells into graded, calcium-dependent release of the excitatory neurotransmitter, glutamate. At the photoreceptor's dark resting potential of −40 mV [55], Ca channels are tonically activated, producing a constant influx of Ca into the synaptic terminal. Light hyperpolarizes photoreceptors, which closes Ca channels and reduces glutamate release. The operating range of mammalian photoreceptor is about 10–20 mV [56], with the absorption of a single photon inducing a hyperpolarization of ~1 mV in a rod photoreceptor, a signal that is reliably transmitted to the rod bipolar cell [57]. The calcium channels that modulate the release of glutamate at ribbon synapses must faithfully track these small voltage changes and impart them to the synaptic vesicle release machinery, implying high-voltage sensitivity of the channels and close coupling to the synaptic release machinery [58]. The effect

produced by adding Mg ions to the solution bathing the retina is unknown. Because it acts competitively with Ca, Mg tends to inhibit the release of transmitter substances at chemically mediated synapses—for example, the cholinergic neuromuscular junction [59]. If Mg exerts the same effect at the receptor terminal, then the fact that the S-unit hyperpolarizes (a reaction similar to that produced by light) suggests that the receptor discharges a depolarizing agent in the dark and that light actually suppresses the release of this agent. Because aspartate (one of the excitatory, short-chain amino acids) has a strong depolarizing effect on both the horizontal cell [60] and the bipolar cell [61], it is one of the substances considered to be a putative transmitter at the receptor terminal.

It is not yet possible to assess fully the functional integrity of the RPE with remote recording techniques. Nevertheless, the results of recent studies suggest that the light rise in the eye's standing potential originates at the basal membrane of the RPE [62], whereas the major component of the electroretinogram c-wave is produced by a hyperpolarization of the apical membrane of the RPE in response to a light-induced decrease in extracellular K [63]. But even more impressive than its structural elegance are the dynamic properties of the visual cells and the mechanisms available for signal generation, intercellular communication, and molecular renewal. The influx of sodium ions constitutes the receptor's dark current [64], the movement of an internal transmitter that is mobilized by light to close the channels of entry [65], the shuttling of vitamin A between the rod and the pigment epithelium to support the rhodopsin cycle [66], the shedding of disk membranes and their incorporation into phagosomes [67], the translocation of proteins and other macromolecules to the outer segment where new disks are assembled and to the receptor terminal for replenishment of its chemical messenger [68], and finally, the Ca-dependent discharge of transmitter by exocytotic release [69]. Each of these activities, as well as many others, is essential to the functional integrity of the cell. In view of this remarkable diversity and the degree of metabolic activity required to sustain normal function, it is perhaps not too surprising that the visual cell is particularly susceptible to injury and that each of its various dynamic mechanisms appears to be selectively affected by different forms of inherited night-blinding disease [70].

Lead, cadmium, and arsenic were found in all of the pigmented ocular tissues (e.g., retinal pigment epithelium/choroid, ciliary body) that we studied. The importance of melanin binding of heavy metals in pigmented ocular tissues is unclear. Melanin may confer tissue protection by acting as a filter or detoxicant for heavy metals from the adjacent neural retina and photoreceptor cells [70, 71]. Similar to the retinal pigment epithelium, the choroid plexus of the brain sequesters lead, acting as a defensive barrier to prevent entry of toxic elements into the brain [72]. Conversely, melanin binding of heavy metals throughout the life of an individual produces a local reservoir of potentially toxic elements that ultimately could reach a concentration that is destructive to the retinal pigment epithelium and adjacent retina.

Low-level lead exposure produces scotopic vision deterioration and rod and bipolar apoptotic cell death [36, 73]. In rabbits, lead poisoning causes swelling of the retinal pigment epithelium [74], which leads to degeneration of the photoreceptors [75]. Visual loss in humans after systemic lead poisoning is usually related to encephalopathy and optic neuropathy because of the toxic effect of lead on the brain and optic nerve. Recent evidence, however, indicates that lead, cadmium, and nickel can exert oxidative stress by producing reactive oxygen species that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems [71]. Oxidative stress and free radical damage are thought to play a significant role in age-related macular degeneration [76]. Arsenic affects mitochondrial enzymes and impairs tissue respiration [77], which seems to be

related to the cellular toxicity of arsenic. Arsenic can also cause a range of neurological effects, including headaches and vision problems [78]. Almost every physiological system in the human including the respiratory, immune, gastrointestinal, genitourinary, reproductive, and nervous systems has been reported to be adversely affected by prolonged exposure to high doses of arsenic [79, 80].

The toxic metals present in environment and in the food chains facilitate metal poisoning of the human body. They interfere with the normal functioning of many essential trace elements and enzyme systems. By replacing nutritional minerals in the enzymes, toxic metals cause those enzymes to become inactive. In turn, a higher dietary level of essential minerals and vitamins helps to prevent toxic metal toxicity, as well as helps to eliminate them from the body [81]. The pigmented tissues of the eye, such as the retinal pigment epithelium, choroid, iris, and ciliary body, have a high affinity for metal ions [82]. Melanin within the pigment granules binds metal ions [83]. Metal ions are bound by melanosomes according to atomic weight and volume (e.g., the percentage binding of calcium 30%, zinc 37%, lead 62%, iron 65%, and mercury 72%). Metals such as zinc, copper, calcium, manganese, molybdenum, and iron are found in ocular melanosomes, particularly within the retinal pigment epithelium [84]. Heavy metals can effectively compete for the same binding sites as other metal ions [85] and have the capacity to replace previously bound metals and alter ocular metal concentrations [86]. Once bound, heavy metals are not easily amenable to displacement [87].

Conclusion

The results of this study revealed that children having ocular problems have a different pattern of essential trace and toxic elements in their biological samples than controls/referents. However, higher levels of As, Cd, and Pb, as well as a lower level of Ca, K, Mg, and Zn, correlated well with the consequences of night blindness. The deficiency of the essential elements, which are replaced by TEs (As, Cd, and Pb), may result in abnormal physiology disorders, and, in addition to other factors, this may have a role in night blindness. This study provides some support for the hypothesis that dietary intake and inhalation of TEs (As, Cd, and Pb), most probably through diet, may increase the risk of night blindness and related disorders.

It is also necessary to provide nutritionally adequate diets via food supplements, which have enough protein, vegetables, fats, and carbohydrates. Such diets would necessarily also provide any missing minerals and vitamins. The results of this study should alert clinicians and other health professionals on the need to consider deficiencies of essential minerals as potentially contributing causes of night blindness in children.

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