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Chromium and Manganese Levels in Biological Samples of Normal and Night Blindness Children of Age Groups (3–7) and (8–12) Years

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Abstract This study was designed to compare the levels of chromium (Cr) and manganese (Mn) in scalp hair, blood, and urine of night blindness in 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 Cr and Mn 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 digests of all biological samples were analyzed for Cr and Mn by electrothermal atomic absorption spectrometry. The results indicated significantly higher levels of Cr, whilst low level of Mn in the biological samples (blood

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and scalp hair) of male and female night blindness children, compared with control subjects of both genders. These data present guidance to clinicians and other professional investigating deficiency of Mn and excessive level of Cr in biological samples (scalp hair and blood) of night blindness children.

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

Introduction

Night blindness may be an early sign of vitamin A deficiency. Such a deficiency may result from a diet low in animal foods, such as eggs, dairy products, organ meats and fish [1]. Christian et al. 2001 and Brody et al., 1999 [1, 2] showed that Vitamin A deficiency is widespread in developing countries. Human cells employ metals, such as copper, zinc, manganese, 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, chromium, cadmium, mercury, and thallium [2].

Trivalent chromium (Cr^{3+}) has been marketed to consumers for use in weight loss, increasing muscle mass and lowering serum cholesterol [3]. However, its safety has recently been questioned, especially with regard to its ability to act as a clastogen [4]. Many studies have suggested that Cr-picolinate supplements may cause renal impairment and cell toxicity when ingested in excess [5, 6]. Nevertheless, no available data were found regarding the effects of Cr on the eye.

Manganese (Mn) superoxide dismutase gene polymorphism is associated with age-related macular degeneration [7]. Microsomal epoxide hydrolase is another enzyme that may be associated with the disease. The age-related macular degeneration may have genetic risk factors against oxidative stress and/or effects of xenobiotics [7]. Manganese superoxide dismutase (MnSOD), the mitochondrial enzyme critical in regulating O_2^- levels within the cell, was the first nitrated protein to be unambiguously identified [8]. MnSOD was found to be tyrosine nitrated and inactivated during human chronic renal allograft rejection [8]. Additionally, studies demonstrated that complete inactivation of MnSOD initiated by peroxynitrite can occur independent of tyrosine nitration of residues located in the enzyme's active site. Complete inactivation requires not only nitration of critical tyrosine residues, but also tyrosine oxidation and subsequent formation of dityrosine [9, 10].

In view of the facts discussed above, it is important to determine the essential trace elemental concentrations in biological samples of humans having physiological disorders, such as myocardial infarction. Various biopsy materials such as serum, scalp hair, urine, and other body fluids may be used as bio-indicators for these purposes [11]. Blood elemental analysis provides information about what the body has recently (hours to days in some cases weeks) absorbed. Blood levels are largely independent of tissue deposition [12]. Whole blood analysis measures total element levels that circulate extracellularly (serum/plasma) as well as intracellularly (function within blood cells). Intracellular elements are those that have specific functions within circulating blood cells [13]. This homeostatic response illustrates the effective clearance mechanisms in the blood and largely explains the short-term utility of blood analysis. The importance of exploring the depot-storage capacities of various elements, particularly the toxic ones, remains a vital aspect in elemental analysis largely met by urine and hair testing [14]. Urine analysis can provide important information to the clinician that may not be readily available with blood analysis.

Levels of nutrient elements in the blood and the excreted urine are tightly controlled via metabolic, reabsorptive, and excretory mechanisms. Consequently, most urine testing is not helpful in nutritional element assessment [15, 16]. Hair analysis is a simple diagnostic technique, based on the idea that hair provides vital clues about nutritional imbalances elsewhere in the body [17, 18].

Compared with blood or urine analysis, a couple of factors such as simplicity of matrix, relatively high concentration of trace elements, easy sampling, transfer, and storage should be considered. It is also used to detect environmental toxins before overt symptoms appear because metal concentrations in hair are usually tenfold higher than in other biological material [19]. Atomic absorption spectrometric methods are frequently used for the specific determination of very low elemental concentrations in biological samples [18]. 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 [11]. The main advantage of microwave-assisted pretreatment of samples is the small amount of mineral acids required and reduced production of nitrous vapors.

The main objectives of our study were : (1) to assess the concentrations of chromium and manganese in the biological samples (scalp hair, blood, and urine) of night blindness and normal children of both genders and with ages ranging between (3–7) and (8–12) years old and, (2) to evaluate the variation of these elements in night blindness children was related with age and sex factors, and (3) 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

The analysis of elements was carried out by means of a double-beam Perkin–Elmer atomic absorption spectrometer model 700 (Perkin Elmer, Norwalk, CT, USA) equipped with a flame burner and graphite furnace HGA-400 (Perkin Elmer), a pyrocoated graphite tube with an integrated platform and an autosampler AS-800 (Perkin Elmer). The instrumental parameters are shown in Table 1. Chromium and manganese were measured under optimized operating conditions using ETAAS. Signals were measured as absorbance peaks in the flame absorption mode, where as integrated absorbance values (peak area) were determined in the graphite furnace. A Pel (PMO23, 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 Standard Solutions

Ultrapure water obtained from ELGA lab water system (Bucks, UK) was used throughout the work. Concentrated nitric acid (65%) and hydrogen peroxide (30%) from Merck (Darmstadt, Germany) were checked for possible trace metal contamination. Working standard solutions of chromium and manganese were prepared immediately prior to their use by stepwise dilution of certified standard solutions (1,000 ppm) Fluka Kamica (Bush, Switzerland), with 0.2 M HNO_3. All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, certified samples (certified reference materials (CRMs))

Table 1 Measurement conditions for electrothermal atomization AAS 700

Parameters	Chromium	Manganese
Lamp current (mA)	7.5	7.5
Wave length (nm)	357.9	279.5
Slit-width (nm)	0.7	0.2
Drying temp (°C/ramp/hold(s))	140/15/5	140/15/15
Ashing temp (°C/ramp/hold(s))	1,400/10/20	1,400/10/20
Atomization temp (°C)/ramp/hold(s))	2,500/0/5.0	2,200/0/5.0
Cleaning temp (°C)/ramp/hold(s))	2,600/1/3	2,600/1/3
Chemical modifier	$Mg(NO_3)_2$	$Mg(NO_3)_2$

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

of human hair (BCR 397, Brussels®, Belgium), Clincheck control-lyophilized® human urine (®Recipe, Munich, Germany), and Clincheck control-lyophilized® human whole blood (Recipe, Munich, Germany) were used. All glassware and plastic materials used were previously soaked for 24 h in 2 M nitric acid, washed with distilled water, finally rinsed with Milli-Q water, dried, and stored in class 100 laminar flow hoods.

Sample Collection and Pretreatment

The study was conducted on 150 children aged ranged between 3–12 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. The biochemical tests were described in Table 3. Both vitamin A and carotene were analyzed by the method of Kimble [20]. 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 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

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

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 2–3 cm above the nape of the neck. The scalp hair samples were washed as in the previous study [21]. After washing, hair samples were dried at 80°C for 6 h. Hair samples were put into separate plastic envelopes with an identification (ID) 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; 5 mL of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, *v*/*v*) were added to each flask, which were heated on a electric hot plate at 80°C, for 2–3 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. 2 mL of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, *v*/*v*) was added to each flask, 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 2–3 min for blood and urine, and 4–5 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 the 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 Cr and Mn by electrothermal 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

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	
Mass (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
Haematocrit (%)	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	
Mass (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
Haematocrit (%)	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

method was checked with those obtained from conventional wet acid digestion method [22].

Analytical results of the certified samples, Clincheck control-lyophilized® human whole blood, Clincheck control-lyophilized® human urine and Certified Human hair ®BCR 397, 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 both elements in CRM samples obtained by conventional digestion method varied between 97.8% and 99.3%, but it was time consuming. The microwave-assisted digestion method was efficient and take less than 10 min to complete the digestion of the three certified biological samples. Mean values for all the elements differed less than 1–2% from the certified values. The coefficient of variation differed less than 2% for the different elements.

Non-significant differences were observed ($p>0.05$) when comparing the values obtained by both methods (paired t test) (Table 4).

Result

The results reported in Table 5 shows that the concentrations of trace metals (chromium and manganese) were altered in all 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.

The concentrations of Mn in the scalp hair samples of control children of both age groups, 3–7 and 8–12 years, were found in the range of (2.74–4.65 μg/g) and (2.95–5.62 μg/g) while the level of Mn in male night blindness children of both age groups have in the range of 1.91–3.74 μg/g and 1.69–4.37 μg/g, the same trend was observed in females. Range of Mn levels in blood samples of referent subjects of both gender (21.9–37.3 μg/l) and (26.9–41.3 μg/l), were significantly higher as compared with the range of Mn values observed in blood samples of night blindness subjects (11.6–27.3 μg/l) and (19.1–36.8 μg/l) in males and females, respectively. The excretion of Mn was high in night blindness patients of both genders.

The elevated level of Cr was observed in scalp hair of night blindness children of both genders (Table 5). The level of Cr in blood samples of night blindness children is higher (104–130 μg/l) and (112–165 μg/l) as compared to values of Cr found in blood samples of referents subjects (79.7–100 μg/l) and (75.5–97.3 μg/l) of male and females respectively of both age groups. The excretion of Cr was high in night blindness children of both genders.

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. Metal ions

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

Elements	Conventional digestion method CDM	Microwave digestion method MWD	T value[a]	% recovery[b]	Certified values
Certified sample of whole blood (μg/L)					
Cr	1.940±0.09 (4.64)	1.98±0.11 (5.55)	6.25×10^{-7}	97.98	2.00±0.5
Mn	24.67±1.3 (5.27)	24.80±1.8 (7.26)	0.0017	99.5	25±5
Certified sample of urine (μg/L)					
Cr	14.57±0.78 (5.35)	14.31±0.93 (6.49)	8.92×10^{-8}	98.2	14.60±3.0
Mn	5.54±0.47 (8.48)	5.56±0.51 (9.17)	0.0006	98.9	5.6±1.5
Certified sample of human hair (μg/g)					
Cr	89.230±6.53 (7.32)	90.94±5.99 (6.59)	1.13×10^{-5}	98.2	91.00 ± 10[c]
Mn	11.09±0.85 (7.66)	10.88±0.95 (8.73)	0.0012	98.1	11.2±0.3

Values in parenthesis are RSD

[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 : $\frac{[MDM]}{[CDM]}\times 100$

[c]Informative value

Table 5 Trace element 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
Manganese						
Scalp Hair (μg/g)						
3–7	3.7±0.9	2.8±0.8	0.027	4.3±0.64	3.2±0.79	0.015
Range[a]	2.74–4.65	1.91–3.74		3.77–5.02	2.55–4.08	
8–12	4.3±1.3	3.5±0.8	0.031	4.4±1.2	3.63±1.1	0.022
Range[a]	2.95–5.62	1.69–4.37		3.14–5.71	2.52–4.82	
Blood (μg/l)						
3–7	29.5±7.5	19.8±7.4	0.021	31.2±5.3	19.9±5.87	0.015
Range[a]	21.9–37.3	11.6–27.3		25.9–36.5	13.6–24.7	
8–12	33.2±6.7	27.8±8.5	0.005	36.2±4.87	28.2±5.63	0.009
Range[a]	26.9–39.9	19.1–36.8		31.6–41.3	22.3–34.2	
Urine (μg/l)						
3–7	1.2±0.4	1.98±0.17	0.003	1.3±0.3	2.31±0.15	0.002
Range[a]	0.7–1.63	1.77–2.22		0.75–1.62	2.14–2.47	
8–12	1.4±0.3	2.0±0.37	0.009	1.3±0.4	2.37±0.32	0.001
Range[a]	0.87–1.75	1.63–2.47		0.84–1.74	1.93–2.73	
Chromium						
Scalp Hair (μg/g)						
3–7	3.5±0.7	6.3±1.5	0.001	3.7±1.17	6.5±0.9	0.001
Range[a]	2.7–4.3	4.7–7.9		2.54–4.9	5.5–7.3	
8–12	3.8±0.62	7.4±0.5	0.001	3.8±0.72	7.5±0.8	0.001
Range[a]	3.14–4.52	6.6–7.95		3.0–4.58	6.8–8.4	
Blood (μg/l)						
3–7	88.9±9.4	116.5±12.5	0.001	86.3±9.9	115±9.65	0.001
Range[a]	79.7–98.2	105–130		75.5–97.3	104–126	
8–12	91.6±8.2	139±23.9	0.001	86.1±7.2	136±19.7	0.001
Range[a]	81.4–100	112–165		79.2–94.6	117–159	
Urine (μg/l)						
3–7	21.6±2.7	29.5±5.3	0.001	19.5±1.75	28.7±3.87	0.001
Range[a]	18.3–24.9	26.7–32.2		17.5–22.3	24.6–32.5	
8–12	16.4 ±3.98	23.4±3.2	0.002	14.9±3.2	25.6±4.31	0.001
Range[a]	12.6–20.8	20.2–25.9		11.4–18.3	20.7–30.2	

[a]Minimum–maximum values

are integral functional components of many enzymes and transcriptional regulatory proteins.

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. 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, copper, and manganese [23].

Analyses of Cr and Mn in scalp hair, blood and urine samples of both age groups of control and NB persons indicate significant difference (Table 5). The children with a history of NB had a higher concentration of Cr, whilst lower concentrations of Mn in scalp hair, and blood samples than the normal persons of both age groups, but difference was less significant (p=0.005–0.031).

The effect of Cr exposures on health has been reviewed by Schrauzer et al. [24], Vincent [25], and Althuis [26]. American Conference of Governmental Industrial Hygienists 1999 [27] reported that Cr exists in a series of oxidation states from −2 to +6 valence, the most important stable states are 0 (elemental metal), +3 (trivalent) and +6 (hexavalent). Trivalent Cr^{3+} and hexavalent Cr^{6+} compounds are thought to be the most biologically significant. Certain compounds of Cr^{6+} appear to be carcinogenic, but insufficient evidence exists to determine whether Cr^{3+} or Cr metal can be human carcinogens [28]. Chromium Cr^{6+} is generally considered 1,000 times more toxic than Cr^{3+} [28]. Chromium Cr^{3+} is found in a wide range of foods, including egg yolks, whole-grain products, high-bran breakfast cereals, coffee, nuts, green beans, broccoli, meat and brewer's yeast. Chromium is also present in many multivitamin/mineral supplements, and there are also specific Cr-picolinate (CrP) supplements that contain 200–600 μg Cr per tablet [28]. Chromium Cr^{6+} rapidly penetrates into the red blood cells, where it binds to the β-chain of hemoglobin. Within the erythrocyte, hexavalent Cr is enzymatically reduced to Cr^{3+} [28].

Stearns et al. [29] stated that organic forms of Cr^{3+} such as Cr-picolinate and nicotinate have much higher absorption compared with chromium(III) chloride and phosphate salts. However, increased absorption of organic-complexed chromium caused toxicity due to the concentration of this metal in the tissues [30]. Because of its neutral charge and hydrophobic character allow it to readily pass through hydrophobic barriers. Ironically, once the Cr in the form of Cr-picolinate is absorbed by cells and because of its ligand composition and the resulting redox potential, the complex can be reduced readily by abundant biological reductants and generate hydroxyl radical via Haber–Weiss and Fenton cycles, leading to lipid peroxidation, mitochondrial, and DNA damage [31]. The keratocytes appeared as swollen dense mass and some of them contained vacuoles, these results were in accordance with Shrivastava et al. [32] who studied the effect Cr(III) complex on human dermal fibroblasts and demonstrated surface morphological damage evidenced by cellular blebbing and spike formation accompanied by nuclear damage which reflecting the cell death. These authors suggested that Cr^{5+} formed as a result of oxidation of Cr^{3+} by cellular oxidative enzymes in the cytotoxic response. Consequently, Cr^{3+} complex, which is oxidized to Cr^{5+}, must be considered as a potential carcinogen.

The result shows that the level of Mn in blood and scalp hair samples of night blindness patients was lower, while higher in urine samples. The functions of manganese present in the eye tissues may well be displayed at other sites in the body [33]. Manganese has something to do with certain enzymes involved in carbohydrate metabolism [34], some amino acid metabolizing pathways [35] and mucopolysaccharide synthesis [36, 37]. Manganese itself seems to be a mutagenic initiator of cancer in some tumors. However, except for the pancreas, manganese specificity for tissues or organs is little known [38]. Although manganese has been detected chemically in various parts of the eye, its significance in ocular tissues is unclear [39]. Tauber and Krause [40] found manganese in various bovine ocular tissues: conjunctiva, cornea, lens, iris, choroid, retina, and optic nerve. The retina has a high content of manganese: 31.8 μg/100 g wet tissue and 260 μg/100 g dry tissue. Serum manganese levels have been reported to decrease after prolonged

depletion [41]. Bell and Hurley [42] demonstrated that manganese deficiency in mice results in changes in the integrity of their cell membranes, swollen and irregular endoplasmic reticulum, and elongated stacked cristae of mitochondria in liver, heart, and kidney cells. A biochemical aberration caused by manganese deficiency is the diminished enzymatic activity of galactosyl transferase reactions related to chondroitin sulfate synthesis, which results in decreased polymerization. In ocular tissues, a deficiency of manganese may result in decreased contact between photoreceptor outer segments and retinal pigment epithelium. This assumption is consistent with the role of manganese in mucopolysaccharide synthesis, because acid mucopolysaccharides are present between the photoreceptor outer segments and retinal pigment epithelium [43]. Another effect of manganese deficiency is a decrease of superoxide dismutase (SOD) activity. Two kinds of SOD are known; CuZn SOD in the cytosol and MnSOD in the mitochondrial matrix. The function of SOD is thought to be associated in part with the protection of the cell from the toxic effects of free superoxide radicals [44]. MnSOD would scavenge the O_{2-} generated by mitochondrial respiratory activity. If the activity of MnSOD is depressed by a dietary deficiency, changes of membrane structures may be the result of accumulation of O_{2-} radicals in the cell with consequent peroxidation of membrane components. Lipid peroxidation increases as MnSOD activity decreases [45]. A manganese-deficient diet decreased MnSOD activity in the kidneys and hearts of Sprague–Dawley rats [46]. Photoreceptor outer segments are composed of lipid membrane accumulation, and photoreceptor inner segments contain many mitochondria. Thus, the disappearance of photoreceptor cells and mitochondrial membrane changes in the current study may be caused by depressed MnSOD activity, resulting in increased lipid peroxidation in biomembranes. In addition, protein metabolism may be disturbed [46]. Lowered protein and glycogen metabolism may prevent the renewal of photoreceptor outer segment discs. Damage of the photoreceptor cells caused by manganese deficiency was specific probably because of mitochondria- and membrane-rich special structures. The increase of mitochondria in the retinal pigment epithelium may be die result of compensation for mitochondrial dysfunction caused by manganese deficiency [46]. Karyopyknosis of photoreceptor cell nuclei occurred after 1 year of manganese deficiency and may be related to lowered DNA polymerase activity resulting from manganese deficiency. Although manganese is a mysterious trace element in living organisms, because of the rarity of its deficiency in humans, it is an important micronutrient in the maintenance of photoreceptor cells [46].

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 and helps to eliminate them from the body [47]. The pigmented tissues of the eye, such as the retinal pigment epithelium, choroid, iris, and ciliary body, have a high affinity for metal ions [48]. Melanin within the pigment granules binds metal ions [49]. 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 [50, 51]. Heavy metals can effectively compete for the same binding sites as other metal ions [53] and have the capacity to replace previously bound metals and alter ocular metal concentrations [52, 53]. Once bound, heavy metals are not easily amenable to displacement [54].

Conclusion

The results of this study revealed that the night blindness children disturbed essential TEs (Cr and Mn) balance in biological samples (blood, scalp hair, and urine) as compared with referents. The results of the present study show that night blindness children tend to exhibit higher hair and blood levels of Cr, whilst lower level of Mn. 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 metals-related illness in individuals. Children are particularly susceptible to essential elemental deficiency due to their increased these minerals needs for rapid growth and the relatively low essential minerals (iron and zinc contents) 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 professional investigating deficiency of essential trace metals and excessive level of toxic metals in biological samples of healthy and night blindness children.

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