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Assessment of Minerals in Obesity-related Diseases in the Chandigarh (India) Population

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Abstract Excessive Zn but normal Cu and Mg in the staple food consumed by the people of Chandigarh (Union territory and capital of Punjab and Haryana States of India) has been considered to be the major risk factor for the prevalence of obesity (33.15%) and obesity-related diseases in this region. Therefore, in the present investigations, in obesity-related diseases, the status of these minerals was estimated in their tissues, including hair, nails, and blood serum and urine, and compared with those of normal subjects. They were grouped as: normal subjects in control Group A, middle-aged diabetics in Group D_M, older diabetics in Group D_O, and diabetics with osteoarthritis in Group D+OA, osteoarthritis in Group OA and rheumatoid arthritis in Group RA, respectively. The results evaluated in the order as: hair Zn, group D+OA>D_M>OA>A (control)>RA>D_O ($p<0.001$); hair Cu, group A (control)>D_M>OA> D+OA>D_O>RA ($p<0.001$); hair Mg, group A (control)>D_M>OA>D+OA>RA>D_O ($p<$ 0.001, 0.01); hair Mn, group A (control)>RA>OA>D-OA>D_M>D_O ($p<0.001$); nail Zn, group D_M>D+OA>OA>A (control)>RA>D_O ($p<0.001$, 0.05); nail Cu, group A (control)> OA>D_M>D+OA>RA>D_O ($p<0.001$); nail Mg, group A (control)>OA>D_M>D_O>D+OA>RA ($p<0.001$); nail Mn, group A (control) >RA>OA>D+OA>D_M>D_O ($p<0.01$); urine Zn, group D_O>D_M>D+OA>A (control)>RA>OA ($p<0.01$); urine Cu, group RA>D+ OA>D_O>OA> D_M>A (control) ($p<0.001$); urine Mg, group RA>OA>D+OA>D_O>D_M>A (control; $p<$ 0.001); urine Mn, group D_O>D_M>OA>D+OA>RA>A (control; $p<0.001$), respectively. The analysis of the mineral status in serum of diabetics further showed their highly significant rise from lower mean age subgroup to higher mean age subgroup than their control counter parts ($p<0.001$, 0.01, and 0.05) with coincident deficiencies of Cu, Mg, and Mn in their tissues. This study would be helpful considering the status of minerals in these obesity-related diseases depending on the choice of the food consumed to improve the quality of life and prognosis for the diseases.

Keywords Minerals (Zn, Cu, Mg and Mn) · Obesity · Diabetes · Osteoarthritis · Rheumatoid arthritis · Osteoarthritis with diabetes

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Introduction

Obesity epidemic is mainly responsible for the rising prevalence of metabolic syndrome-X and cardiovascular diseases [1] resulting in increased mortality especially if obesity begins at an early age in adolescents [2]. Obesity strongly correlates with traditional risk factors, such as diabetes, hypertension, and hyperlipidemia. When women aged 30–35 years were monitored for 14 years, the additional risk of developing non-insulin-dependent diabetes mellitus (NIDDM) for those who were obese was over 40 times greater than for women who remained slim [3]. Overweight is associated with a two to six-fold increase in risk of developing hypertension [4]. Overweight individuals are also further at increased risk of developing joint diseases leading to osteoarthritis (OA) and rheumatoid arthritis (RA) especially in the women [5–11].

The underline event in the obesity is the inflammation when it was discovered that inflammatory cytokines are overproduced in the adipose tissue and muscle tissues of obese humans due to overproduction of reactive oxygen species (ROS) [12–17]. Available data strongly suggest that inflammation is a primary cause of obesity-linked diseases such as NIDDM, hyperglycemia, hyperlipidemia, osteoarthritis, and rheumatoid arthritis rather than merely a consequence [18].

The prevalence of overweight/obesity in the Chandigarh population (Union territory of Panjab and Haryana of India) was reported as 33.15% (42.1% in women and 20.9% in men), and this prevalence of obesity has nearly become double among the urban population of Chandigarh in the last 30 years. The high prevalence of obesity in this region has led to an increase in hypertension as 82.5% among overweight elderly in comparison to 45.87% non-overweight elderly subjects [19]. It seems that dietary factors and activity patterns are more involved than any other factor in the etiology of the higher prevalence of hypertension in this region.

Many theories have been proposed to explain the mechanism involved in the devolvement of the obesity. Primary factors are dietary factors, and it was reported in our previous studies that the diets used in this region is rich in Zn content that has been reported to induce obesity [20, 21]. Kang [22] observed that the amount of fat deposition in tissues of mice depends on the amount of Zn present in diet, suggesting the importance of Zn/fat ratio as an essential criterion in the etiology of obesity. The proposed potential mechanisms for the development of obesity due to high Zn intake in the experimental animals have been already suggested [23, 24]. A higher intake of Zn results in an increase in subcutaneous fat in the animals and humans, and a possible factor favoring obesity has been suggested by many [25–28]. Leptin, a product of ob gene, is expressed mainly in adipose tissue and participates in diabetes and hypertension in obese individuals [29–35]. Leptin levels were directly correlated with the higher Zn supplementation in rats [35].

In the recent years, further, a number of studies have investigated the possible role of Zn, Cu, Mg, and Mn in the obesity, diabetes, osteoarthritis, and rheumatoid arthritis [36–42]. Higher Zn and Cu levels in the urine of diabetic patients were reported [43–46]. Diabetic humans have been reported to have hypo- [47], normo- [48–51], and hyper-zincemia [44, 52, 53] as reported in obese [21]. The role of Zn and Cu in chronic inflammatory disease is of interest because they are co-factors of important enzymes involved in collagen and bone metabolism [54–57] and antioxidant protection [58]. Patients with rheumatoid arthritis were also found to have elevated urinary Cu excretion rates, but the Zn levels in serum and urine did not differ significantly from control [59]. Serum Zn and Cu levels were observed to decrease more in RA than OA [60]. Serum Mg and Mn were found to be non-significantly lower in RA than the control, but higher Cu and lower Zn were found in RA than control [61].

In the previous epidemiological studies in this region, higher Zn levels were observed in nails, hairs, and urine of obese and in the patients of diabetics and myocardial infarction with low Cu status than the normal subjects indicating Cu deficiency in these patients. This greater bioavailability of Zn in adults over a period of years as reflected in our previous study on Chandigarh population is significant enough to reflect the changes in body weight leading to obesity [21, 28, 62–68].

The rising prevalence of obesity and obesity-related disease during the past two decades in this region, therefore, may not essentially be due to the change in lifestyle but may also be associated with the excessive Zn intake through food chain [21]. To elucidate further the link between excessive Zn in diet and diabetes mellitus and joint diseases in the Chandigarh population, investigations were carried out on these patients, and the results of this study are reported in this paper.

Materials and Methods

Present study was conducted on the population residing in Chandigarh. It is the first well-planned modern city of India, situated at the foot of the Shivalik range of Himalayas in the Northwest India. Coordinates of Chandigarh (North India) are latitude, 30°45′0″N/30.75°, longitude, 76°46′48″E/76.78°, and altitude, 304–365 m above MSL and area of 114 km^2. It is bounded on the north and west by Punjab State and on the east and south by Haryana State; therefore, it serves as the capital of these two states, and the triangle of these three cities is collectively called as the Chandigarh Tricity. The surrounding districts are Mohali and Ropar in Punjab and Panchkula in Haryana. Other surrounding areas are Derabassi, Kharar, Lalru, Ambala, Rajpura, and Patiala (all within 70 km range).

Subjects In the present investigations, the study was conducted on the Chandigarh population. About 950 subjects on the basis of the type of the disease were taken and grouped as normal subjects in Group A (control), patients of diabetes with middle age in Group D_M, older diabetics in Group D_O, diabetics with osteoarthritis in Group D+OA, osteoarthritis patients in Group OA, and rheumatoid arthritis patients in Group RA matched

Table 1 Mean Age Group, Body Weight, Type, and Time Duration of the Disease in Groups DM, DO, D+OA, OA, and RA and Normal Subjects in Group A in the Chandigarh Population

Groups	Type of Disease	Mean Age (Years)	Body Weight (Kg)	Time Duration of the Disease (Years)
Group A	Control subjects	53.30±1.43	62.30±1.02	–
Group D_M	Middle age diabetics	51.90±1.62	75.30±3.70	10.52±2.53
Group D_O	Old age diabetics	73.40±2.30	53.90±2.73	23.53±3.62
Group D+OA	Diabetic patients* with osteoarthritis[&]	54.90±2.23	80.35±1.34	11.64±3.56*6.25±2.12[&]
Group OA	Osteoarthritis patients	54.20±1.58	77.50±2.44	7.52±1.35
Group RA	Rheumatoid arthritis patients	52.90±1.90	65.10±0.71	4.26±2.51

Values are mean±SEM, $n=100$ (each).

* Time duration of diabetes in diabetic patients (diabetes onset, as 11.64 years).

[&] Time duration for the onset of osteoarthritis in the same patients (6.25 years) having diabetes in advance.

with number and age (Table 1, except Group D_o including older diabetic patients). Mean age and body weights were calculated and measured in each group with duration of the disease (Table 1). Diabetic patients of different mean age subgroups SG-I to IV of Group B and control subjects of different mean age subgroups SG-I to V of control Group A were taken, and the study was done by taking their blood samples (Table 2, only in diabetic patients).

Procurement of Requisite Details of the Subject The personal and medical history along with relevant details of the subjects were recorded for the present study through a questionnaire. The information required to be filled in the proforma included patients with the type of disease, sex, age, samples taken (blood, urine, hair, and nails), place of residence, occupation, and type of eating habits. Regarding their eating habits, most of them were vegetarians (approximately 80%), and only few were occasional meat eaters (20%) consuming not more than 200 g of fresh chicken or goat meat/week. All of them consumed 250 ml milk/day either full fat milk or skimmed milk. Intake of meat in occasional meat eaters was not significant enough; therefore, the data of vegetarians and occasional meat eaters were pooled for the present investigations. Samples were collected from the Orthopediatric clinic, Sector-16-D, Chandigarh, under the guidance of Dr. H. C. Gupta (Orthopedics and General Surgeon, Former head of the Department of Surgery and Orthopedics, General hospital, Sector-16-D, Chandigarh) and of diabetic patients from Tarun Clinic, Sector-46C, Chandigarh.

Diagnosis of the Disease Diabetic patients were diagnosed by measuring the serum glucose concentrations (serum glucose>250 mg/dl). Osteoarthritis patients were taken having the knee osteoarthritis and rheumatoid arthritis patients as having at least three of the following: morning stiffness for more than 45 min, five swollen joints, five tender joints, and erythrocyte sedimentation rate more than 45 mm/h [69].

Sampling of Blood and Urine The fasting blood samples (10 ml) were taken from each subject from subgroup SG-I to V of control Group A and diabetics from SG-I to V of Group B using a plastic syringe fitted with stainless steel needle (Table 2). The blood was collected into a metal-free plastic tube, allowed to clot, and then centrifuged at 2500 rpm for 15 min. The blood serum was separated and transferred into the metal-free plastic vials. The morning urine samples (spot urine) were collected in the sterilized vials. The samples were collected from each healthy subject and from the diseased subject (from Groups A, D_M, D_O, D+OA, OA, and RA, Table 1) in the collection vials after every 3 days for three times and stored in the vials (using conc. HNO_3). The timing for the spot urine sample was standardized. The blood serum samples were stored in the plastic vials at −20°C and urine samples at 2–4°C for further analysis. However, long storage was avoided in the present study.

Table 2 Grouping of Normal Subjects and Diabetic Patients on the Bases of Different Mean Age Subgroups (SG)-I, II, III, IV, and V (in years) in the Control Group A and Diabetic Patients in Group B in the Chandigarh Population

Groups	SG-I	SG-II	SG-III	SG-IV	SG-V
Group A (control)	35.11± 0.52	44.74±0.39	53.30±1.43	65.18±0.42	75.46±0.46
Group B	36.06±0.47	46.93±0.43	51.90±1.62	64.13±0.66	73.40±2.30

Values are mean±SEM, n=50 (each).

Collection of Hair and Nail Samples For the collection of hair and nail samples, the diseased patients in Groups D_M, D_O, D+OA, OA, RA, and control subjects in Group A (Table 1) were asked to wash their hands and hairs thoroughly with distilled water and medicated soap (detole) devoid of any metal contamination, followed by drying with clean towel or tissue paper to remove any external contamination. Then, with the help of clean stainless steel scissors, fingernails and hairs from the neck region were collected in the plastic polythene bags.

Washings The hair and nail samples were scrapped and cut with stainless steel scissors, and both were cleaned of dust particles with nonionic detergent Triton X-100 following the standardized washing procedures [70]. Subsequently, the hair and nail samples were soaked in acetone to remove external contamination, then in alcohol, rinse five times with deionized water, dried in an oven, and stored in desiccators.

Analysis of Mineral Status in Serum, Urine, Hair, and Nails of Control and Diseased Subjects using Atomic Absorption Spectrophotometer Before subsampling for analysis, the samples of blood serum and urine were shaken vigorously for 1 min to ensure a homogeneous suspension. Samples of blood serum (1 ml), urine (pooled=1 ml), hair (50 mg), and nails (50 mg) were digested separately in 3:1 nitric acid and perchloric acid in triplicates. The ash formed was dissolved in 6 ml of 10 mM nitric acid and filtered through ash-free filter paper before analysis. They were analyzed for Zn, Cu, Mg, and Mn levels on the atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad, AAS 4139) using hollow cathode lamps (213.9, 324.8, 285.2, and 279.5 nm for Zn, Cu, Mg, and Mn, respectively) in triplicates with dilutions. Standards for Zn, Cu, Mg, and Mn (Sigma) were prepared in triple distilled deionized water.

Statistical Analysis The data was subjected to statistical analysis applying Student's *t* test and analysis of variance (ANOVA). Results were expressed as mean±SEM at the significance level $p<0.001$ (highly significant), 0.01 (significant), and 0.05 (almost significant) in the tabular form and graphical forms, respectively.

Results

Mineral Concentrations in the Hairs

Hair Zn Concentrations The data on hair mineral status revealed a highly significant increase and decrease in hair Zn concentrations in the diseased groups than that of control Group A (Table 3). Significant elevations in hair Zn concentrations was evaluated as maximum in Group D+OA, followed by Group D_M, and Group OA than that of the control Group A (Table 3, $p<0.001$, 0.05). However, a highly significant reduction in hair Zn was recorded in Group RA and Group D_O (least concentrations) than that of the control Group A [Table 3, $p<0.001$; order of decrease, Group D+OA>D_M>OA>A (control)>RA>D_O (minimum); Fig. 1a].

Hair Cu Concentrations The data on hair Cu concentrations shows a highly significant decrease in hair Cu concentrations in all the groups than that of the control Group A (Table 3). Highly significant reductions were evaluated as maximum in Group RA, followed by Group

Table 3 Mean Zn, Cu, Mg, and Mn Concentrations (μg/g) in the Hair of Control [a]Group A and Diseased Patients in [a]Groups D_M, [a]D_O, [a]D+OA, [a]OA, and [a]RA in the Chandigarh population

Parameters	Hair Zn	Hair Cu	Hair Mg	Hair Mn
[a]Group A (control)	54.60±1.81	295.66±24.69	34.85±1.05	91.28±7.91
[a]Group D_M	62.58±2.73*	163.70±8.44*	27.67±0.59**	33.98±0.46*
[a]Group D_O	14.04±0.44*	105.18±4.95*	13.02±1.79*	27.30±2.07*
[a]Group D+OA	71.96±4.81*	125.50±3.82*	22.52±1.61*	47.96±3.47*
[a]Group OA	59.61±3.14***	153.63±5.49*	25.24±3.43*	53.91±3.81*
[a]Group RA	24.72±0.72*	101.52±0.54*	15.90±0.48*	59.64±1.33*

Values of Groups D_M, D_O, D+OA, OA, and RA were compared with control Group A. Values are mean± SEM, $n=100$ (each).

[a] [Group A, control; Group D_M, middle-aged diabetic patients; Group D_O, old-aged diabetic patients; Group D+OA, diabetic patients with osteoarthritis; Group OA, osteoarthritis patients; Group RA, rheumatoid arthritis patients].

*$p<0.001$.

**$p<0.01$.

***p<0.05.

D_O, D+OA, OA, and least in Group D_M than that of the control Group A [Table 3, $p<0.001$; order of decrease, Group A (control)>D_M>OA>D+OA>D_O>RA (maximum); Fig. 1b].

Hair Mg Concentrations The status of hair Mg revealed a significant decrease in Mg concentrations in Group D_M, followed by Group OA, D+OA, RA, and maximum in Group D_O than that of control Group A [Table 3, $p<0.001$ and 0.01; order of decrease, Group A (control)>D_M>OA>D+OA>RA>D_O (maximum); Fig. 1c].

Hair Mn Concentrations Hair Mn concentrations were also observed to decrease in all the groups than that of control group (Table 3). Highly significant reductions were evaluated as minimum in Group RA, followed by Group OA, D+OA, D_M, and maximum in Group D_O than that of control Group A [Table 3, $p<0.001$; order of decrease, Group A (control)> RA>OA>D+OA>D_M>D_O (maximum); Fig. 1d].

Mineral Concentrations in the Nails

Nail Zn Concentrations The status on nail Zn concentrations showed increase and decreases in Zn concentrations. Significant elevations were evaluated as maximum in Group D_M, followed by Group D+OA and OA than that of control Group A (Table 4, $p<$ 0.001 and 0.05). Significant reduction in nail Zn concentrations was recorded in Group RA, followed by Group D_O (least) than that of control Group A [Table 4, $p<0.001$ and 0.05; order of decrease, Group D_M>D+OA>OA>A (control)>RA>D_O (maximum); Fig. 2a].

Nail Cu Concentrations A highly significant reductions in nail Cu concentrations were evaluated in all the decreased groups than that of control Group A (Table 4). The reduction in nail Cu was evaluated as minimum in Group OA, followed by Group D_M, D+OA, and RA and maximum in Group D_O [Table 4, $p<0.001$; order of decrease, Group A (control)> OA>D_M>D+OA>RA>D_O (maximum); Fig. 2b].

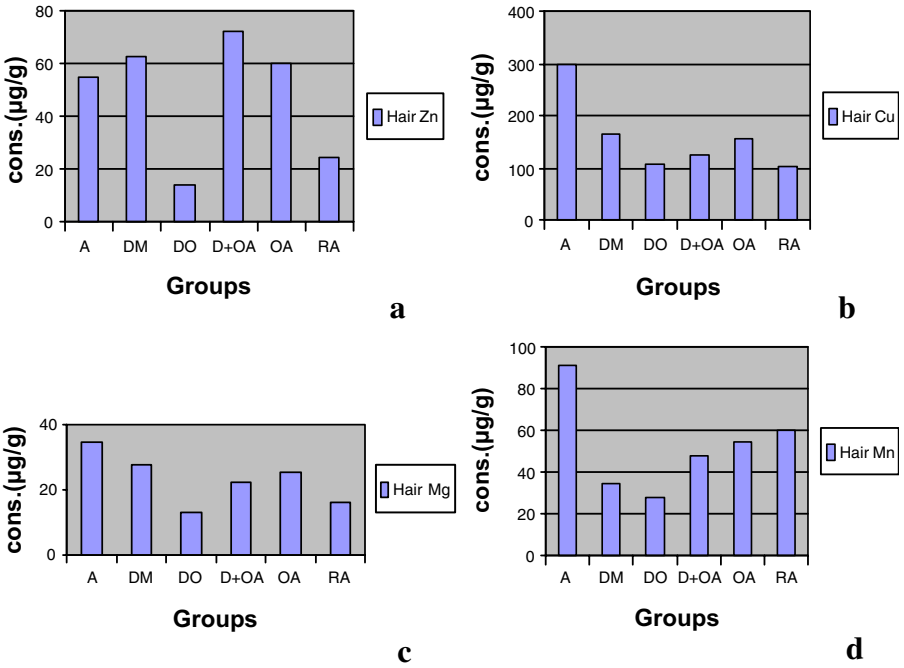

Fig. 1 Status of minerals (Zn, Cu, Mg and Mn) in the hair in Group A (control), normal subjects; Group D_M, middle-aged diabetics; Group D_O, old-age diabetics; Group D+OA, diabetics with osteoarthritis; Group OA, osteoarthritis and Group RA, rheumatoid arthritis patients. Order of decrease in hair mineral status: **a** Hair Zn, Group D+OA>D_M>OA>A (control)>RA>D_O; **b** Hair Cu, Group A (control)>D_M>OA>D+OA>D_O>RA; **c** Hair Mg, Group A (control)>D_M>OA>D+OA>RA>D_O; **d** Hair Mn, Group A (control)>RA>OA>D+OA>D_M>D_O

Nail Mg Concentrations A highly significant decrease in nail Mg concentrations were reported in all the diseased groups than that of the control group (Table 4). The reductions were evaluated as minimum in Group OA, followed by D_M, D_O, and D+OA and maximum in Group RA than that of control Group A [Table 4, $p<0.001$; order of decrease, Group A (control)>RA>D_M>D_O>D+OA>RA (maximum); Fig. 2c].

Nail Mn Concentrations Highly significant reduction in nail Mn concentrations were reported in all the groups than that of control group (Table 4). The reductions were evaluated as minimum in Group RA, followed by Group OA, D+OA, and D_M and maximum in Group D_O [Table 4, $p<0.001$; order of decrease, Group A (control)> RA>OA>D+OA>D_M>D_O (maximum); Fig. 2d].

Mineral Levels in the Urine

Urine Zn Levels Both the elevation and reductions in urine Zn levels was reported in all the diseased groups than that of control group (Table 5). Highly significant elevations in urine Zn were evaluated as maximum in Group D_O, followed by Group D_M and D+OA than that of the control Group A (Table 5, $p<0.001$). But a significant and non-significant reductions

Table 4 Mean Zn, Cu, Mg, and Mn Concentrations (μg/g) in Nails of [a]Control Group A and Diseased Patients in [a]Groups D_M, [a]D_O, [a]D+OA, [a]OA, and [a]RA in the Chandigarh Population

Parameters	Nails Zn	Nails Cu	Nails Mg	Nails Mn
[a]Group A (control)	32.27±8.97	480.16±21.76	63.89±3.59	152.19±7.08
[a]Group D_M	98.97±5.44*	321.91±0.46*	29.39±1.25*	41.86±1.83*
[a]Group D_O	19.03±0.85*	155.35±4.36*	27.92±0.51*	37.47±1.24*
[a]Group D+OA	90.71±6.77*	316.92±12.81*	20.93±2.00*	65.80±5.70*
[a]Group OA	60.35±3.82*	323.28±18.80*	50.75±2.24**	106.15±5.33*
[a]Group RA	29.97±0.45***	292.42±11.80*	16.66±1.22*	123.90±2.16*

Values are mean±SEM, $n=100$ (each). Values of group D_M, D_O, D+OA, OA, and RA were compared with control group A.

[a] [Group A, control; Group D_M, middle-aged diabetic patients; Group D_O, old-aged diabetic patients; Group D+OA, diabetic patients with osteoarthritis; Group OA, osteoarthritis patients; Group RA, rheumatoid arthritis patients].

*$p<0.001$.

**$p<0.01$.

***<0.05.

were evaluated in Groups OA and RA than that of control Group A (Table 5, $p<0.01$; order of decrease, Group D_O>D_M>D+OA>A (control)>RA>OA (least); Fig. 3a].

Urine Cu Levels Highly significant elevations in urine Cu levels were reported in all the diseased groups than that of the control group (Table 5). The elevations in urine Cu was evaluated as maximum in Group RA, followed by Groups D+OA, D_O, and OA and least in Group D_M than that of control Group A [Table 5, $p<0.001$; order of decrease, Group RA> D+OA>D_O>OA>D_M>A (control); Fig. 3b].

Urine Mg Levels Highly significant elevations in urine Mg levels were reported in all the diseased groups than that of control group (Table 5). The elevations were evaluated as maximum in Group RA, followed by Groups OA, D+OA, and D_O and least in Group D_M than that of control Group A [Table 5, $p<0.001$; order of decrease, Group RA>OA>D+ OA>D_O>D_M>A (control); Fig. 3c].

Urine Mn Levels Urine Mn levels were also reported to increase significantly in all the diseased groups than that of control group (Table 5). The elevations in urine Mn levels was evaluated as maximum in Group D_O, followed by Group D_M, OA, and D+OA and least in Group RA than that of control Group A [Table 5, $p<0.001$; order of decrease, Group D_O>D_M>OA>D+OA>RA>A (control); Fig. 3d].

Mineral Status in the Serum of Diabetic Patients

Serum Zn, Cu, Mg, and Mn Levels A highly significant rise in serum Zn, Cu, Mg, and Mn concentrations were evaluated in the diabetics with subgroup from SG-I to IV in Group B than their control counterparts as SG-I to IV in control Group A (Table 6). The elevations were evaluated as values rising from SG-I to IV of Group B diabetic patients, and thereafter, the concentrations decreased in SG-V but still were high than control SG-V in Group A control, respectively (Table 6, $p<0.001$, 0.01, and 0.05).

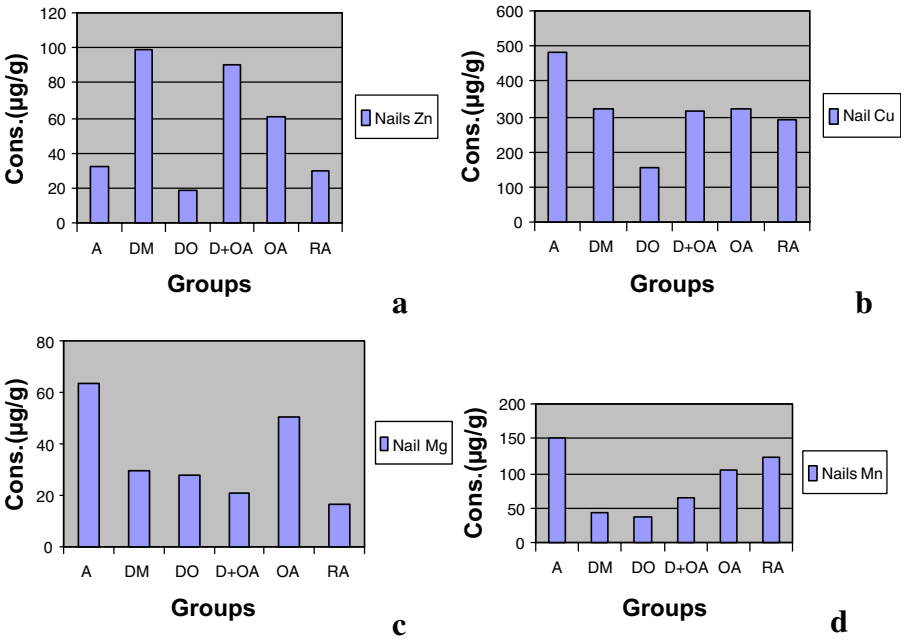

Fig. 2 Status of minerals (Zn, Cu, Mg, and Mn) in the nails in Group A (control), normal subjects; Group D_M, middle-aged diabetics; Group D_O, old-age diabetics; Group D+OA, diabetics with osteoarthritis; Group OA, osteoarthritis; and Group RA, rheumatoid arthritis patients. Order of decrease in nail mineral status: **a** Nail Zn, Group D_M>D+OA>OA>A (control)>RA>D_O; **b** Nail Cu, Group A (control)>OA>D_M>D+OA>RA>D_O; **c** Nail Mg, Group A (control)>RA>D_M>D_O>D+OA>RA; **d** Nail Mn, Group A (control)>RA>OA>D+OA>D_M>D_O

Discussion

The data showed a highly significant rise of Zn concentrations in hair and nails of Group D_M (middle aged diabetics) and Group D+OA (diabetics with osteoarthritis) patients, followed by Group OA (osteoarthritis), but a significant decrease in Zn concentrations was observed in Group D_O (older diabetics) and Group RA patients (rheumatoid arthritis) than that of control Group A (Tables 3 and 4; Figs. 1a and 2a). The more rise in hair and nail Zn levels in Group DM (middle-aged diabetics) and Group D+OA (diabetics with osteoarthritis) was observed, as they are genetically predisposed to absorption and retention of more Zn in the body [71].

These results are significant in terms of Chandigarh population who are predominantly vegetarian. The recent survey on trace metal status of different vegetables in State of Punjab around Chandigarh revealed that the concentrations of Cu and Mg are within prescribed limit, but that of Zn concentration is significantly higher (40 mg/kg or more in above ground vegetables and 120 mg/kg or above in underground vegetables) in underground-water-irrigated vegetables. The reason for its higher concentration in vegetables has been linked to different inputs used in the fields by farmers during the growth of vegetables [20]. Recent data on Zn levels estimated in cereals and other food stuffs was also found to be high. The higher level of Zn in tissues of overweight and obese women than those with body mass index of 25 kg/m^2 observed in Indians, more so in Punjabis [62, 63], is on account of high Zn in food [72] due to the liberal use of Zn as $ZnSO_4$ as a micronutrient in

Table 5 Mean Zn, Cu, Mg, and Mn levels (mg/dl) in the Urine of Control [a]Group A and Diseased Patients in [a]Groups D_M, [a]D_O, [a]D+OA, [a]OA, and [a]RA in the Chandigarh Population

Parameters	Urine Zn	Urine Cu	Urine Mg	Urine Mn
[a]Group A (control)	0.29±0.01	3.74±0.13	0.59±0.05	1.37±0.06
[a]Group D_M	0.57±0.04*	5.01±0.14*	0.89±0.03*	2.68±0.09*
[a]Group D_O	0.74±0.03*	5.61±0.12*	0.94±0.06*	3.01±0.12*
[a]Group D+OA	0.40±0.01*	6.18±0.14*	1.27±0.09*	2.14±0.11*
[a]Group OA	0.24±0.01**	5.09±0.17*	1.58±0.05*	2.21±0.14*
[a]Group RA	0.28±0.01[b]	7.29±0.30*	1.84±0.04*	1.82±0.08*

Values are mean±SEM, $n=100$ (each). Values of group D_M, D_O, D+OA, OA, and RA were compared with control group A.

[a] [Group A, control; Group D_M, middle-aged diabetic patients; Group D_O, old-aged diabetic patients; Group D+OA, diabetic patients with osteoarthritis; Group OA, osteoarthritis patients; Group RA, rheumatoid arthritis patients].

[b] Nonsignificant.

*$p<0.001$.

**$p<0.01$.

***<0.05.

agricultural practices employed by the farmers and the low amount of Zn-binding factor such as phytates and fibers in their diet. It is further supported by epidemiological studies where in spite of hyperzincuria, the diabetic patients have not appeared to be Zn deficient [73]. This is further evident by elevated Zn concentration in serum, urine, and mononuclear cell in diabetic patients after high dose Zn supplementation, which has a greater ramification in diabetes [71]. The concentration of metals in blood plasma at a given time represents a total of bound, exchangeable, and catabolic components of metals. They, therefore, lead to confusing results and predictions, more so in case of chronic diseases where urinary losses are high. The metal concentrations in tissues are more dependable indicator of assessing metal deficiencies/excesses, as their concentrations are maintained through dynamic equilibrium between tissue metals and exchangeable metal components of the blood plasma. The metals concentrations in tissues therefore were estimated in the present investigations as better indicators of mineral status in the body than that of blood plasma.

The loss of glucose in urine and hyperzincuria increased with increase in time duration and amount of Zn present in diet. Urinary Zn excretion has also been correlated with the degree of either glucosuria [45, 74] or albuminuria [75, 76]. Cunningham et al. reported that increased Zn mass action increases more Zn levels in the body and urine of both diabetic and normal individuals that coincided well with the present investigations as more increased Zn concentrations were evaluated in the urine of diabetic patients and even with osteoarthritis (Table 4; Fig. 3a), which may have link to increased Zn intake through food stuffs in this region (Chandigarh population), as increased Zn concentrations were observed in the vegetables consumed by this population [20]. Chausmer [42] has postulated that hyperglycemia interferes with the active transport of Zn back into the renal tubular cell and results in hyperzincuria. Further, previous studies have also suggested elevated excretions of Zn in urine of diabetic patients as observed in the present investigations [43, 45].

Cu concentrations in the hair and nails were evaluated to decrease significantly in all the groups than that of control Group A (Tables 3 and 4; Figs. 1b and 2b). Similarly, Mg and Mn concentrations in hair and nails were also reduced significantly in all the groups than that of control Group A (Tables 3 and 4; Figs. 1c,d and 2c,d). Use of excessive Zn in free-

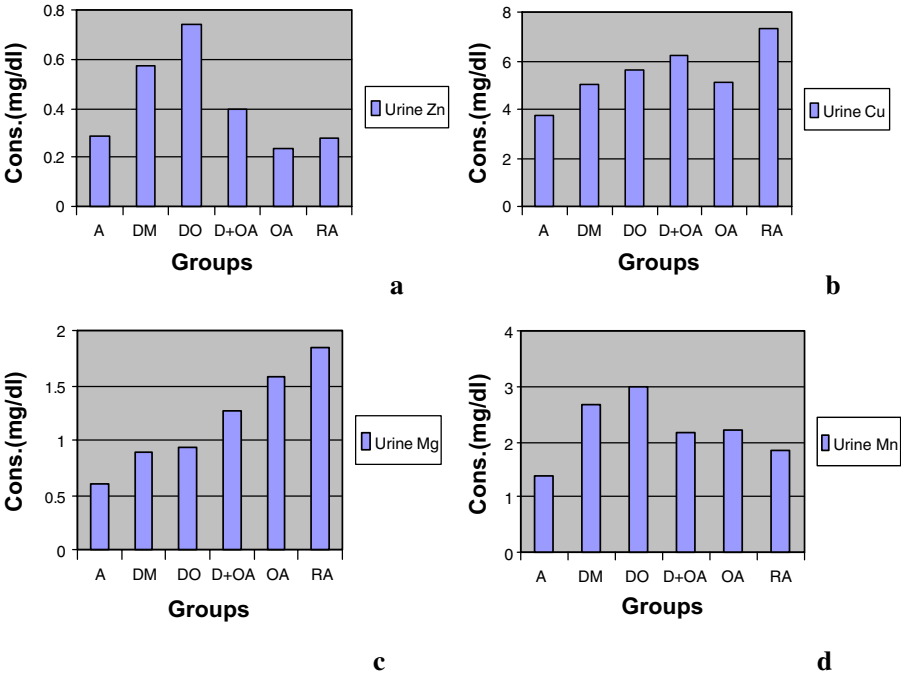

Fig. 3 Status of minerals (Zn, Cu, Mg and Mn) in the urine of Group A (control), normal subjects; Group D_M, middle-aged diabetics; Group D_O, old-age diabetics; Group D+OA, diabetics with osteoarthritis; Group OA, osteoarthritis and Group RA, rheumatoid arthritis patients. Order of decrease in urine mineral status: **a** Urine Zn, Group D_O>D_M>D+OA>A (control)>RA>OA; **b** Urine Cu, Group RA>D+OA>D_O>OA>D_M>A (control); **c** Urine Mg, Group RA>OA>D+OA>D_O>D_M>A (control); **d** Urine Mn, Group D_O>D_M>OA>D+OA>RA>A (control)

living population has been discouraged because it results in Cu-deficiency. Zn and Cu are essential elements that exhibit important interactions and possibly inhibition of each other's transport and bioavailability [77]. The excess Zn in the vegetable food stuffs (vegetarians) and meat food stuffs (non-vegetarians) consumed by the population in this region made them Cu deficient, as decreased Cu concentrations in hair and nails were observed in the all the diseased groups in the present investigations [65–68]. Further, increased Zn concentrations in the hair and nails in Group D_M (middle-aged diabetics) and Group D+OA (diabetics with osteoarthritis) also coincide well with respect to their increased body weights (Tables 1, 3, 4; Figs. 1a and 2a) because it is already reported in ours and other previous epidemiological studies where a positive relationship between Zn concentrations in hair with obesity, diabetics, and myocardial infarction but an inverse correlation with copper concentrations in hard tissues, such as hair and nails, were observed that support the present findings [21, 62–64].

Increase and decrease in hair and nail Zn levels in Groups OA and RA patients were reported due to the involvement of more cytokines, as RA is a chronic inflammatory disease. Higher serum IL_1, IL-6, and TNFα were found to be elevated in RA patients [78] and is involved in the alteration of trace metals metabolism. Synthesis of metallothioneins was observed to be induced by IL-1 in the liver that causes marked accumulation of Zn in the liver and inflamed joints [79], and consequently, due to more inflammatory action in RA, more reduction in hair and nails Zn concentrations were evaluated in Group RA than

Table 6 Mean Serum Zn, Cu, Mg, and Mn Levels (mg/dl) in the Different Mean Age Subgroup [a]SG-I–V in Control [a]Group A and [b]SG-I to V (with Different Mean Age, in Years) in Diabetic Patients in [b]Group B in the Chandigarh Population

Groups	SG-I	SG-II	SG-II	SG-IV	SG-V	Parameters
[a]Group A	0.23±0.01	0.33±0.02	0.41±0.02	0.40±0.02	0.34±0.02	Serum Zn
[b]Group B	0.51±0.03[a]	0.61±0.06[a]	0.67±0.17[b]	0.94±0.12[a]	0.46±0.02[b]	
[a]Group A	0.06±0.01	0.07±0.01	0.06±0.01	0.06±0.01	0.06±0.01	Serum Cu
[b]Group B	0.33±0.04[a]	0.66±0.14[a]	0.75±0.14[a]	1.61±0.25[a]	0.43±0.04[a]	
[a]Group A	0.24±0.02	0.35±0.03	0.40±0.05	0.42±0.04	0.35±0.011	Serum Mg
[b]Group B	0.59±0.04[b]	0.99±0.14[a]	1.38±0.27[a]	1.62±0.10[a]	0.51±0.06[b]	
[a]Group A	2.00±0.05	2.42±0.13	2.37±0.15	2.26±0.14	1.98±0.05	Serum Mn
[b]Group B	2.80±0.09[a]	3.16±0.10[a]	3.37±0.18[a]	4.61±0.31[a]	2.09±0.13[c]	

Values of [b] SG-I, II, III, IV, and V in [b] Group B were compared with their control counter parts ([a] SG-I to V) in control [a] Group A, respectively. Values are mean±SEM, $n=50$ (each).

[a] [SG-I, mean age: 35.11±0.52 years; SG-II, mean age: 44.74±0.39; SG-III, mean age: 53.30±1.43; SG-IV, mean age: 65.18±0.42; SG-V, mean age: 75.46±0.46 years in Group A[a] control].

[b] [SG-I,,mean age: 36.06±0.47 years; SG-II, mean age: 46.93±0.43; SG-III, mean age: 51.90±1.62; SG-IV, mean age: 64.13±0.66; SG-V, mean age: 73.40±2.30 years in [b] Group B diabetic patients].

*$p<0.001$.

**$p<0.01$.

***$p<0.05$.

OA (Tables 3, 4; Figs. 1a and 2a). Secondly, osteoarthritis patients in Group OA were having more body weight than the rheumatoid arthritis patients in Group RA, which may cause more Zn concentrations in the hair and nails of Group OA (Tables 2, 3, 4; Figs. 1a and 2a) than Group RA patients [21].

Highly significant elevation in urinary Cu concentrations in Group RA (rheumatoid arthritis) than Group OA (osteoarthritis), even larger than Group D_M (middle aged diabetics), Group D_O (older diabetics), and Group D+OA (diabetics with osteoarthritis) further explained the effect of acute chronic inflammatory process hypothesized to increase the body requirement for Cu [59, 80]. The non-significant decrease in urinary Zn levels in Group RA (rheumatoid arthritis) in the present investigations are again in accordance with previous finding [59] but with a significant decrease in Group OA patients (osteoarthritis). Further, more reduction in hair and nails Cu in rheumatoid arthritis patients in Group RA suggests severe Cu deficiency in RA than OA (Tables 3 and 4; Figs. 1b and 2b). Increased urinary Cu excretions in RA observed in previous studies may be due to increased plasma Cu concentrations in RA than OA and control, leading to more excretions in the urine [81]. Secondly, Zn depressed Cu absorption even if the intake was in normal physiological ranges, a study done in the patients of rheumatoid arthritis previously [82]. Zn-rich diet consumed in this region may depress the Cu absorption resulting in Cu deficiencies as observed in all the groups than that of control Group A. Urinary Cu levels further increased in the patients of diabetics with osteoarthritis in Group D+OA, but values were observed to be still less than the Group RA patients (rheumatoid arthritis) showing the significance of more inflammatory response on trace metal status in rheumatoid arthritis as compared to other groups (Tables 2, 3, 4; Fig. 3b). Decrease in Zn concentrations in nail and hair of rheumatoid arthritis patients in Group RA shows a redistribution of trace elements due to inflammatory character [83].

The concentration of antioxidant enzymes in the liver to eliminate free radicals is very important. Cu, Zn, and Mn are the key components of the major antioxidants, i.e.,

superoxide dismutase enzymes (SOD), which have been shown to fight against the reactive oxygen intermediates that are linked to joint damages in arthritis [11]. Mitochondrial manganese superoxide dismutase (Mn-SOD) is the primary cellular defense against damaging superoxide radicals generated by aerobic metabolism. As in inflammatory diseases, its elevated levels were observed to provide protection against arthritic inflammation. This may be the reason for the elevation in Mn concentrations in hair and nails of Group RA (rheumatoid arthritis) patients as compared to other diseased groups but were less than the control Group A (Tables 3 and 4; Figs. 1d and 2d) and well coincide with its less urinary excretions in Group RA (less excretion) than the other groups [84] but still larger than control Group A (Table 5; Fig. 3d).

Mg in the body is primarily located in bone and cartilage [85], and up to a third of Mg in the body is intracellular [86]. It is suggested that decreased Mg concentrations observed in the present investigations in all the diseased groups especially in arthritis conditions (Tables 3, 4; Figs. 1c and 2c) may impair the expression and activity of the receptors causing cell damage, cell death, and ECM degeneration, which may ultimately lead to cartilage damage and joint lesions [87].

Low level of Cu in hair and nails of rheumatoid arthritis patients in Group RA may be due to low plasma total antioxidant capacity than Group OA patients (OA) suggesting more inflammatory character in RA, as severely decreased SOD activities were observed in RA than OA in the previous study [88]. It also suggests a more dietary Cu requirement to maintain hepatic Cu–Zn SOD activity [89] in RA than OA. The findings of previous study suggest that cumulative oxidative stress leads to a decreased antioxidant capacity in articular cartilage resulting in chondrocyte genomic instability especially telomere shortening, regardless of cell proliferation [90] linking overproduction of ROS to acute inflammatory response [91–93]. Cu status could affect bone breakdown in young adult women via at least two mechanisms: firstly, lysyl oxidase activity, a Cu enzyme that cross-links collagen, involved in bone structure, and secondly, superoxide dismutase activity that eliminates superoxide radicals stimulating bone resorption. It was confirmed in young adult women who were screened for Cu status based on erythrocyte superoxide dismutase activity. In women with Cu/Zn SOD activities<65% values, when supplemented with Cu (2 mg/day as copper glycine amino acid chelate, Albion) or a placebo for 6 weeks, the Cu but not the placebo raised superoxide dismutase activities and urinary ratio of collagen cross-links to collagen protein. Similarly, manganese glycine amino acid chelate supplementation led to significant increase in MnSOD activity, which showed increased requirement of Cu and Mn in arthritic conditions [94]. Lysyl oxidase enzyme is required for the inflammations of pyrodoline cross-links between collagen fibrils. It was suggested that Cu deficiency via inadequate lysyl oxidase leads to poorly cross-linked and weakened cartilage susceptible to subsequent fragmentation. Cu is reported to have anti-arthritic effects [95, 96]. Studies in 1996 demonstrated the stimulatory effects of Cu supplementation to chondrocytes in vitro on proteoglycan synthesis and abrogation of proteoglycan depletion after exposure to inflammatory synovium [97, 98].

Further, when Group D_M (middle-aged diabetics) and Group D+OA (diabetics with osteoarthritis) were compared, more Zn and Mn concentrations were observed with less Cu and Mg concentrations in Group D+OA (diabetics with osteoarthritis) than Group D_M (middle-aged diabetics) with respect to control Group A (Tables 3, 4; Figs. 1a,d and 2a,d). Less urinary Zn and Mn, but more Cu and Mg levels were observed in Group D+OA patients (diabetics with osteoarthritis) than Group D_M (middle aged diabetics) with respect to control Group A (Table 5; Fig. 3a,d). This alteration may be associated with more degenerative conditions in cartilage, such as in osteoarthritis (OA), that occur after

coincidently with metabolic dysfunction, nutrient imbalance, and diabetes mellitus [99–101]. Clinical and epidemiological survey of OA patients with diabetes mellitus supports the hypothesis that hyperglycemia affects matrix macromolecules and may be related to the development of degenerative joint and bone diseases [102] and may be the possible reason for the increase in Zn and Mn concentrations and reduction in Cu and Mg levels in hair and nails of Group D+OA (diabetics with osteoarthritis) than Group D_M patients (middle-aged diabetics) with respect to control Group A (Tables 2, 3, and 4; Figs. 1a–d and 2a–d).

Lastly, the mineral status in older diabetic patients showed a significant reduction in Zn, Cu, Mg, and Mn in hair and nails of Group D_O (older diabetics) compared to other groups and with respect to control Group A (Tables 3 and 4; Figs. 1a–d and 2a–d). It was coincided well with their higher increase in urine Zn, Mn, Cu, and Mg levels than the other groups and control Group A (Table 5; Figs. 3a–d). Urinary Zn excretion has been reported to correlate with the degree of glucosuria [45, 74]. These studies suggest that hyperzincuria is a component of pathology of IDDM. Hyperzincuria of IDDM was accompanied by measurements of plasma Zn that was elevated [45, 71, 74, 76, 103–107]. Earlier works have also demonstrated elevated serum Cu [108] and an unexplained hyperzincuria in this metabolic disease [46]. It revealed that diabetic conditions in old age becomes more damaging and leads to more deficiency of Zn and Mn, followed by Cu and Mg in Group D_O than the diabetes during middle age in Group D_M patients. It was further confirmed by measuring the serum mineral status in diabetic patients, which further revealed a highly significant rise in serum Zn, Cu, Mg, and Mn levels from SG-I to IV in Group B except SG-V (older diabetics) where a reduction in serum mineral status was recorded as compared to other diabetic patients in Group B that showed less mineral concentrations in the body of older diabetic patients as observed in Group D_O than the middle aged diabetics in Group D_M, respectively (Tables 5 and 6; Figs. 1a–d and 2a–d).

Conclusion

Although obesity, NIDDM, IDDM, osteoarthritis, and rheumatoid arthritis are considered to be the genetic disorder, their patients appear to have genetic predisposition of excessive absorption and retention of Zn in their tissues. Excessive bioavailability of Zn in the food chain exacerbates its deposition causing leaching of other physiologically important elements, including Cu, Mn, and Mg, from their tissues in spite of their adequate concentrations in food items. For Cu, Mn, and Mg, being components of enzymes involved in antioxidant defense system, there deficiencies at young age due to excessive Zn in diet lead to an early onset of pro-inflammatory response resulting their early onset, relatively at a younger age, which appears to be the possible cause for these obesity-related diseases in the population of the Chandigarh.

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