LEVELS OF SERUM Cu, Fe AND Zn ALONG WITH SERUM TOTAL PROTEINS AND FRACTIONS IN DISEASED MALNOURISHED CHILDREN OF 4-12 YEARS OF AGE

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SUMMARY: This study was conducted to uncover the differences in the serum Cu, Fe, Zn and proteins in children of different degrees of malnutrition. For this purpose 85 malnourished and 15 normal children of school age (4-12 years) were chosen. Results revealed that serum Fe and Cu were significantly or relatively higher in malnourished than apparently healthy children irrespective of age, sex, socio-economic status or area of living. The levels of serum Zn, however, showed statistically non-significant differences between malnourished and apparently healthy children. Serum levels of total proteins and globulins were significantly or relatively lower in malnourished compared to the children of control group irrespective of age, sex, socio-economic status and area of living. The results of albumin were variable in these groups. While serum total proteins and globulins in male malnourished children were significantly lower compared to the male children of the control group, it was non-significantly different among females. The means of serum Cu, Zn and Fe in severely malnourished males and females were 48.61, 80.40; 50.00, 48.20; 227.15, 229.40 µg/dL, respectively. These parameters in children of low and middle socio-economic status were 58.30, 90.00; 47.80, 58.67; 240.15, 148.00 µg/dL, respectively while in severely malnourished children of three age groups (4-6, 7-9 and 10-12 years) were 38.25, 81.00, 71.55; 50.50, 46.66, 49.77; 224.88, 155.00, 279.78 µg/dL, respectively. It can be concluded from the present study that serum Cu and Fe levels are increased, while serum total proteins and globulins are decreased in children of 4-12 years of age suffering from disease malnutrition.

Key Words: Copper, zinc, total protein.

INTRODUCTION

The fundamental objective of nutritional assessment in general clinical practice is to learn more about overall nutritional status. Methods of nutritional assessment of patients involve anthropometrical observations, biochemical tests, clinical observations and diet evaluations. The individual nutritional status varies on the basis of person's living conditions, available food supply, health and socio-economic status (22). Although human body has a great capacity to adapt to lower nutritional conditions, it can only sustain certain amount of physiological stress before

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Table 1: Comparison of Cu, Zn and Fe (means ± SD) in children of different ages of different degrees of malnutrition.

Age groups (year)	Malnutrition degree			Control
	First	Second	Third	
Cu (μg/dL)				
4–6	62.00 ± 18.40a	59.77 ± 28.06a	38.25 ± 33.40ab	14.67 ± 10.26b
7–9	55.20 ± 31.13	58.72 ± 28.66	81.00 ± 108.64	23.50 ± 23.63
10–12	65.75 ± 44.47	43.28 ± 22.02	71.55 ± 53.50	33.40 ± 20.58
Overall mean	62.43 ± 34.53	54.13 ± 27.08	62.43 ± 66.47	25.42 ± 19.68
Zn (μg/dL)				
4–6	60.50 ± 5.00	50.44 ± 11.78	50.50 ± 14.37	47.33 ± 6.35
7–9	52.00 ± 11.22	55.09 ± 15.17	46.66 ± 4.32	52.50 ± 8.54
10–12	47.50 ± 14.37	55.00 ± 15.17	49.77 ± 8.33	55.60 ± 8.04
Overall mean	51.88 ± 12.43	54.13 ± 13.07	49.21 ± 9.88	52.50 ± 7.48
Fe (μg/dL)				
4–6	298.50 ± 125.31a	274.66 ± 145.23ab	224.88 ± 142.65ABab	100.66 ± 36.01b
7–9	333.20 ± 149.01a	354.18 ± 154.49a	155.00 ± 62.72Bb	147.00 ± 65.32b
10–12	290.25 ± 201.51	245.14 ± 95.65	279.78 ± 87.30A	163.60 ± 59.85
Overall mean	304.82 ± 163.19	304.36 ± 143.06	228.13 ± 112.67	142.33 ± 58.22

Values with different small letters in a row and with different capital letters in a column are statistically significant at $p<0.05$.

signs of malnutrition appear. Malnutrition appears when nutritional reserves including macronutrients (protein) and micronutrients (Zn^{++}, Cu^{++} and Fe^{++}) are depleted.

Cu, Zn and Fe are not only essential for development and growth but they are necessary for immune system and metabolism of anti-oxidants. It has been reported that in protein calorie malnutrition, serum Cu levels can be used as indicator of severity of malnutrition (11). Serum Cu and Zn levels decrease in malnourished children, therefore, these must be taken into account when treating diseased malnourished children (12). Cu is required for infant growth, defense mechanisms, bone strength, quality and quantity of red and white blood cells, Fe transport, glucose metabolism and brain development. Most common clinical manifestations of acquired Cu deficiency are anemia, neutropenia, bone abnormalities and impaired growth (14), while drowsiness, rapid pulse and respiration are caused by deficiency of Fe. Deficiency of Zn is associated with abnormal taste acuity and growth retardation (9).

Recent studies suggest that normal infant's energy and protein requirements might be substantially of a lower than previous estimates. Net protein deposition in growing children results from protein synthesis being higher than protein break down. However, higher protein breakdown occurs in conditions of acute as well as chronic inflammatory processes leading to severe protein malnutrition, which is not always amenable to nutritional support (4).

Data regarding serum Zn, Cu, Fe along with serum total proteins including fractions is lacking with reference

Table 2: Comparison of serum total proteins, albumin and globulins (mean ± SD) in children of different ages of different degrees of malnutrition.

Age groups (year)	Malnutrition degree			Control
	First	Second	Third	
Total proteins (g/dL)				
4–6	8.28 ± 2.70b	9.52 ± 1.15b	9.16 ± 2.84b	13.35 ± 2.32a
7–9	9.43 ± 3.78b	10.24 ± 2.11b	11.20 ± 1.57b	15.80 ± 3.97a
10–12	10.70 ± 1.49b	10.75 ± 1.60b	11.56 ± 2.05b	18.16 ± 6.27a
Overall mean	9.76 ± 2.62	10.34 ± 1.82	10.63 ± 2.42	16.17 ± 4.86
Albumin (g/dL)				
4–6	3.04 ± 0.31ab	2.66 ± 0.50Bb	2.99 ± 0.92ab	3.72 ± 0.40a
7–9	4.02 ± 0.77	3.26 ± 0.76A	3.47 ± 0.98	3.76 ± 0.76
10–12	3.49 ± 0.84	3.48 ± 0.62A	3.27 ± 0.64	3.13 ± 1.32
Overall mean	3.54 ± 0.78	3.21 ± 0.72	3.23 ± 0.82	3.49 ± 0.96
Globulins (g/dL)				
4–6	5.24 ± 2.67b	6.86 ± 1.17b	6.16 ± 2.16b	9.62 ± 2.21a
7–9	5.41 ± 3.98b	7.16 ± 1.98b	7.73 ± 1.59b	12.03 ± 4.20a
10–12	7.20 ± 1.66b	7.26 ± 1.51b	8.29 ± 1.91b	15.02 ± 5.31a
Overall mean	6.21 ± 2.72	7.13 ± 1.68	7.40 ± 2.07	12.67 ± 4.60

Values with different small letters in a row and with different capital letters in a column are statistically significant at $p<0.05$.

to local children of 4–12 years of age suffering from malnutrition. This research work was planned to study the serum Fe, Cu, Zn, total proteins and fractions in children of 4–12 years suffering from different degrees of diseased malnutrition with special reference to socio-economic status, sex, age and area of living.

MATERIALS AND METHODS

The study was conducted on children admitted to Pediatrics Wards of Allied and National Hospitals of Faisalabad. Children, included in the study were those suffering from some common diseases and had developed signs of malnutrition. Children from Outdoor Patient Department of Allied Hospital were also included.

A total of 100 children of 4-12 years of age were randomly selected for the study. Of these, 85 were suffering from some common diseases including diarrhea, pneumonia, jaundice and gastroenteritis, while 15 apparently healthy children selected as controls were free of symptoms. These subjects were grouped according to socio-economic status, age, sex and area of living. The area of living included residency in industrial or non-industrial areas. They were divided into low socio-economic status, i.e., low (maximum earning, Rs. 5.000 per month) or middle class (monthly earning between Rs. 5.000 and 15.000).

The children were also divided into three groups on the basis of age, i.e., 4-6, 7-9 and 10-12 years. History from the close family members of these children was obtained including age,

Table 3: Comparison of Cu, Zn and Fe (means ± SD) in children of different sex of different degrees of malnutrition.

Sex	Malnutrition degree			Control
	First	Second	Third	
Cu (μg/dL)				
Male	60.54 ± 26.66a	59.22 ± 31.09a	48.61 ± 31.11ab	29.00 ± 21.17b
Female	64.00 ± 48.82ab	50.74 ± 24.00ab	80.40 ± 94.21a	14.66 ± 10.26b
Zn (μg/dL)				
Male	54.72 ± 7.70ab	59.44 ± 11.35Aa	50.00 ± 7.91b	53.11 ± 8.43ab
Female	46.66 ± 23.05	50.59 ± 13.13B	48.20 ± 12.30	50.66 ± 4.16
Fe (μg/dL)				
Male	284.18 ± 120.79a	286.11 ± 132.44a	227.15 ± 113.17ab	156.22 ± 60.69b
Female	342.66 ± 231.05a	316.31 ± 150.94a	229.40 ± 118.11ab	100.66 ± 20.81b

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sex, disease suffering, duration of illness, family income, *etc.* Patients were classified into three degrees of malnutrition according to their weight by using Gomez classification (10).

First degree of malnutrition: If weight is 75-90% of the target or expected weight.

Second degree of malnutrition: If weight is 60-75% of the target or expected weight.

Third degree of malnutrition: If weight is below 60% of the target or expected weight.

About 3 ml of blood samples were collected from these children using disposable syringes. Blood samples were allowed to clot, then were transferred into test tubes and centrifuged to obtain the serum. The serum thus collected was stored at -20°C for further studies. Serum total protein was determined by Biuret method as described by Oser (17). The serum albumin was determined by the method of Gowenlock *et al.* (8). The globulin was estimated by subtracting albumin from total protein and results were expressed in gm globulin/100 ml of samples. Serum Zn, Cu and Fe were determined by atomic absorption spectrophotometer (Z-8200 polarized Zeeman) at 329, 324.8 and 24.3 nm wavelength (8).

Data obtained were studied by analysis of variance technique (GLM procedure) and means were compared by LSD and DMR tests by using SAS 6.1.2 statistical program.

RESULTS AND DISCUSSIONS

Age

Among the trace elements, Cu was significantly ($p<0.05$) higher in malnourished than apparently healthy children of 4-6 years of age, while in other age groups, it was relatively higher (Table 1). Serum Fe was again significantly or relatively higher in malnourished compared to apparently healthy children, in each age group (Table 1). Serum Zn showed a non-significant difference between malnourished and apparently healthy children in each age group. These results suggest that the level of Zn was not affected, while serum Cu and Fe were higher in diseased malnourished children. This might be due to release of Cu and Fe from damaged tissues in the form of Cu and Fe containing enzymes, leading to higher levels of these elements or perhaps the utilization of these were lowered while availability was increased due to disease.

Table 4: Comparison of serum total proteins, albumin and globulins (means ± SD) in children of different sex of different degrees of malnutrition.

Sex	Malnutrition degree			Control
	First	Second	Third	
Total proteins (g/dL)				
Male	8.81± 2.44Bb	10.21 ± 1.70b	10.40 ± 2.89b	17.62 ± 4.53a
Female	11.49 ± 2.12A	10.44 ± 1.93	10.93 ± 1.76	11.83 ± 3.14
Albumin (g/dL)				
Male	3.66 ± 0.91a	3.30 ± 0.61ab	3.03 ± 0.73b	3.69 ± 0.67a
Female	5.15 ± 2.53	3.15 ± 0.80	3.49 ± 0.90	2.90 ± 1.61
Globulins (g/dL)				
Male	5.15 ± 2.53Bb	6.90 ± 1.62b	7.37 ± 2.60b	13.93 ± 4.63a
Female	8.17 ± 1.95A	7.28 ± 1.73	7.44 ± 1.21	8.92 ± 1.54

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Further research is needed to uncover the real reasons of these changes. Chan and Subramanium (5) and Litzman *et al.* (13) reported an increase in Cu in leukemic children and in conditions of common variable immune deficiency disease, respectively. Ece *et al.* (6) and Onerci *et al.* (16) observed increase in children with Fe deficiency anemia

Table 5: Comparison of Cu, Zn and Fe (means ± SD) in children of different degrees of malnutrition of different socio-economic status.

Socio-economic status	Malnutrition degree			Control
	First	Second	Third	
Cu (μg/dL)				
Low	60.25 ± 35.07a	53.60 ± 28.17a	58.30 ± 17.34a	8.00 ± 3.46b
Middle	86.00 ± 11.56	58.40 ± 17.34	90.00 ± 47.03	33.40 ± 20.58
Zn (μg/dL)				
Low	51.00 ± 12.28	54.85 ± 11.03	47.80 ± 9.28	48.00 ± 3.46
Middle	66.00 ± 12.26	48.40 ± 22.68	58.67 ± 10.06	55.60 ± 7.66
Fe (μg/dL)				
Low	296.38 ± 164.66a	305.85 ± 134.59a	240.15 ± 114.93ab	138.67 ± 86.17b
Middle	440.00 ± 59.63a	292.40 ± 219.77a	148.00 ± 55.46ab	144.40 ± 50.74b

Values with different small letters in a row are statistically significant at $p<0.05$.

Table 6: Comparison of serum total proteins, albumin and globulins (means ± SD) in children of different degrees of malnutrition of different socio-economic status.

Socio-economic status	Malnutrition degree			Control
	First	Second	Third	
Total proteins (g/dL)				
Low	9.73 ± 2.70	10.40 ± 1.80	11.13 ± 2.03A	11.79 ± 3.12
Middle	10.18 ± 1.26b	9.92 ± 2.20b	7.32 ± 2.55Bb	18.42 ± 3.97a
Albumin (g/dL)				
Low	3.59 ± 0.78	3.15 ± 0.67	3.38 ± 0.76A	2.96 ± 1.64
Middle	2.72 ± 1.25	3.68 ± 1.07	2.22 ± 0.38B	3.95 ± 0.82
Globulins (g/dL)				
Low	6.14 ± 2.79b	7.24 ± 1.63ab	7.75 ± 1.85Aab	8.83 ± 1.48a
Middle	7.46 ± 1.36b	6.24 ± 2.01b	5.10 ± 2.39Bb	14.47 ± 4.11a

Values with different small letters in a row and with different capital letters in a column are statistically significant at $p<0.05$.

and tonsillitis, respectively. Reunanen *et al.* (19) reported that higher serum Cu and lower serum Zn were significantly associated with an increased mortality from all cardiovascular diseases.

The results also show that serum total proteins and globulins were significantly ($p<0.05$) lower in malnourished children than apparently healthy children in each age group (Table 2). This suggests that lower globulins resulted in decrease of serum total proteins, while albumin was not affected. The decrease in globulin might be due to immunosuppression or decrease synthesis of other types of globulins (α and β globulins). In cases of protein malnutrition, the decrease in total protein has already been reported (7) with lower serum globulin in diabetic children (21).

Sex

Serum Cu and Fe were significantly ($p<0.05$) higher in malnourished children which were irrespective of sex and age. The difference, however, in severe degree of malnutrition and apparently healthy children was non-significant in each sex (Table 3). This suggests that the levels of these elements increase during the early phase of disease. It has been reported that the malnutrition in childhood cancer was commonly a serious problem and serum Zn levels decrease while serum Cu levels increase in leukemia (20). Similarly, higher serum Cu in Schizophrenic females has also been reported (15). Results of present and earlier studies indicate that Cu increases in disease conditions, its real reasons, however, need to be further investigated.

These results suggest that higher reduction in serum total proteins, albumin and globulins occurred in male malnourished children compared to females. Table 4 shows that the changes in males were more pronounced and reduction of protein might be due to the higher catabolism as has been reported that protein breakdowns occur in inflammatory diseases of acute and chronic diseases (4). However, no effect of protein status in female might be related with the difference of genetic and metabolic reasons.

Table 7: Comparison of Cu, Zn and Fe (means ± SD) in children of different areas of living of different degrees of malnutrition.

Area of living	Malnutrition degree			Control
	First	Second	Third	
Cu (μg/dL)				
Industrial	75.60 ± 41.96b	51.50 ± 13.34b	136.00 ± 31.11a	31.63 ± 13.48c
Non-industrial	56.00 ± 48.82a	54.70 ± 24.00a	59.06 ± 94.21a	25.41 ± 10.26b
Zn (μg/dL)				
Industrial	44.80 ± 15.59	50.00 ± 18.29	68.00 ± 7.91	54.48 ± 8.25
Non-industrial	54.83 ± 10.21	55.02 ± 11.80	48.36 ± 9.20	52.50 ± 7.48
Fe (μg/dL)				
Industrial	228.80 ± 147.21b	385.25 ± 142.65a	180.00 ± 113.17c	139.46 ± 49.62d
Non-industrial	336.50 ± 164.73a	286.86 ± 138.88ab	230.31 ± 114.82bc	142.33 ± 58.22c

Values with different small letters in a row are statistically significant at $p<0.05$.

Socio-economic status

As were findings of Cu, Zn and Fe with respect to age, a similar pattern was observed between malnourished and apparently healthy children in each socio-economic status (Table 5). In a previous study by Ahmed and Maqbool (3), the levels of Zn in children of 5-15 years of age of high socio-economic status were 62 μg/dL (males) and 64 μg/dL (females). However, during the present study, almost similar levels were observed in diseased malnourished children while these were much lower in control subjects. The difference could be due to the geographical or genetic factors. Ahmed and Maqbool (3) reported that high relationship existed between levels of Zn and Cu. They encountered even lower Zn levels in children of low socio-economic status. Such a change was not observed during this study which might be due to the fact that research was undertaken on diseased malnourished children. It might be also possible that these patients were treated at different levels for deficiencies of the same.

Likewise for age and sex, almost a similar pattern was observed of slightly lower level of total proteins and globulins in malnourished children compared to apparently healthy children (Table 6). However, the effect of albumin was non-significant. This suggested that these parameters were relatively influenced by malnutrition, irrespective of age, sex and socio-economic status. These findings were also in line with the findings of Ahmad and Gilani (2), who reported that the effect of malnutrition in males of 6-14 years of age with respect to albumin was not significant. Ahmad *et al.* (1) however, reported significantly higher serum protein levels in children of high socio-economic status with respect to children of low income families. This was not confirmed by our study because our subjects were diseased malnourished children.

Area of living

The levels of Cu and Fe were significantly ($p<0.05$) higher in malnourished than apparently healthy children of each industrial and non-industrial area (Table 7). This showed that Cu and Fe were related to disease malnutrition but were independent of area of living. Likewise in

Table 8: Comparison of total proteins, albumin and globulins (means ± SD) in children of different ages of different degrees of malnutrition.

Area of living	Malnutrition degree			Control
	First	Second	Third	
Total proteins (g/dL)				
Industrial	10.17 ± 2.05bc	11.48 ± 1.51b	9.46 ± 2.89c	17.19 ± 3.67a
Non-industrial	9.59 ± 2.89b	10.10 ± 1.81b	10.68 ± 2.47b	16.17 ± 4.86a
Albumin (g/dL)				
Industrial	3.52 ± 0.19a	3.53 ± 0.76a	2.11 ± 0.80b	3.51 ± 0.20a
Non-industrial	3.55 ± 0.94	3.14 ± 0.71	3.28 ± 0.80	3.49 ± 0.96
Globulins (g/dL)				
Industrial	6.64 ± 1.88b	7.95 ± 1.79b	7.35 ± 2.60b	14.63 ± 4.36a
Non-industrial	6.04 ± 3.06b	6.95 ± 1.62b	7.40 ± 2.12b	12.67 ± 4.60a

Values with different small letters in a row are statistically significant at $p<0.05$.

other groups Zn showed non-significant differences. The reduction of serum Zn concentration, observed previously in malnourished children, was related to the severity of clinical condition (18). Such a change was however, not observed during the present study as the level in diseased malnourished children remained close to normal subjects.

The levels of total proteins and globulins were lower in malnourished than apparently healthy children of each industrial and non-industrial area which suggested that the changes in these were independent of area of living (Table 8). The albumin levels were, however, significantly ($p<0.05$) lower in children of industrial area in third degree of malnutrition, which suggested that the albumin synthesis was interfered only in severely malnourished children and that synthesis was probably less and breakdown was more in children of industrial area due to the environmental factors. Lower level of albumin has also been previously reported in children with diabetic stress and was related with lowered synthesis of serum proteins in diabetics (21).

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