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Levels of Serum Copper, Iron and Zinc along with Serum Total Proteins and Fractions in Diseased Malnourished Children of 4-12 Years of Age

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The study was conducted to find out the differences in serum copper (Cu), Iron (Fe), Zinc (Zn) and serum proteins in children of different degrees of malnutrition as a result of some disease. For this purpose a total of 100 children of school going age (4-12 years) having 85 malnourished and 15 apparently healthy were selected. 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 non-significant difference between malnourished and apparently healthy children. Serum levels of total proteins and globulins were lower (significantly or relatively) in malnourished children than control irrespective of age, sex, socio-economic status and area of living. The results of albumin were variable in these groups. The decrease in serum total proteins and globulins in male malnourished children were significant than male control while it were non-significant 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 100 ml $^{-1}$, respectively. These in children of low and middle socio-economic status were 58.30; 90.00; 47.80; 58.67; 240.15; 148.00 μg 100 ml^{-1}, 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 100 ml^{-1} respectively. It can be concluded that serum Cu and Fe increases, while serum total proteins and globulins decreases in children of 4-12 years of age suffering from disease malnutrition.

Key words: Serum, Cu, Zn, total protein, albumin, age, sex, socio-economic status

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Introduction

The fundamental objective of nutritional assessment in general clinical practice is to find out the overall nutritional status. Methods of nutritional assessment of patients involve anthropometrical and clinical observations, biochemical tests and diet evaluation. The individual nutritional status varies on the basis of person's living situation, available food supply, health and socio-economic status (Williams, 1994). Although human body has a great capacity to adapt lower nutritional status, it can only sustain certain amount of physiological stress before 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 for anti-oxidant effects also. It has been reported that in protein calorie malnutrition, serum Cu levels can be used as an indicator of severity of malnutrition (Houssani *et al*., 1997). Serum Cu and Zn levels decreases in malnourished children, therefore, these must be taken into account when treating diseased malnourished children (Khaldi *et al*., 1995). Cu is required for infant growth, defense mechanism, bone strength and blood cells (red and white), iron transport, glucose metabolism and brain development. Most common clinical manifestations of acquired Cu deficiency are anaemia, neutropenia, bone abnormalities and impaired growth (Olivares and Uauy, 1996), while drowsiness, rapid pulse and respiration are caused by deficiency of iron. Deficiency of Zn is associated with abnormal taste acuity and growth retardation (Hambidge, *et al*., 1972). Recent studies suggest that normal infant's energy and protein requirements might be substantially lower than previous estimates. Net protein deposition in growing child 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 amendable to nutritional support (Bresson, 1998).

Data regarding serum Zn, Cu, Fe along with serum total proteins including fractions is lacking with reference to local children of 4-12 years of age suffering from malnutrition. The 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 reference to socio-economic status, sex, age and area of living.

Materials and Methods

The study was conducted on children admitted in paediatric wards of Allied and National Hospitals of Faisalabad and children from Outdoor Patient, Department of Allied Hospital were also included. Children. A total of 100 children (had developed signs of malnutrition) of 4-12 years of age were randomly selected out of these, 85 were suffering from some common diseases including diarrhoea, pneumonia, jaundice, gastroenteritis, while 15 apparently healthy children were also selected. 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 tow socio-economic status, i.e., low (maximum earning Rs. 5,000 per month) or middle class (monthly earning between Rs. 5,000 to 15,000). The children were also divided on the basis of age into i.e., 4-6, 7-9 and 10-12 years groups. History from the close family members of these children was obtained including age, sex, disease suffering, duration of illness, family income, etc. Children were classified into three degrees of malnutrition according to their weight by using Gomez classification (Hamill *et al*., 1979).

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

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

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

Blood samples of about 3ml were collected from these children with the help of a disposable syringes and they were allowed to clot, then were transferred into test tube 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 (1976). The serum albumin was determined following the method of Gowenlock *et al*. (1988). The globulin was estimated by subtracting albumin from total protein and results were expressed in gm globulin 100 ml^{-1} of samples. Serum Zn, Cu and Fe were determined by atomic absorption spectrophotometer (Z-8200 polarized Zeeman) at 329, 324.8 and 24.3nm wavelength (Gowenlock *et al*.,1988). Data obtained were analyzed by analysis of variance technique (GLM procedure) and means were compared by LSD and DMR test by using SAS 6.1.2 statistical programme.

Results and Discussions

Age: Among the trace elements, Cu and Fe were significantly ($P < 0.05$) higher in malnourished than apparently healthy children (Table 1). Serum Zn showed a non-significant difference between malnourished and apparently healthy children in each age group. These results suggested that level of Zn was not affected, while serum Cu and Fe were higher in diseased malnourished children and 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 or may be the utilization of these were lowered while availability was increased due to disease. Further research is needed to know the real reasons about these changes. Chan and Subramanium (1998) and Litzman *et al*., (1995) reported that an increase in Cu in leukaemic children and conditions of common variable immune deficiency disease, respectively. Ece *et al*., (1997) and Onerci *et al*. (1997) observed increase in children with Fe deficiency anaemia and tonsillitis, respectively. Reunanen *et al*., (1996) reported that higher serum Cu and lower serum Zn were significantly associated with an increased mortality from all cardiovascular diseases. 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 suggested that lower globulins resulted in decrease of serum total proteins, while albumin was not affected. Decrease in globulin might be due to immuno-suppression or decreased synthesis of other types of globulins (α and β globulins). In cases of protein malnutrition, decrease in total protein has already been reported (Flodin, 1953) with lower serum globulin in diabetic children (Tuvemo *et al*., 1997).

Sex: Like in age, serum Cu and Fe were significantly ($P < 0.05$) higher in malnourished children which were independent 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 suggested that the levels of these elements increases during the early phase of disease.

Table 1: Comparison of Cu, Zn and Fe (μg 100 ml^{-1}) (mean $\pm$SD) in children of different ages of different degrees of malnutrition.

Age Groups	Malnutritions degree			
	1st	2nd	3rd	Control
Cu				
4-6 years	62.00 $\pm$ 18.40a	59.77 $\pm$ 28.06a	38.25 $\pm$ 33.40ab	14.67 $\pm$ 10.26b
7-9 years	55.20 $\pm$ 31.13	58.72 $\pm$ 28.66	81.00 $\pm$ 108.64	23.50 $\pm$ 20.58
10-12 years	65.75 $\pm$ 44.47	43.28 $\pm$ 22.02	71.55 $\pm$ 53.50	33.40 $\pm$ 20.58
Overall Mean	62.43 $\pm$ 34.53	54.13 $\pm$ 27.08	62.43 $\pm$ 66.47	25.42 $\pm$ 19.68
Zn				
4-6 years	60.50 $\pm$ 5.00	50.44 $\pm$ 11.78	50.50 $\pm$ 14.37	47.33 $\pm$ 6.35
7-9 years	52.00 $\pm$ 11.22	55.09 $\pm$ 15.17	46.66 $\pm$ 4.32	52.50 $\pm$ 8.54
10-12 years	47.50 $\pm$ 14.37	55.00 $\pm$ 15.17	49.77 $\pm$ 8.33	55.60 $\pm$ 8.04
Overall Mean	51.88 $\pm$ 12.43	54.13 $\pm$ 13.07	49.21 $\pm$ 9.88	52.50 $\pm$ 7.48
Fe				
4-6 years	298.50 $\pm$ 125.31	274.66 $\pm$ 145.23ab	224.88 $\pm$ 142.65ABab	100.66 $\pm$ 36.01b
7-9 years	333.20 $\pm$ 149.01a	354.18 $\pm$ 154.49a	155.00 $\pm$ 62.72Bb	147.00 $\pm$ 65.32b
10-12 years	290.25 $\pm$ 201.51	245.14 $\pm$ 95.65	279.78 $\pm$ 87.30A	163.60 $\pm$ 59.85
Overall Mean	304.82 $\pm$ 163.19	304.365 $\pm$ 143.06	228.13 $\pm$ 112.67	142.33 $\pm$ 58.22

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

Table 2: Comparison of serum total proteins, albumin and globulins (g 100 ml^{-1})(means $\pm$SD) in children of different ages of different degrees of malnutrition

Age Groups	Malnutritions degree			
	1st	2nd	3rd	Control
Total proteins				
4-6 years	8.28 $\pm$ 2.70a	9.52 $\pm$ 1.15b	9.16 $\pm$ 2.84b	13.35 $\pm$ 2.32a
7-9 years	9.43 $\pm$ 3.78b	10.24 $\pm$ 2.11b	11.20 $\pm$ 1.57b	15.80 $\pm$ 3.97a
10-12 years	10.70 $\pm$ 1.49b	10.75 $\pm$ 1.60b	11.56 $\pm$ 2.05b	18.16 $\pm$ 6.27a
Overall Mean	9.76 $\pm$ 2.623	10.34 $\pm$ 1.82	10.63 + 2.42	16.17 $\pm$ 4.86
Albumin				
4-6 years	3.04 $\pm$ 0.31ab	2.66 $\pm$ 0.50Bb	2.99 $\pm$ 0.92ab	3.72 $\pm$ 0.40a
7-9 years	4.02 $\pm$ 0.77	3.26 $\pm$ 0.76A	3.47 $\pm$ 0.98	3.76 $\pm$ 0.76
10-12 years	3.49 $\pm$ 0.84	3.48 $\pm$ 0.62A	3.27 $\pm$ 0.64	3.13 $\pm$ 1.32
Overall Mean	3.54 $\pm$ 0.78	3.21 $\pm$ 0.72	3.23 $\pm$ 0.82	3.49 $\pm$ 0.96
Globulins				
4-6 years	5.24 $\pm$ 2.67b	6.86 $\pm$ 1.17b	6.16 $\pm$ 2.16b	9.62 $\pm$ 2.21a
7-9 years	5.41 $\pm$ 3.98b	7.16 $\pm$ 1.98b	7.73 $\pm$ 1.59b	12.03 $\pm$ 4.20a
10-12 years	5.41 $\pm$ 1.66b	7.26 $\pm$ 1.51b	8.29 $\pm$ 1.91b	15.02 $\pm$ 5.31a
Overall Mean	7.20 $\pm$ 2.72b	7.13 $\pm$ 1.68	7.40 $\pm$ 2.07	12.67 $\pm$ 4.60

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

Table 3: Comparison of Cu, Zn and Fe (μg100 ml^{-1}) (means $\pm$SD) in children of different sex of different degrees of malnutrition

Sex	Malnutritions degree			
	1st	2nd	3rd	Control
Cu				
Male	60.54 $\pm$ 26.66a	59.22 $\pm$ 31.09a	48.61 $\pm$ 31.11ab	29.00 $\pm$ 21.17b
Female	64.00 $\pm$ 48.82ab	50.74 $\pm$ 24.00ab	80.40 $\pm$ 94.21a	14.66 $\pm$ 10.26b
Zn				
Male	54.72 $\pm$ 7.70ab	59.44 $\pm$ 11.35Aa	50.00 $\pm$ 7.91b	53.11 $\pm$ 8.43ab
Female	46.66 $\pm$ 23.05	50.59 $\pm$ 13.13B	48.20 $\pm$ 12.30	50.66 $\pm$ 4.16
Fe				
Male	284.18 $\pm$ 120.79a	286.11 $\pm$ 132.44a	227.15 $\pm$ 113.17ab	156.22 $\pm$ 60.69b
Female	342.66 $\pm$ 231.05a	316.31 $\pm$ 150.94a	229.40 $\pm$ 118.11ab	100.66 $\pm$ 20.81b

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

Table 4: Comparison of serum total proteins, albumin and globulins (g 100 ml^{-1}) (means ± SD) in children of different sex of different degrees of malnutrition

Age Groups	Malnutritions degree			
	1st	2nd	3rd	Control
Total proteins				
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				
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				
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

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

Table 5: Comparison of Cu, Zn and Fe (μg 100 ml^{-1})(means ± SD) in children of different of malnutrition of different socio-economic status.

Socio-economic	Malnutritions degree			
Status	1st	2nd	3rd	Control
Cu				
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				
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				
Low	296.38 ± 164.66a	305.85 ± 134.59a	240.15 ± 114.93ab	138.67 ± 86.17b
Middle	440.00 ± 59.33a	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 (g 100 ml^{-1})(means ± SD) in children of different degrees of malnutrition of different socio-economic status.

Socio-economic	Malnutritions degree			
Status	1st	2nd	3rd	Control
Total Proteins				
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				
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				
Low	6.14 ± 2.79b	7.24 ± 1.63ab	7.75 ± 1.85Aab	8.83 ± 1.48a
Middle	7.46 ± 1.36	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 $P < 0.05$

Table 7: Comparison of Cu, Zn and Fe (μg 100 ml^{-1}) (means ± SD) in children of different areas of living of different degrees of malnutrition

Sex	Malnutritions degree			
	1st	2nd	3rd	Control
Cu				
Industrial	75.60 ± 41.96b	51.50 ± 13.34b	136.00 ± 31.11a	31.63 ± 13.48c
Non industrial	56.00 ± 48.82a	54.780 ± 24.00a	59.06 ± 94.21	25.41 ± 10.26b
Zn				
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				
Industrial	228.80 ± 147.21b	385.25 ± 142.65a	180.00 ± 113.17	139.49 ± 49.62
Non industrial	336.50 ± 164.73	286.86 ± 138.88ab	230.31 ± 114.82bc	142.33 ± 58.22

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

Table 8: Comparison of serum total proteins, albumin and globulins (g 100 ml^{-1}) (mean ± SD) in children of different area in different degrees of malnutrition

Socio-economic	Malnutritions degree			
Status	1st	2nd	3rd	Control
Total Proteins				
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 ± 1.81	10.68 ± 2.47b	16.17 ± 4.86a
Albumin				
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				
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 and with different capital letters in a column are statistically significant at $P < 0.05$

It has been reported that malnutrition in childhood cancer was commonly a serious problem and in leukemia serum Zn levels decreases while serum Cu levels increases (Sgarbieri *et al.*, 1999). Similarly, higher serum Cu in Schizophrenic females has also been reported (Olatunbosun *et al.*, 1975). The results of this study and of earlier are indicative that Cu increases in disease conditions.

The results suggested that decrease in serum total proteins, albumin and globulins occurred in male malnourished children than females (Table 4). This showed that the changes in males were more pronounced and protein decreased in these might be due to the higher catabolism as has been reported that protein breakdowns occur in inflammatory diseases of acute and chronic nature (Bresson, 1998). However, no effect of protein status in female might be related with the difference of genetic and metabolism.

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). The study by Ahmed and Maqbool (1993), the levels of Zn in children of 5-15 years of age of high socio-economic status observed were 62 (boys) and 64 μg100 ml^{-1} respectively (girls). However, during this study, almost similar levels were observed in diseased malnourished children while these were much lower in control subjects. The difference could be due geographical or genetic differences. Ahmed and Maqbool (1993) reported that high relationship existed between levels of Zn and Cu and they observed lower Zn levels in children of low socio-economic status. Such a change was not observed during this study which might be due to that the research was undertaken on diseased malnourished children. It might also be 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 slightly lower total proteins and globulins in malnourished children than 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 (1988), who reported that the effect of malnutrition in boys of 6-14 years of age with respect to albumin was not significant. Ahmad *et al.* (1992) however, reported significantly higher serum protein level in children of high than low socio-economic status which was not the case during our study because this was undertaken on 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 have relation with disease malnutrition and was independent of area of living. Likewise in other groups Zn showed non-significant difference. The decrease observed previously in malnourished children in serum Zn concentration was related with the severity of clinical condition (Pras *et al.*, 1983). Such a change was however, not observed during this 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 3rd degree of malnutrition, which suggested that the albumin synthesis was interfered only in severely malnourished children and the synthesis was less and probably breakdown was more in children of industrial area being a stressful area, keeping in view 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 Tuvemo *et al.* (1997).

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