

# Correlation of Serum Biochemical Parameters and Oxidative Stress in Malnourished Children: A Case-control Study

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## ABSTRACT

**Introduction:** There are evidence regarding enhanced oxidative stress in the form of serum Malondialdehyde (MDA) and depleted activities of serum zinc and iron in malnourished children. The deficiency of trace elements predisposes the susceptibility to various infections. Changes in oxidant and antioxidant levels may be responsible for grading in Protein Energy Malnutrition (PEM).

**Aim:** To correlate the levels of serum biochemical parameters such as proteins, zinc, iron with oxidative stress in the form of Malondialdehyde (MDA), in malnourished children of 1-5 years of age and also to compare the findings with age and gender matched well-nourished children.

**Materials and Methods:** This case-control study was conducted in the Biochemistry Department at Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, India, from January to December 2014. A total of 202 children (aged 1-5 years) were included in the study. Out of these, 103 children were undernourished and 99 children were healthy control. Out of 103 undernourished children 46 were severe malnourished

and 57 were moderate malnourished. Blood samples were analysed for the estimation of serum zinc, iron, MDA, albumin and total protein. T-test, one-way Analysis of Variance (ANOVA) and Pearson correlation were used for statistical analysis and p-value <0.001 was considered significant.

**Results:** Mean weight of moderate malnourished (10.44±2 kg) and severe malnourished children (9.36±1.54 kg) were significantly low (p-value <0.001) when compared with control group (12.73±2.36 Kg). Compared to controls (0.71±0.18 nmol/mL) the serum MDA level were significantly high (p-value <0.001) in both moderate (1.84±0.38 nmol/mL) and severe (3.44±0.59 nmol/mL) malnourished children. Serum zinc and serum iron levels, serum total protein and albumin of malnourished cases were significantly low (p-value <0.001) as compared to the control subjects.

**Conclusion:** Compared to control, the malnourished children had significant high level of serum MDA while low levels of serum zinc, iron, albumin, globulin and total proteins. Early replacement of antioxidants, nutrients, proteins, zinc and iron supplement could be useful in the therapy of this disease.

**Keywords:** Antioxidants, Iron, Malondialdehyde, Protein energy malnutrition, Zinc

## INTRODUCTION

Protein Energy Malnutrition (PEM) is one of the common health problems among children of developing countries including India [1]. It is a wide spread deficiency disease among children of low socio-economic groups. Growth retardation due to this condition occurs in children of post weaning age which may be a result of dietary deficiency of specific nutrients [2].

World Health Organisation (WHO) has defined PEM as a range of pathological conditions arising from coincident lack in varying proportions of protein and calories occurring most frequently in infants and young children and commonly associated with infections. PEM includes severe and mild forms of PEM. The severe forms of PEM are kwashiorkor, marasmus and marasmic kwashiorkor [3]. India, like other developing countries has a high burden of micronutrient deficiencies with almost 75% of its children suffering from iron deficiency anaemia and over 50% of children from zinc deficiency [4,5].

The clinical features of PEM are well defined but its pathophysiology is still poorly understood. Antioxidant role of some trace elements such as Zinc (Zn), Copper (Cu) and Selenium (Se) has been shown in some studies apart from the deficiency of calories and proteins [6-8]. Recently free radicals have been implicated in pathophysiology of PEM [9]. In malnutrition there is excessive oxidative stress in the form of production of reactive oxygen intermediates within the erythrocytes. Malondialdehyde (MDA), a product of lipid peroxidation is generated in excess amounts [10].

The oxidative stress may contribute to pathophysiology in malnutrition. Uttar Pradesh is geographically a large state of India. Review of the published articles on malnutrition in children from

Uttar Pradesh and other states of India has shown limited number of studies and none of them have combined the assessment of the parameters of oxidative stress, the trace elements in serum such as zinc and iron and the serum proteins, albumin and globulin in a single study [11-13]. Keeping the above fact in mind, the present study was aimed to assess the intensity of oxidative stress in undernourished children one-five years of age by measuring MDA level along with serum zinc, iron and protein levels and to compare with age and gender matched well-nourished children.

## MATERIALS AND METHODS

This case-control study was conducted in the Department of Biochemistry, Rohilkhand Medical College and Hospital (RMCH) Bareilly, Uttar Pradesh, India, from January to December 2014. The study was conducted after getting approval from Institutional Ethical Committee (IEC/32/2013). After explaining the aim and objectives informed consent was taken from parents of children for participation in this study.

**Inclusion criteria:** The moderate and severe undernourished children, aged between 1-5 years, attending Outpatients Department of Paediatrics of the study institute, were included as cases. Age and sex matched healthy well-nourished children were included as control.

**Exclusion criteria:** Child with sepsis or any acute illness, any systemic illness/liver disease, nephrotic syndrome, thalassaemia, children on micronutrient supplementation {Zn, Se, Cu, Magnesium (Mg)}, children on vitamin C or vitamin E supplementation, those parents' not giving consent/child refusal were excluded from the study.

**Sample size calculation:** Sample size was calculated by using the formula  $\{z^2pq/d^2\}$

Where  $z=1.96$ ,  $p$  is 48%,  $q=1-p$  and  $d=10\%$ , the calculated sample size comes to be 96, which are rounded up to 100 [14]. A total of 103 undernourished children aged 1-5 years were recruited as cases and 99 healthy well nourished children as control.

The cases were divided into two groups:

- Severe malnourished children (n=46)
- Moderate malnourished children (n=57)

### Data Collection

The requisite information were collected from the mother (or the guardian of the child if mother unavailable) about the biosocial profile and this was followed by general physical examination of the child including anthropometry and signs of malnutrition.

The moderate acute malnutrition and severe acute malnutrition were adopted from WHO classification.

- Normal child in the age group 1-5 years was defined as the weight-for-height z-score (WHZ) between  $\geq -2$  to  $\leq +2$  or mid-upper arm circumference (MUAC)  $>135$  mm.
- Moderate Acute Malnutrition (MAM) was defined as weight-for-height z-score (WHZ) between -2 and -3 or MUAC between 115 mm and  $<125$  mm [15].
- Severe Acute Malnutrition (SAM) was defined as WHZ  $<-3$  or MUAC  $<115$  mm, or the presence of bilateral pitting oedema, or both [16].

### Anthropometry

**Weight:** Weight was measured using the weighing scale especially for children under five years of age, supplied to the aganwadi workers under Integrated Child Development Services (ICDS) by Government of India. The weighing scale used was standardised to measure the maximum weight of 25 kg, closest to 100 gm. The children were weighed with minimal clothing.

**Height:** For children aged  $>2$  years, height was measured by stadiometer. Recumbent length from top of head to the bottom of heels was measured in children below two years.

**Assessment of nutritional status:** The nutritional status of the children was assessed by plotting the weight and height of the children on WHO 2006 Growth Standards growth charts using z-scores. Weight for age and height for age assessment was done by plotting the study subject's weight and height on different growth charts for boys and girls. Weight for height assessment was done by plotting on different graphs for 0-2 years and 2-5 years, as per the study subject's age, separately for boys and girls. Nutritional status of the children was classified according to the WHO classification into healthy, moderate acute malnutrition and severe acute malnutrition as given in the inclusion criteria [15, 16].

### Study Procedure

A 5 mL of venous blood was aseptically taken from each subject by using peripheral vein through venipuncture. After allowing 30 minutes for spontaneous blood clotting, the serum was separated from the blood cells by centrifugation at 2000 rpm for 10 minutes at 37°C. The removed serum was stored at 2-8°C until analysis in a capped disposable serum tubes. The investigations were performed in the laboratory of Biochemistry Department, of the study Institute on daily basis.

**Biochemical parameters and their respective methods included in the study were:**

- (i) Assay of serum zinc performed by a double beam spectrophotometer (Systronics), using a commercial kit (Randox laboratories).
- (ii) Malondialdehyde (MDA) estimated by Thiobarbituric acid assay (TBA) method as described by Satho's K, [17].
- (iii) Iron was estimated by Ferrozine method [18].

(iv) Total protein was measured by Biuret method as described by Reinhold JG [19].

(v) Albumin was estimated by Bromocresol Green (BCG) method as described by Hill PG, [20].

The reference range of normal levels of these parameters were [21]:

- 60-80 µg/dL for serum zinc,
- 60-160 µg/dL for serum iron,
- 6-8 g/dL for total proteins,
- 3.5-5.5 g/dL for albumin,
- 2.0-3.5 g/dL for globulin,
- 1-2 for A/G ratio

### STATISTICAL ANALYSIS

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Incorporation, Chicago, IL, USA). The collected data were expressed as frequencies, percentage and Mean±SD. Statistical comparison was done by using Chi-square test, t-test, one-way Analysis of Variance (ANOVA) and Pearson correlation. A p-value  $<0.001$  were considered highly significant also p-value  $<0.05$  was considered as statistically significant.

### RESULTS

Out of the total 202 participants in this study, the moderate malnourished, severe malnourished and the age and gender matched healthy children were comprised of 57 (28.2%), 46 (22.8%) and 99 (49%) participants respectively. In this study 101 male and 101 female children were included. Gender distribution among the moderate malnourished, severe malnourished children and healthy controls was statistically non significant (p-value=0.52) [Table/Fig-1].

Gender	Control n (%)	Moderate malnourished children n (%)	Severe malnourished children n (%)	Total n (%)	p-value
Female	53 (26.2%)	28 (13.9%)	20 (9.9%)	101 (50%)	0.523326
Male	46 (22.8%)	29 (14.4%)	26 (12.9%)	101 (50%)	

**[Table/Fig-1]:** Distribution of study population.  
Test of significance: Chi-square test  
The Chi-square statistic was 1.2951. The p-value was 0.523326

The mean ages of moderate malnourished, severe malnourished and healthy children were  $34.22 \pm 13.22$  months,  $33.99 \pm 12.61$  months and  $36.67 \pm 11.14$  months respectively and this difference in age was also not significant (p-value-0.325). Mean weight of moderate malnourished children was  $10.44 \pm 2$  Kg while that of severe malnourished children was  $9.36 \pm 1.54$  Kg. Weight of malnourished children compared to control subjects were significantly low (p-value  $<0.001$ ) [Table/Fig-2].

Parameters	Control Mean±SD	Moderate malnourished children Mean±SD	Severe malnourished children Mean±SD	p-value
Weight (kg)	12.73±2.36	10.44±2	9.36±1.54	<0.001
Age (months)	36.67±11.14	34.22±13.22	33.99±12.61	0.325
Height (cm)	91.69±9.02	86.75±15.02	88.17±9.54	0.020

**[Table/Fig-2]:** Anthropometric measurements in the different groups of study population.  
Test of significance: One way ANOVA

Compared to control, malnourished children have high serum MDA level which was statistically significant (p-value  $<0.001$ ). Serum zinc, iron level, Serum total protein, albumin and globulin level of malnourished children were significantly low (p-value  $<0.001$ ) as compared to control subjects [Table/Fig-3].

A negative and statistically significant correlation was found between the Body Mass Index (BMI) with serum MDA. A significantly positive correlation of BMI was found with iron and zinc [Table/Fig-4].

Parameters	Control (Mean±SD)	Moderate malnourished children (Mean±SD)	Severe malnourished children (Mean±SD)	p-value (ANOVA)
Zinc (µg/dL)	104.36±14.56	72.66±6.78	53.95±12.70	<0.001
Iron (µg/dL)	139.31±20.19	99.49±10.05	75.71±10.92	<0.001
MDA (nmol/mL)	0.71±0.18	1.84±0.38	3.44±0.59	<0.001
Protein (g/dL)	7.14±0.64	6.17±0.47	5.39±0.59	<0.001
Albumin (g/dL)	3.82±0.45	3.11±0.39	2.59±0.36	<0.001
Globulin (g/dL)	3.31±0.47	3.06±0.39	2.79±0.50	<0.001
A/G ratio	1.18±0.20	1.04±0.23	0.97±0.26	<0.001

**[Table/Fig-3]:** Serum zinc, iron, MDA, total protein, albumin, globulin and A/G ratio in the different groups of study population.

X-axis vs Y-axis	Coefficient of correlation 'r'	p-value of 'r'
Body mass index	MDA	-0.318
	Zinc	0.201
	Iron	0.301
	Total protein	0.079
	Albumin	0.101
	Globulin	0.0
	A/G ratio	0.076

**[Table/Fig-4]:** Correlation of BMI with various biochemical indices in the malnourished children.

Statistical test applied: Coefficient of correlation 'r' using Pearson's correlation

A statistically significant positive correlation was observed between serum MDA level with zinc, iron, total protein, globulin, A/G ratio and albumin. There was a significant positive correlation between zinc and total protein, albumin and globulin. A strong positive correlation was observed when serum iron correlated with total protein, globulin and albumin [Table/Fig-5].

## DISCUSSION

Undernutrition is a major public health problem worldwide particularly in developing countries [2,4]. Undernutrition impairs physical, mental and behavioural development of millions of children and is a major cause of child death. Protein and energy malnutrition

X-axis vs Y-axis	Coefficient of correlation 'r'	p-value
MDA	Zinc	0.7
	Iron	0.694
	Total protein	0.473
	Albumin	0.542
	Globulin	0.152
Zinc	A/G ratio	0.232
	Iron	0.771
	Total protein	0.560
	Albumin	0.524
	Globulin	0.293
Iron	A/G ratio	0.122
	Total protein	0.614
	Albumin	0.561
	Globulin	0.319
	A/G ratio	0.114

**[Table/Fig-5]:** Correlation and significance levels of various biochemical indices in the malnourished children.

Statistical test applied: coefficient of correlation 'r' using Pearson's correlation

and deficiencies of specific micronutrients (including iron, zinc and vitamins) increase the susceptibility to infection [2,4]. An overview of the comparison of few studies conducted on malnourished children with the present study on the similar parameters is shown in the [Table/Fig-6] [5,11,12,22-28].

The zinc requirement of growing children is high so they are more vulnerable to zinc depletion. In the present study the children with severe and moderate malnutrition had significantly low levels of serum zinc (p-value <0.001). Results of present study correlate well with previous studies done by Jain A et al., Ghone RA et al., Khare M et al., and Ugwuja El et al., [5,11,12,22]. Iron is an important integral component or essential cofactor for several metabolic processes which is deranged in PEM. In the present study, serum iron level of malnourished children was significantly low (p-value <0.001) when compared with normal children. Similar observation was also seen in study of Shaheen B et al., and Ejaz MS and Latif N [23,24]. Present findings did not agree with the study of Naomi E et al., that showed

Authors	Region of study	Publication year	Parameters included	Results and findings
Present study	Uttar Pradesh, India	2022	Serum Malondialdehyde (MDA), iron, zinc, albumin, globulin and proteins	Significant increase in the level of serum MDA and decrease in the level of serum iron, zinc, albumin, globulins and proteins in malnourished children.
Jain A et al., [5]	Madhya Pradesh, India	2008	Serum zinc and MDA	Significant increase in the level of serum MDA and decrease in the level of serum zinc.
Ghone RA et al., [11]	Maharashtra, India	2013	Serum zinc, MDA, vitamin E, erythrocyte superoxide dismutase	Significant increase in the level of MDA, decrease in the level of zinc, vitamin E and erythrocyte superoxide dismutase.
Khare M et al., [12]	Eastern Uttar Pradesh, India	2012	Serum micro-mineral levels	Low levels of zinc in children with PEM.
Ugwuja El et al., [22]	North Central Nigeria	2007	Serum zinc, copper, serum proteins, albumin, hemoglobin and total WBC count	Significant decrease in all parameters except the total WBC count.
Shaheen B et al., [23]	India	2013	Iron profile	The mean serum iron, ferritin and TIBC were significantly low in children with PEM than in the control group.
Ejaz MS and Latif N [24]	Karachi, Pakistan	2010	Height/length, serum iron, vitamin A and D level	Stunting, iron deficiency anaemia, deficiency of vitamin A and D
Naomi E et al., [25]	Calabar, Nigeria	2013	Serum iron transferrin saturation, serum ferritin, packed cell volume and haemoglobin	Significant decrease in all parameters except serum iron and transferrin saturation.
Rahman MA et al., [26]	Bangladesh	2007	Serum albumin, globulin, total proteins, serum iron and Total Iron Binding Capacity (TIBC)	Mean serum total protein and albumin level in normal children were significantly higher than that of malnourished children. But mean serum globulin level was higher in malnourished children than that of normal children. The mean serum iron and transferrin saturation level were significantly high in severely malnourished children than in normal children, whereas serum TIBC had no significant difference between these two groups.

Abdullah SF et al., [27]	Sudan	2014	Serum albumin	Serum albumin levels were significantly lower in malnourished subjects with edema compared to those with malnutrition but no edema.
Nwosu DC et al., [28]	Rural population of IMO state, Nigeria	2015	Vitamin A, serum proteins and albumin	Significant difference in the mean values of the vitamin A, protein and albumin, between boys and girls of malnourished children. Vitamin A level is significantly lowered in protein energy malnutrition children.

**[Table/Fig-6]:** An overview of the few studies conducted on malnourished children and the biochemical parameters involved in the oxidative stress [5,11,12,22-28].

the higher mean values the serum iron and transferrin saturation of the test group than that of the control [25]. The present study was also not correlated with study of Rahman MA et al., which showed normal level of iron and Total Iron-Binding Capacity (TIBC) in severe malnourished children [26]. The possible mechanisms of decrease in serum iron levels in present study may be due to poor diet, elevated needs and chronic loss from parasitic infections.

The MDA is the oxidised byproduct often used as a reliable marker of lipid peroxidation in malnutrition. In the present study, serum MDA level in severe and moderate malnourished children were extremely higher ( $p$ -value  $<0.001$ ) as compared to that in control subjects. Several mechanisms could contribute to enhanced oxidative stress in severe acute malnutrition. Inadequate dietary intake of nutrients such as carbohydrates, proteins and vitamins could be one of the important mechanisms that lead to accumulation of Reactive Oxygen Species (ROS). The second mechanism for increased oxidative stress in malnutrition may be chronic activation of the immune system due to chronic inflammation. Deficiency of antioxidants along with trace elements has also been reported in malnutrition [5,9,10]. The study conducted by Ghone RA et al., found significant increase in the level of MDA along with decrease in the level of zinc, vitamin E and erythrocyte superoxide dismutase [11]. Similar result was seen by Jain A et al., which showed that MDA concentration in malnourished children was significantly higher ( $p$ -value  $<0.001$ ) as compared to the control [5]. According to the study by Khare M et al., stress is created as a result of PEM which is responsible for the overproduction of ROS [12]. These ROS will lead to membrane oxidation and thus an increase in lipid peroxidation byproducts such as MDA and product of protein oxidation.

The present study showed a significantly low level of total protein and albumin ( $p$ -value  $<0.001$ ) in malnourished children compared to control. The A/G ratio of malnourished children were significantly low ( $p$ -value  $<0.001$ ) when compared with control. The findings of present study were consistent with the studies conducted by Abdullah SF et al. and Nwosu DC et al., [27,28]. So, the results of this study were consistent to show the biochemical and oxidative stress among the moderate and severe malnourished children of 1-5 years of age.

### Limitation(s)

In the present study, the measurements of association between the biochemical parameters of oxidative stress and the antioxidant mechanism were done. But authors could not investigate the comparative evaluation after antioxidants and micronutrient supplementation to the malnourished children. Further studies should be conducted in different locations with large sample sizes.

### CONCLUSION(S)

The findings in present study provide reasonable evidence for oxidative stress in undernourished children as shown by reduced levels of antioxidants such as serum zinc and iron and excess levels of lipid peroxidation metabolites such as MDA. Further studies should be conducted in a large geographical area with inclusion of more sample sizes. Comparative studies could evaluate the roles of micronutrients and antioxidants supplementation on the biochemical parameters of oxidative stress. The preventive measures for PEM such as exclusive breastfeeding for six months, food fortification and supplementation of micronutrients, trace elements and antioxidants

should be implemented to reduce the deleterious effects of PEM and under five mortality of children in the countries like India.

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**PLAGIARISM CHECKING METHODS:** [Jain H et al.]

- Plagiarism X-checker: Jun 18, 2022
- Manual Googling: Aug 30, 2022
- iThenticate Software: Sep 06, 2022 (15%)

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