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The study of liver function test in Malnutrition patients in Indore District

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Abstract:
Background: Malnutrition causes nutritional, metabolism, and biochemical disorders and finally leads to mortality. Several studies have highlighted that serum liver enzymes are increased and serum total proteinis decreased in patients with malnutrition.
Objectives: This study aimed to evaluate the relationship between malnutrition and liver biochemical marker (serum total protein , albumin, globulin, bilirubin, ALP, AST and ALT) in MP, India.
Materials and Methods: Present study comprises 120 participants of matched gender and age from the same population, 60 participants served as healthy controls, and 60 participants as cases of Malnutrition, with an age group ranging from 6 months- 5 years. serum (serum total protein , albumin, globulin, bilirubin, ALP, AST and ALT)was estimated by *Biosystems A-25 Fully Automated analyser* was used for measurement**.**
Result: The liver biomedical marker were significantly ($p>0.05$) raised in clinically diagnosed hypothyroid patients as compared to age and gender match healthy control subjects.
Conclusions: The findings indicated that there was Significantly low levels of total protein, albumin and globulin are present in our study population, No significant difference in the serum bilirubin values in malnourished children as compared to controls and there was no significant alteration in enzyme markers in the two groups. However, AST and ALT levels increased, and ALP levels decreased compared with the control. Different degrees of malnutrition, including mild, moderate, and severe, can probably change the levels of hepatic enzymes in under-nourished children. Alteration of these liver enzymes could be due to the metabolic modification, which can be the result of protein deficiency.

Kew words: Malnutrition, liver, Nutritional, SAM

Introduction

Malnutrition is one of the major health problems in developing countries, including India. It is also one of the biggest factors suppressing India's spectacular growth. In the current Indian population of 1100 million, there would be about 132 million under five-children (about 12% of population), of which about 8 million can be assumed to be suffering from Severe Acute Malnutrition (SAM).[1]

Geographically, Madhya Pradesh is the second largest state in India and has a population of about 72 million. Many of these reside in rural areas where they subsist on tiny farm plots. There are also a large number of tribal communities sustaining on agro-forestry and other meagre resources. Almost 40 per cent of the state's inhabitants live below the poverty line.

While high rates of malnutrition, child and maternal mortality have challenged this state, UNICEF, National and the State Government are making a positive impact with a range of programs. These include training thousands of village health workers to recognize and treat sick babies and encouraging women to rest & eat well-balanced meals during pregnancy. New hospital units for sick newborns are also saving lives.[2]

The World Health Organization (WHO) defines malnutrition as "the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions"[3] Malnutrition is classified into *severe acute malnutrition (SAM)* and *moderate acute malnutrition (MAM)* according to the degree of wasting and the presence of edema. WHO and United Nations Children's Fund (UNICEF) have proposed diagnostic criteria for severe acute malnutrition in children aged 6 to 60 months.[4, 5]

In present context, malnutrition is synonymous with 'Protein-Energy Malnutrition'. It develops in children whose consumption of protein and energy is insufficient to satisfy their nutritional needs. Number of biochemical parameters gets altered during protein energy malnutrition. They prove as early & sensitive indicators of development of PEM and provide valuable information for overall management. From the biochemical values, we can trace the exact nutritional status and also help in optimal, specific and precise management of PEM. So it can be inferred that biochemical markers belong among the basic examination in the diagnosis of malnutrition. Formation of serum proteins in the liver is connected with the nutritional status of the organism. In malnutrition, proteo-synthetic liver function is usually decreased. According to half-life of individual plasma proteins, duration of malnutrition can be determined.

Alterations in biochemical parameters play an important role in pathophysiology, development of clinical features and further complications in PEM. With this background knowledge, the present study aims to evaluate the various biochemical markers in young patients of malnutrition.

Materials and Methods

Present study was conducted in the Department of Biochemistry, Index Medical College Hospital And Research Center, Indore after the approval of Research Advisory Committee (RAC) and Institutional Ethics Committee (IEC).

The research protocol was in agreement with the Helsinki declaration. This study consists of malnourished children who were clinically diagnosed in the department of Paediatrics, during the period of 2020 – 22.

Selection of study subjects:

Present study included 120 children between the ages of 6 months to 5 years. Out of 120, sixty children were diagnosed with the help of paediatrician and were found suffering from severe acute malnutrition according to the diagnostic criteria proposed by WHO[4] and admitted to the nutritional rehabilitation centre, Index Medical College Hospital And Research Center, Indore. Sixty samples of healthy children were taken as controls. Controls were age and sex matched to cases, ratio of case to control was 1:1.

Inclusion and exclusion criteria:

Malnourished children having no clinical evidence of any infectious disease at the time of blood collection were taken as subjects. Children taking antioxidant supplements were excluded from the study.Informed consent was taken from parents of study participants.

Collection of blood samples:

Five ml of venous blood was withdrawn from each subject, collected in plain bulb and allowed for spontaneous blood clotting for 20-30 minutes. Samples were then centrifuged at 3000 rpm for 10 minutes at room temperature to separate serum from the sample. Separated sera were stored in deep freezer at -20^0C in Eppendrof tube vials until assay.

Observations and Results

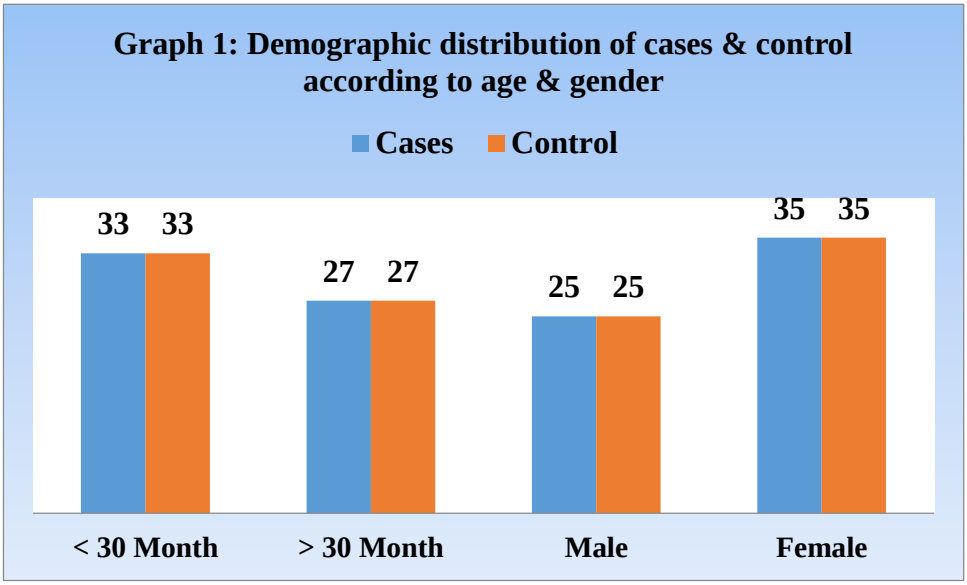
The present study included a total of 120 samples out of which 60 blood samples were of previously diagnosed severe acute malnourished children and 60 samples were of healthy controls. Controls were age and sex matched to cases with ratio of case to control 1:1.All cases as well as controls belonged to the age group 6 months to 5 years. Comparison between mean values of anthropometric and biochemical parameters of different groups were determined using *student t test*.

Table 1: Age wise distribution of study subjects

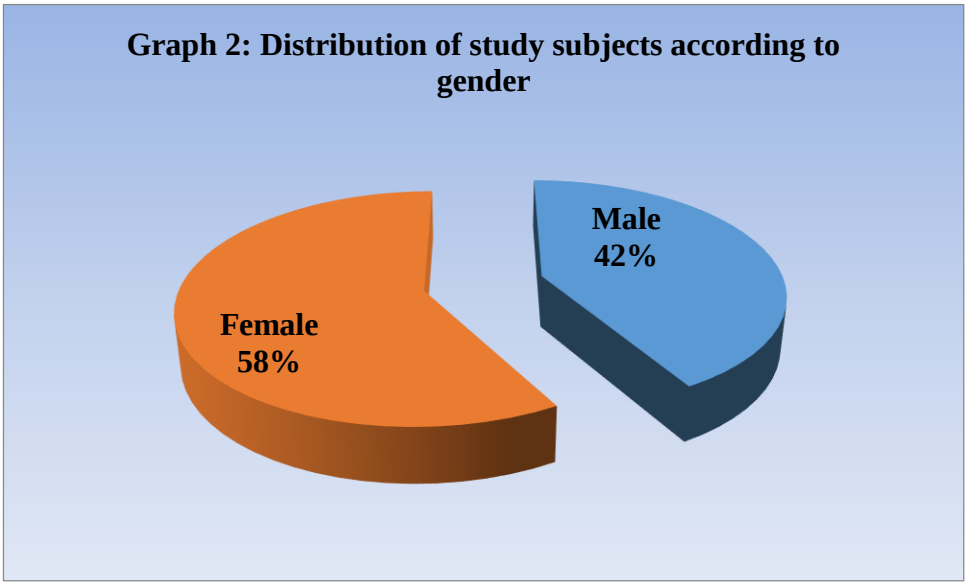
Age Groups	Cases	Controls	Total
< 30 Months	33	33	66
> 30 Months	27	27	54
Total	60	60	120

Table 2: Gender wise distribution of study subjects

Gender	Cases	Control	Total
Male	25	25	50
Female	35	35	70
Total	60	60	120



Among 120 subjects, 25 cases + 25 controls are males and 35 cases + 35 controls are females. Incidence of female is 58 % and male is 42 %. (Table-2, Graph-2)



When controls and cases were distributed according to the age, 66 were <30 Month (55%) and 54 were > 30 Month (45%). (Graph-3)

Table 3: Distribution of parameters of Liver Function Tests in Cases & Controls

PARAMETERS	Group	MEAN	SD	Student 't' test	p Value
Tot. Protein (g/dl)	**Cases**	5.10	0.23	30.218	<0.001
	Controls	7.17	0.47		
Albumin (g/dl)	**Cases**	3.04	0.11	49.282	<0.001
	Controls	4.52	0.20		
Globulin (g/dl)	**Cases**	2.06	0.19	11.927	<0.001
	Controls	2.65	0.33		
A/G Ratio	**Cases**	1.48	0.11	8.838	<0.001
	Controls	1.72	0.17		
Tot. Bilirubin (mg/dl)	**Cases**	0.68	0.16	0.454	0.651
	Controls	0.69	0.16		
AST (IU/L)	**Cases**	78.39	9.06	31.454	<0.001

	Controls	28.45	8.31		
ALT (IU/L)	**Cases**	68.61	8.71	20.351	<0.001
	Controls	30.48	11.60		
ALP (IU/L)	**Cases**	168.46	5.66	23.072	<0.001
	Controls	264.84	31.85		

Table showing the results of biochemical parameters measured in malnourished children and healthy controls. Mean values of various parameters of cases (n= 60) are compared with controls (n=60). Student's t-test was applied at 95% CI and results are expressed as mean ± standard deviation. P values< 0.05 are considered to be significant.Total protein, Serum albumin, Serum Globulin, A/G ratio, ALP as observed were significantly lower and AST, ALT are significantly higher in malnourished children ($p < 0.001$) as compared with healthy controls.

Table 4: Genderwise distribution of Liver Function Tests in Cases

Variables	**Group**	**MEAN**	**SD**	**Student 't' test**	**p Value**
Tot. Protein (g/dl)	**Male**	5.09	0.16	0.335	0.738
	Female	5.11	0.28		
Albumin (g/dl)	**Male**	3.06	0.10	0.772	0.443
	Female	3.03	0.12		
Globulin (g/dl)	**Male**	2.03	0.09	0.872	0.387
	Female	2.08	0.23		
A/G Ratio	**Male**	1.50	0.07	1.060	0.293
	Female	1.47	0.13		
Tot. Bilirubin (mg/dl)	**Male**	0.69	0.15	0.583	0.562
	Female	0.67	0.16		
AST (IU/L)	**Male**	78.23	8.88	0.114	0.910
	Female	78.50	9.32		
ALT (IU/L)	**Male**	67.74	8.60	0.650	0.518
	Female	69.23	8.86		
ALP (IU/L)	**Male**	169.21	4.15	0.86	0.393
	Female	167.93	6.53		

Table showing mean values of Total Protein, Albumin, Globulin, AST & ALT lower in males, while mean values of ALP higher, when compared with female malnourished cases. But difference was not statistically significant.

Table 5: Genderwise distribution of Liver Function Tests in Controls

Variables	**Group**	**MEAN**	**SD**	**Student 't' test**	**p Value**
Tot. Protein (g/dl)	**Male**	7.16	0.50	0.151	0.880
	Female	7.18	0.45		
Albumin (g/dl)	**Male**	4.51	0.21	0.310	0.758
	Female	4.52	0.19		
Globulin (g/dl)	**Male**	2.65	0.36	0.026	0.979
	Female	2.65	0.31		
A/G Ratio	**Male**	1.72	0.18	0.002	0.998
	Female	1.72	0.17		
Tot. Bilirubin (mg/dl)	**Male**	0.69	0.18	0.000	1.000
	Female	0.69	0.15		
AST (IU/L)	**Male**	28.22	8.50	0.175	0.862
	Female	28.61	8.28		
ALT (IU/L)	**Male**	29.09	12.34	0.781	0.438
	Female	31.47	11.11		
ALP (IU/L)	**Male**	264.00	34.61	0.172	0.864
	Female	265.44	30.23		

Table showing mean values of Total Protein, Albumin, AST, ALT & ALP lower in males, when compared with female healthy controls. But the difference was not statistically significant.

Discussion

Malnutrition is one of the most serious and large scale health problems allover India. The National Family Household Survey (2006) put the number of malnourished children in Madhya Pradesh at a whopping 6 million which is over 60 per cent of its total number of children under 5 years of age. Out of these 6 million malnourished children, 1.3 million have Severe Acute Malnutrition (SAM) and another 1 million have Moderate Acute Malnutrition (MAM).[6]

Severe forms of malnutrition are the leading killers of our paediatric population. Malnourished children particularly those with severe acute malnutrition, have a higher risk of death from common childhood illness such as diarrhoea, pneumonia and malaria. Nutrition-related factors contribute to about 45% of deaths in children under five years of age.[7]

Total protein and serum albumin can be considered as useful indicators of the nutritional status of the malnourished children and are so proposed as good markers of protein energy malnutrition. Many researchers reported total protein and serum albumin to be significantly lower in malnourished children in comparison to normal controls. Different fractions of serum proteins were also found to be useful for assessing malnutrition due to significant changes observed in their value. Albumin and beta fraction were significantly reduced while alpha 1, alpha 2 and gamma globulin fractions were significantly raised in malnourished children in comparison to controls. So the study of different fractions of serum protein electrophoresis is also a good marker of protein energy malnutrition. In this study, it has been observed that the Mean serum total protein and albumin level in normal children were 7.17 ± 0.47gm/dl & 4.52 ± 0.20gm/dl respectively which was significantly higher ($p < 0.001$) than that of malnourished children 5.10 ± 0.23gm/dl & 3.04 ± 0.11gm/dl. Mean serum globulin level (total protein – albumin) was 2.06 ± 0.19gm/dl which is significantly lower in malnourished children than that of normal children 2.65 ± 0.33gm/dl ($p < 0.001$).

This well expected finding was very much similar to the observations of the previous studies reported by Ugwuja et al (2007), Perampalli et.al (2010) & Suliman et al (2011).Proteins are important part of a balanced diet. They are the building blocks for body tissues. Daily requirement of protein in children is 1.75g/kg body wt/day. Only 10-15% of total energy is derived from proteins. When enough carbohydrates are present in the diet, proteins are not used for yielding energy. This is called *protein sparing effect* of carbohydrates. During starvation, proteins also act as an energy source.[8]

In our study, serum bilirubin was found to be within the normal range in malnourished children and healthy control group. The mean serum bilirubin level was found to be 0.68±0.16mg/dl in cases and 0.69±0.16 in healthy controls. There was no significant difference found between them ($p=0.651$). Similar findings were reported by Onyeneke EC et al [2003] and Bosnak et al [2010] in their study.

Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) are nonfunctional plasma enzymes and they are present in blood at levels million fold lower than in tissues. Their presence in plasma at levels above normal suggests increase rate of tissue destruction or hepatobiliary disorder. Assay of these enzyme activities in PEM is very useful to detect tissue damage that may occur in PEM before clinical manifestations are established.In this study, it has been observed that the mean serum AST and ALT levels in malnourished children were 78.39±9.06 IU/L & 68.61 ± 8.71 IU/L respectively which was significantly higher than normal healthy controls 28.45 ± 8.31 IU/L & 30.48 ± 11.60 IU/L ($p<0.001$) (Table-4, Graph 29,30). Kumari R et al [1993] has suggested raised AST and ALT may reflect effort by the body to maintain homeostasis through protein synthesis, mobilizing amino acid from tissue breakdown. Many of the abnormalities result from this metabolic readjustment due to protein deficiency. Any grade of PEM can be associated with alteration in the level of serum enzymes.[9]

Onyeneke EC et al [2003] [10] and Tadas AK et al [2012] [11]also reported similar results in their study. Tadas AK et al [2012] are of the view that the moderate rise in aminotransferases found in PEM is not due to damage to the liver. There is increased metabolism of amino acids released from increased tissue breakdown. Thus process of transamination is enhanced leading to increased activity of ALT and AST.

Though Alkaline phosphatase (ALP) is a protein found in all cells of the body, highest concentrations are found in cells of the liver, bones and bile ducts. ALP in blood is primarily derived from excess ALP that is released from these cells. Blood levels of ALP are often measured as part of routine blood tests. Normal level in children is generally less than 350 IU/L. Though many conditions, including liver and bone diseases can cause high levels of ALP, low blood ALP levels are uncommon. Malnutrition is the main cause of low ALP levels.

In the present study, it was observed that mean serum ALP level was found to be 168.46±5.66 IU/L in cases and 264.84±31.85 IU/L in healthy controls. A significant decrease in the level of ALP is found in the

malnourished children as compared to control ($p<0.001$). Edozein[1962][12], Olambiwonno et al [1976][13], Atinmo et al [1982][14], Kumari at al [1993][15]and Jain et al [2008][16]found similar results in their study.

The results of our present work combined with previous studies provide compelling evidence that oxidative stress is present in severe acute malnourished children along with inflammation and hepatic damage to some extent. These findings may have important clinical relevance. Extent of derangement of biochemical parameters can help plan specific treatment for malnourished children. Supplementation of antioxidants in the treatment plan can help in preventing the complications of severe acute malnutrition.

Conclusions

The findings indicated that there was Significantly low levels of total protein, albumin and globulin are present in our study population, No significant difference in the serum bilirubin values in malnourished children as compared to controls and there was no significant alteration in enzyme markers in the two groups. However, AST and ALT levels increased, and ALP levels decreased compared with the control. Different degrees of malnutrition, including mild, moderate, and severe, can probably change the levels of hepatic enzymes in under-nourished children. Alteration of these liver enzymes could be due to the metabolic modification, which can be the result of protein deficiency.

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