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HAEMATOLOGICAL AND IMMUNOLOGICAL ALTERATIONS AMONG PROTEIN ENERGY MALNOURISHED CHILDREN: A COMPARATIVE STUDY

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ABSTRACT

A comparative study was done to analyse the haematological and immunological profile of the protein energy malnourished children to that of the healthy ones. Following anthropometric assessment done on 100 randomly selected samples, a total of 92 children of age 3-12 years bifurcated as 43 healthy and 49 malnourished following WHO scaling were included and rest 8 children were excluded from the study. Further, the samples were subjected to haematological assessments including haemoglobin level, total serum protein and platelet count as well as immunological estimations which included serum IgA and CD_4 cell count. The results were then statistically analysed using unpaired t test at 95% confidence interval. Haematological depression among malnourished subjects showed Hb level (10.94 ±1.28gm/dl, P<0.0001; 10.02±1.39gm/dl, P<0.0001), platelet count (236.94 ±28.53million, P<0.0001; 254.73±23.47million, P<0.0001) and total serum protein (6.87±0.79gm/dl, P<0.001; 5.61±0.25gm/dl, P<0.0001) among male and female subjects respectively to be lower than that of the healthy subjects. Immunological parameters including serum IgA was found significantly higher among the malnourished male (342.73±59.85gm/dl, P<0.0001) and female subjects (328.64±61.90gm/dl, P<0.0001) than detected among healthy ones. On contrary, CD_4 cell count was recorded to be 639.59 ±133.90cell/µl (P<0.0001) and

625.36±118.73 cell/µl (P<0.0001) among malnourished male and female subjects respectively that was lower than the normal ranges. Thus, long-term nutrient deprivation with chronic protein deficits among malnourished children hinders immunoregulation phenomenon by increasing the serum IgA and depressing CD_4 cell count whereas the haematological profile like haemoglobin level, total serum protein and platelet count were decreased remarkably from their normal ranges in the body.

KEYWORDS: Protein Energy Malnutrition, immunodeficiency, undernutrition, haematological profile, immunological status.

INTRODUCTION

Malnutrition is considered as both a cause and consequence of ill health. Protein Energy Malnutrition is a major health problem to be taken care of globally.[1] It is one of the most important risk factor for morbidity and death, especially among the young children.[2] According to the recent estimates provided by WHO, 60% of the gross death of children of age less than five years among developing countries is attributed by malnutrition.[3] Malnutrition is the primary cause of immunodeficiency worldwide.[4] It has been estimated that by 2015, prevalence of malnutrition worldwide will be 17.6% with vast majority belonging to developing countries.

Children belonging to lower socio-economic group and especially of growing age living in countries where the diet is grossly deficient in protein, suffer from severe malnutrition which further hampers the immune function and increases the susceptibility towards infections.[5] Malnutrition has been documented to affect the blood and immunological profile among children remarkably. As mentioned in one of the study done by Viteri et al. (1970), Haematological profile is also hampered which mainly includes decreased haemoglobin levels, low level of platelet counts and decreased serum proteins etc.[6] It has been also concluded in one of the study done by T Rikimaru et al. (1998) that the mean concentrations of serum total protein, albumin, prealbumin were found significantly lower among chronically malnourished children.[7] Perhaps the best indication of altered protein metabolism in PEM is reduction in the level of circulatory plasma protein and the albumin fraction.[8] Various investigative phenomenons are being undertaken among Protein Energy Malnourished children to estimate the degree of depletion of thymolymphatic system and its effect on cell-mediated immunity.[9] The humoral immune system among malnourished children constituting of B lymphocyte subpopulations, serum IgG, and IgA levels,

immunoglobulin synthesis and metabolism are depicted to increase.[10, 11] The most eventual alterations in immune competence during Protein Energy Malnourished condition involves cell-mediated immunity, bactericidal function of neutrophils, the complement system and the secretory immunoglobulin A (IgA) antibody response.[12] The serum IgA level is considered as one of the prominent markers for malnutrition as it increases significantly and distinctly among Marasmic kwashiorkor samples. Serum IgA is considered one of the important aspects of cell mediated immunity and represents by far the largest area of contact between the immune system and the environment.[13] Significant reduction in CD_4 cell count has been noticed during deprived conditions as compared to that of the normal ranges.[14] Malnutrition results in severe immunodeficiency with depletion of CD_4 cells and depressed cell mediated immunity.[15] Our study was implemented with an objective to estimate and analyse the effect of Protein Energy Malnutrition (PEM) on the haematological and immunological aspects of body; and further to compare the results with the blood and immune profile of the healthy children so as to reach to a conclusion.

METHOD AND MATERIALS

A total of 100 samples of age 3-12 years were selected from Sonbhadra district of Eastern Uttar Pradesh through random sampling method. Prior to the study, the designed study protocol was approved by the institution's ethical committee and parents of all the subjects were required to submit a written informed consent and a medical health questionnaire was needed to be filled prior to participation. The samples were then assessed using a pre-formed questionnaire containing various parameters like socio-demographic, anthropometric and clinical status. Socio-demographic assessment included age, gender, parental education, working status and income etc. Anthropometric assessment included BMI and skin fold thickness. Weighing machine, anthropometer and skinfold calliper were used for measurements. The bifurcation of the healthy and malnourished samples was done following WHO standards for BMI (Body Mass Index) according to their weight for height. The research being a comparative study, anthropometric assessments were done on all the study samples as a result of which; a total of 92 children-43 healthy and 49 malnourished were included and rest 8 children were excluded from the study following inclusion and exclusion criteria as mentioned in the study design. The inclusion criteria for study subjects included age varying between 3-12 years; and consent to participate in the anthropometric and biochemical assessments. For biochemical examination, 5ml of venous blood was collected from each subject using sterile disposable syringes under aseptic condition, after cleaning the

venepuncture site with 70% alcohol. Henceforth, the children belonging to the respective groups were assessed for their haematological status which included distinct parameters like haemoglobin count (Hb), platelet count and total serum protein. Haemoglobin level was measured using colorimetric Assay kit. Platelet count was estimated using Automated Impedence Cell Counter and total serum protein was estimated using semi auto-analyzer. Further, IgA and CD_4 cell count were estimated for immunological analysis. Serum IgA was estimated using ELISA kit (Avandor Performances Materials India Ltd.) which is an in-vitro Enzyme-Linked Immunosorbant Assay for quantitative estimation. CD_4 cell count was estimated using flow cytometry (conjugated with FITC). After the determination of immunological as well as haematological estimations, the respective differences between healthy and malnourished children were evaluated statistically using unpaired t test (two-tailed) at 95% confidence interval with significance level at $P<0.05$.

RESULT

After segregating the study population into healthy and malnourished group following WHO standards, successive immunological and haematological analysis were done so as to evaluate the degree of differences between them and eventually result were chalked out. Table 1 depicts the BMI and skinfold thickness obtained through anthropometric assessments among the children of healthy and malnourished groups. It was observed that the prevalence of malnutrition was more among 3-5 years of children and that the BMI of the subjects belonging to malnourished group (13.7±1.03 Kg/m^2, P value <0.0001) was significantly lower than the healthy subjects (15.03±0.91 Kg/ m^2). Although the BMI between the healthy and malnourished groups of age ranging from 6-8 and 9-12 years didn't show any significant difference, though there were observable differences. The skinfold thickness of the malnourished groups belonging to 3-5 and 6-8 years showed significant lower values (3.92±0.73 mm P value<0.0001, 4.89±1.05mm P value<0.001 respectively) in comparison to the healthy ones.

Table.2 depicts the haematological observations among healthy and malnourished children as obtained following standard procedures. The haemoglobin level among malnourished males and females were found significantly lower than the healthy children i.e. 10.94 ±1.28gm/dl (P value<0.0001) and 10.02±1.39gm/dl (P value<0.0001) respectively, although the severity was found more among the girls. The platelet count was also found lower among the malnourished subjects (236.94 ±28.53million in males, P value<0.0001; 254.73±23.47million

in females, P value<0.0001) that showed significant difference when compared to the platelet count of the healthy samples. Total serum protein as obtained showed significant lower values among the malnourished male (6.87±0.79gm/dl, P value<0.001) and female subjects (5.61±0.25gm/dl, P value<0.0001) in comparison to the healthy ones.

Table.3 depicts the estimation of immunological parameters among healthy and malnourished children. The serum IgA level among malnourished male (342.73±59.85gm/dl, P value<0.0001) and female subjects (328.64±61.90gm/dl, P value<0.0001) were found significantly higher than the healthy subjects. The CD_4 cell count was recorded as 639.59 ±133.90cell/µl (P value<0.0001) and 625.36±118.73 cell/µl (P value<0.0001) among malnourished male and female subjects respectively. Therefore, it can be inferred that immunological parameters like serum IgA was found significantly higher in malnourished samples to that of the healthy ones whereas, on contrary the CD_4 cell count was found significantly lower in malnourished samples.

Table 1: Anthropometric study of children with malnutrition and showing evidence of various infections

Age (in years)	Average BMI(Kg/m^2)			Skin fold thickness Triceps(mm)		
	Malnourished sample	Healthy sample	Significance level (P value)	Malnourished sample	Healthy sample	Significance level (P value)
3-5	13.71±1.03 (n=22)	15.03±0.91 (n=12)	P<0.05***	3.92±0.73 (n=22)	6.01±0.87 (n=12)	P<0.05***
6-8	12.90±1.26 (n=16)	13.90±1.97 (n=14)	NS	4.89±1.05 (n=16)	6.16±1.03 (n=14)	P<0.05**
9-12	13.04±1.73 (n=11)	14.42±1.56 (n=17)	NS	5.74±1.14 (n=11)	6.35±1.09 (n=17)	NS

*=P value<0.0, **=P value<0.001, ***P value<0.0001 (at 95% confidence interval)
t=Unpaired T test, two tailed, p<0.05=significant

Table 2. Haematological estimation of malnourished and healthy samples

Hematological parameter	Male children				Female children			
	Healthy (n=25)	Malnourished (n=17)	Significance level (P value)	t	Healthy (n=18)	Malnourished (n=32)	Significance level (P value)	t
Hb(gm/dl)	14.52 ±1.36	10.94 ±1.28	<0.05***	8.57	12.51±2.04	10.02±1.39	<0.05***	5.123
Platelet count(million)	428.73 ±82.77	236.94±28.53	<0.05***	9.16	391.99±64.36	254.73±23.47	<0.05***	10.91
Total serum protein(gm/dl)	7.82 ±0.91	6.87±0.79	<0.05**	3.49	7.29±0.66	5.61±0.25	<0.05***	12.92

*=P value<0.0, **=P value<0.001, ***P value<0.0001 (at 95% confidence interval)
t=Unpaired T test, two tailed, p<0.05=significant

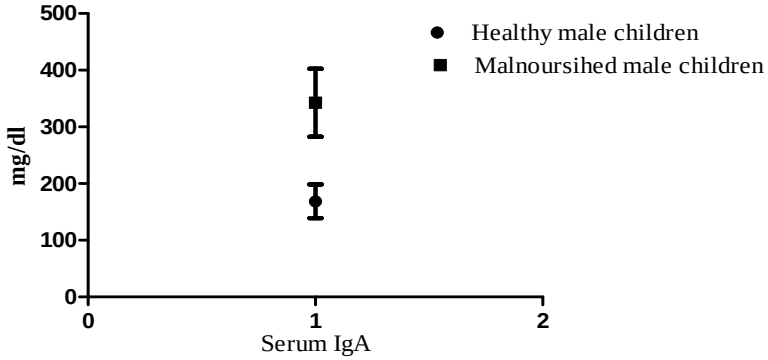
Table 3: Immunological estimation of malnourished and healthy samples

Immunological parameter	Male children				Female children			
	Healthy (n=25)	Malnourished (n=17)	Significance level (P value)	t	Healthy (n=18)	Malnourished (n=32)	Significance level (P value)	t
IgA (mg/dl)	168.73±29.98	342.73±59.85	P<0.05***	12.46	136.90±38.35	328.64±61.90	P<0.05***	11.89
CD_4 count (cell/µl)	924.84±121.86	639.59±133.90	P<0.05***	7.165	875.62±136.04	625.36±118.73	P<0.05***	6.788

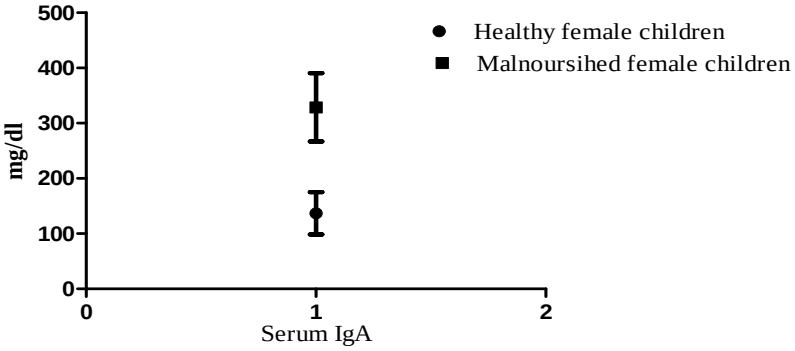
*=P value<0.0, **=P value<0.001, ***P value<0.0001 (at 95% confidence interval)

t=Unpaired T test, two tailed, p<0.05=significant

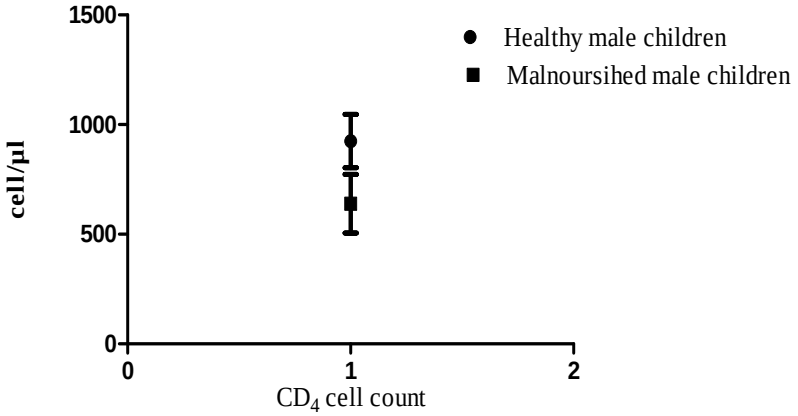
Estimation of serum IgA among healthy and malnourished male children



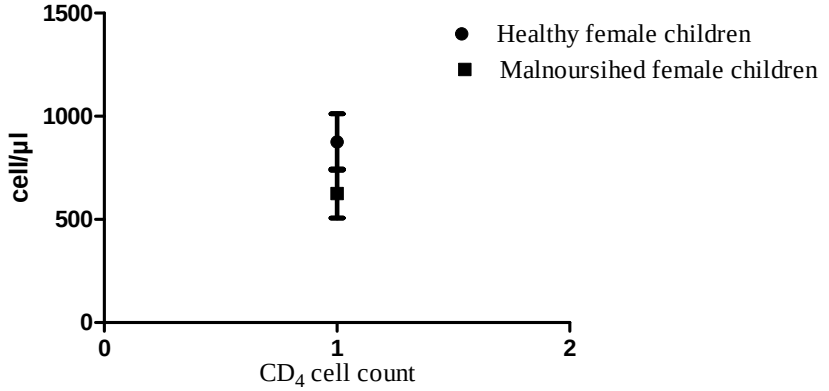
Estimation of serum IgA among healthy and malnourished female children



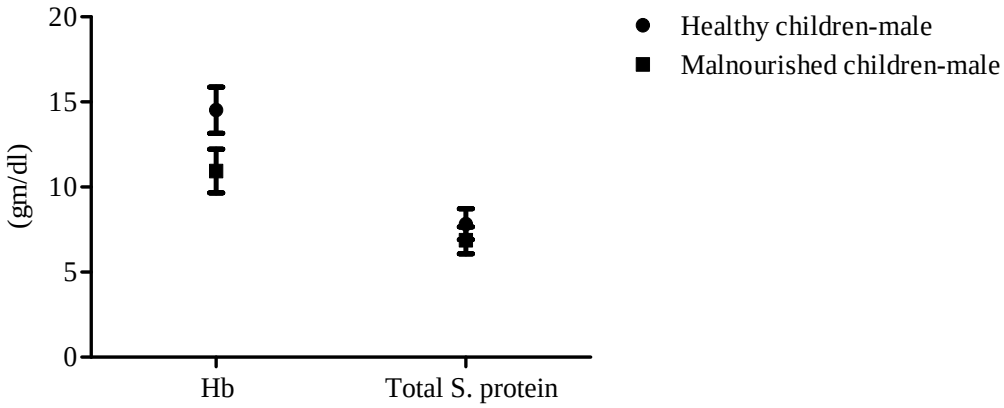
Estimation of CD_4 cell count among healthy and malnourished male children



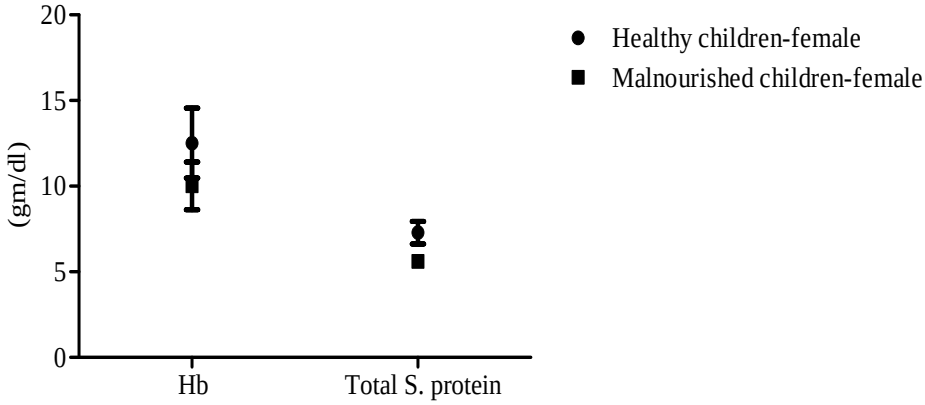
Estimation of CD_4 cell count among healthy and malnourished female children



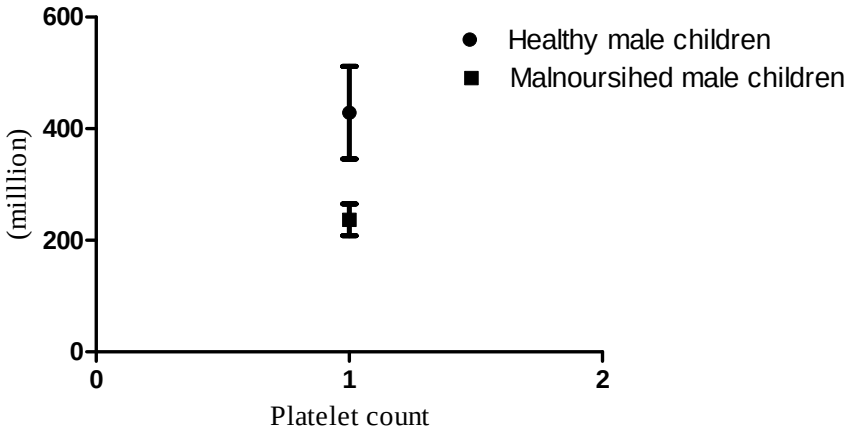
Estimation of serum Hb and total Serum protein in healthy and malnourished male children



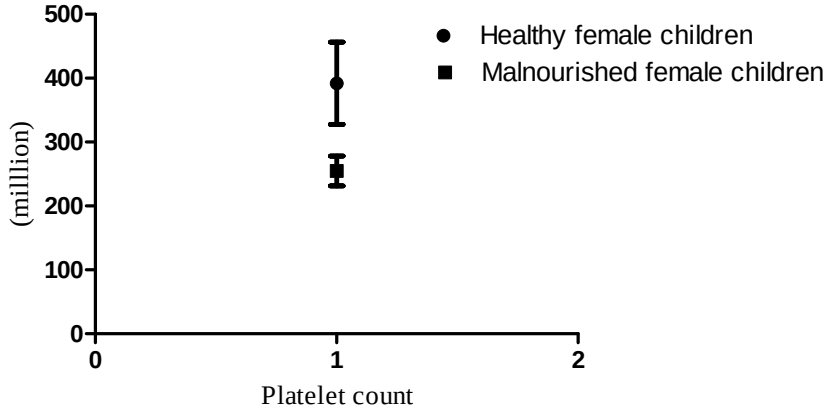
Estimation of serum Hb and total serum protein in healthy and malnourished female children



Estimation of platelet count in healthy and malnourished male children



Estimation of platelet count in healthy and malnourished female children



DISCUSSION

Many authors have demarcated malnutrition as one of the most important causes of immunocompetence globally.[16, 17] The immunological and haematological profiles of children are adversely affected due to malnourished condition. It alters and changes cell-mediated immunity, cell integrity and peripheral-lymphocyte subsets. Immunological depression may aggravate the health condition of children and result into secondary malnutrition that further influence other clinical conditions like inflammatory bowel syndrome, celiac disease, chronic anaemia, renal disorders etc (Susanna et al., 2008).[18]

In accordance to the haematological assessments, it was observed that the total serum protein of the malnourished groups- both males and females were significantly lower than the healthy ones (p value<0.0001). It has been documented by Jelliffe et al. (1990) that the total serum protein and albumin are expected to be below the normal ranges in severely malnourished

and wasted children.[19] Claudia et al. (2010) and Israil et al. have mentioned in their study that total serum protein and albumin among children with Kwashiorkor were significantly lower than those among the marasmic ones; and there was a significant relationship between serum protein, albumin and cellular immunity in the malnourished groups.[20] Hence, previous studies give a similar conclusion in accordance to observed results in our study. Lack of protein and elements like Fe, Zn and Cu may result in lymphopenia where cell proliferation ceases to minimal levels (Frayn et al., 1986).[20, 21]

Heamogolbulin level is also said to be decreased in nutrient deficit condition. Our study reveals that the Hb level in malnourished children was significantly lower than the healthy children at 95% confidence interval (p value<0.0001). Significant fall in Hb level and packed cell volume is explained in many of the previous studies with the reason being protein depletion (Allen and Dean, 1993).[22] It has also been concluded that erythropoetic adaptation occurs in children with PEM rather than anaemia as a result of depletion in the lean body mass. Thus our study results are evidential and are in synchronisation with the previous conclusive studies. One of the study documented by Viteri and Alvardo (1970) as well as (Ramdath and Goklein, 1989) that the circulating haemoglobin decreases proportionally in relation to the degree of depletion or decrease in lean body mass and decreased serum ferritin levels.[23, 24] Omer et al. (2011), in his study inferred that malnourished children were having significantly lower Hb levels as discussed in many of the previously done case studies.[25, 26, 27, 28] Decreased Hb level and Fe deficiency may depress bacterial activity of leucocytes (Sousa et al., 1978).[29] Iron deficiency is more commonly seen in children with Kwashiorkor than those in marasmic ones (Jelliffe, 1989; Suskind, 1990).[30]

Nutritionally deficient diet hinders proper immune function and also diminishes the efficacy of the host defense mechanism, eventually increasing the susceptibility to various infections and nutrient loss (Stekel, 1984).[31] Serum IgA level are considered as one of the remarkable determinant of immune function as malnourished condition may leave a child with deviated IgA levels as a result of repetitive infections. Immunological estimations as done in our research work showed that serum IgA levels in malnourished males (342.73mg/dl) and females (328.64 mg/dl) were significantly higher (p valu<0.001) as compared to the healthy samples. Omer et al. (2011), in his study concluded that marasmic groups were shown to have significantly higher serum IgA and IgG levels in comparison to the other groups which was in accordance to the reports mentioned by other authors.[32] In a previous study

conducted by Tanusree et al.(2014), it was observed that the mean serum IgA levels among undernourished children between 5-10 years of age were higher than the healthy samples,[33] although a significant correlation were drawn between undernutrition and Serum IgA levels (Suskind et al, 1976).[34] It has been mentioned that high levels of immunoglobulins may emphasize decreased catabolism by the impaired liver which is evidential in kwashiorkor samples (Murson et al., 2014).[35] It has been hypothesized by Alvardo et al. (1971) that increased IgA level is probably due to frequent Gastrointestinal Tract Infections.[36] Macpherson et al, in the year 2008 have depicted that high titre of protective antibodies at the mucosal sites is caused by immunization or infection whereas Suskind et al in 1995 documented that IgA levels were higher in malnourished children in comparison to controls and returned to normal with treatment (Suskind, 1995).[37] consistent results have shown that IgA level is elevated in malnutrition irrespective of the type of malnutrition (Watson et al, 1985; Ozkan et al, 1993; McMurray et al, 1981).[38, 39, 40]

The CD_4cell counts were estimated to be significantly lower among malnourished males (639.59 cell/µl) and females (625.59 cell/µl) with respect to the well nourished children. The result was statistically significant at 95% confidence interval (p value<0.0001). In accordance to our study process, previously done study by Meta-Marin and other co-workers (2010) inferred that the CD_4 cell count below 200cells/ mm^3 as one of the important risk factor aggravating anaemia among malnourished subjects.[41] In a study, Ruhinda et al. (2012) concluded that advanced disease conditions along with low CD_4 count occurs during the younger ages among the children surviving in malnourished conditions.[42] It has been said that they are also associated with anaemia which is considered as one of the common haematological abnormalities which has shown a directly proportional relationship with CD_4 cell counts (Leoner et al., 2005).[43] A low level of CD_4 cell count is widely marked as an essential indicator of thymic depression leading to immune competence.[44] Low CD_4 lymphocyte counts are said to be associated with a variety of conditions, including many viral infections, parasitic infections, sepsis, tuberculosis and infections of foreign proteins among malnourished groups (Matt et al., 2001).[45] Authors have also found that reduced CD_4 count were natural physiological effect of malnutrition that leads to a state of anergy alongwith failure to cell-mediated immunity (Hegde et al., 1999).[46]

CONCLUSION

Prolonged nutrient deprivation or starving conditions make children more susceptible to homeostatic alterations by hampering their immune system and defense mechanism. Chronic protein deficit in the diets of underprivileged children who suffer from varying degrees of malnutrition renders the body utilise its own store thus depressing the lymphatic system and blood profile. Haematological parameters like Hb level, total serum protein were estimated to be significantly lower among the malnourished children. Serum IgA was found significantly higher among the malnourished group than the healthy children. On contrary, the CD_4 cell count was found significantly lower among the malnourished study samples. Immunological alterations increases the risk factors and prevalence of various infections and chronic diseases whereas, haematological alterations render weak oxygen carrying capacity, anaemia, hypoglycaemia etc. Hence, immunocompetence during the phase of malnutrition may lead to morbid conditions that may further lead to mortality.

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Author's contribution

The authors contributed significantly towards the research study *i.e.,* (a) conception, design and/or analysis and interpretation of data and to (b) drafting the article or revising it critically for important intellectual content and on (c) final approval of the version to be published. Monalisa Debnath planned the study design and conducted the field study along with related laboratory work; generated data for further analysis presented in the paper in the form of manuscript. Aruna Agrawal (3^{rd} Author) designed the Study Performa to be applied in the field and provided interpretations. G. P. Dubey (4^{th} Author) supervised by giving the idea to chalk out the whole research process.

Conflict of Interest

The authors declare that they have no conflict of interest.

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