Research Article

Evaluation of Positive Relation between Pro-Inflammatory Cytokines and Malnourished Children: Eastern Uttar Pradesh-India

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Abstract

Malnutrition affects more than 95.9% of population in developing nations, and it has a big impact on children's health and their development and also associated with high morbidity impact and it is linked with changes in immunological-cytokines. The researchers needed to look at pro-in lammatory mediators in both healthy and underweight children. The goal of present study was to ind and compare the levels of pro-in lammatory cytokines in the control (healthy) and malnourished children. A total of 151 children were involved in this study, including 96 malnourished children and 55 healthy children of identical age and gender. The clinical, demographic, and plasma levels of Interleukin-1beta (IL-1beta) and 6, Tumor Necrosis Factor-alpha (TNF-alpha) and C-Reactive Protein (CRP) of these subjects were all evaluated using an ELISA kit. When compared to healthy children, malnourished children experienced a considerable reduction in weight and Weight-for-Age Z score (WAZ). Furthermore, the levels of serum interleukin-1 (IL-1) (0.10 *vs.* 0.05) and serum Interleukin-6 (IL-6) (pg/ml), (1.5 *vs.* 1.6), TNF-alpha (pg/ml) (1.7 *vs.* 1.8), and CRP (mg/l) (0.7 *vs.* 1.0) were signi icantly higher in the malnourished group compared to the control group. Pro-in lammatory cytokines (IL-1, Interleukin-6 (IL-6), TNF-alpha and CRP were found at higher concentrations in the blood of malnourished children.

Keywords: Malnutrition; Interleukins; Tumor necrosis factor-alpha; C-reactive protein

Introduction

Undernutrition, frequently known as malnutrition, is caused by nutritional malabsorption, and according to various prevalence-based studies, one out of every ten children in the globe is malnourished, with more than 95.9 percent of those residing in underdeveloped countries [1]. Malnutrition (Under nutrition) is a challenging community health issue that affects roughly one-third of children in impoverished nations. It is responsible for 14-17 percent of all child fatalities worldwide persistently, inadequate food intake and low-grade inflammation during pre-natal and post-natal development involved reducing production of growth hormone axis, early in life, and other factors enhance malnutrition in the child [2-5]. Deficits in micronutrients may contribute to the development of intrauterine growth retardation. Because specific micronutrients are essential for body development, maturation phase, sensorimotor development, and overall wellness. Undernutrition among children is the primary triggers of immunodeficiency those implications for both innate and acquired immunity [6,7]. A number of immune defects have been reported, likely an imbalance in the ratio of "CD_{4+}/CD_{8+} T cells [8,9], low CD_{69} expression levels on lymphocytes [10,11], biased T helper cell responses, reduced antibody responses, impaired phagocytosis by macrophages" [12,13], lower nitrite/nitrate concentrations in

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wound fluid [14] and decreased NF-kappa activation by macrophages [15]. Although, nutrient deficiencies are more common among children under the age of five, caused by insufficient dietary intake, an increased requirement for nutrients for rapid growth, and the onset of infectious disorders. It also has something to do with bone density, morphology, and stiffness. Inadequate food intake during growing phases can cause rickets and interfere with the attainment of genetically programmed height. Poor nutrition associate with malnutrition [5-7]. Although these deficiencies are associated with poor childhood growth, decreased immune competence, chronic diarrhea, anemia, and higher infectious illness morbidity [10]. Micronutrient deficiencies and viral illness frequently coexist and exhibit complex relationships in malnourished children, resulting in "Acute inflammation" that is associate with the activation of other components like-macrophages, natural killer cells, and the complement system and as resulting in the fabrication of "Cytokines" [15-17]. Acquired immune responses are suppressed, but innate immune responses are amplified, and positive acute-phase proteins such as CRP are released, all of which contribute to the inflammatory response. Excessive inflammatory response resulting from an imbalance between pro-inflammatory and anti-inflammatory mediators is a key contributor to the development of Clinical Malnutrition Syndrome. TNF is comprised of two homologous proteins first one-TNF-a and secondly, TNF-b and both are produced primarily by mononuclear phagocytes (TNF-a) and lymphocytes (TNF-b) respect while both cytokines are active in a homotrimer form. However, TNF-a production in malnourished children must be examined because TNF-a deficiency may contribute to the immunological deficits that associated with famine whereas TNF-a overproduction, might exacerbate nutritional problems by causing anorexia and cachexia. Several studies reported that the Interleukin-1beta (IL-1beta) and Interleukin-6 (IL-6) are act as essential inducers for the production of "hepatocyte acute phase protein. Growth problems like growth impairment in the type of children are caused by persistent inflammation phase along with excessive synthesis of IL-1betaand -IL-6, which results in the form of stunting. Consequently, it create hindernce in the synthesis of the "Insulin-Like Growth Factor Binding Protein-3 Gene" in the liver and enhances the proteolytic degradation of the insulin-like growth factor binding protein-3 (IGFBP-3). It was revealed by seder quist and colleagues, that inflammatory cytokines including "TNF-a and IL-1 beta", and interleukin-6, can have an influence on newborn development either alone or in combination. These biochemical substances have the potential to act both systemically and locally at the growth plate of long bones. The presence of Interleukin-6 (IL-6) produces CRP in the liver. On the other hand, severe enteropathy during infancy, has been linked to low levels of IGF-1 in the mother and hyper CRP and 'Alpha1-acid glycoprotein' during infancy, which has been linked to low-grade chronic inflammation. Taking into consideration this information, the current study aims to assess the levels of inflammatory markers IL-1beta, IL-6, TNF-alpha, and CRP in malnourished newborns of both Tribal- Rural areas of Uttar Pradesh.

Materials and Methods

Subjects

The institute institutional ethics committee of medical sciences, Banaras Hindu University, and Varanasi approved the studies. Children were selected from the Indian cities of Sonbhadra, Chandowali, and Varanasi, which are located in Eastern Uttar Pradesh [15]. Teachers at each of the participating schools addressed the parents, and informed agreement was sought from the parents of the children before they were allowed to participate in the study. The present case control study was conducted on 96 malnourished children and 55 healthy children aged 5 to 12 years, and as determined by height-2SD for matched age and sex; According to the World health organization height for age Z score growth chart reference for school aged group children [16]. In order to obtain further information regarding undernourished children in Indians, controls were utilized rather than cases because of the high prevalence of malnutrition within the population. The BMI of a kid was estimated by taking their weight and dividing it by their height squared, which was done with the help of scales and an audiometer.

The subjects were selected on the basis of the following inclusion and exclusion criteria

Inclusion criteria: According to the WHO 2007, both male and female children aged 5 to 12 years with dietary reasons of delayed linear development and height below the 2-curve of standard growth curve of both sexes were included in this study [18].

Criteria for exclusion: Young children, who were experiencing puberty symptoms, having endocrine abnormalities, having chronic or systemic illnesses, were suffering from genetic problems, having short family stature, and have significantly disproportionate short height. Children who were taking any medications that had an effect on their growth or hormones were also banned from taking part in the study. All children were subjected to a thorough examination of their past, which included their "Age, gender, height, and family history" along with other prospect like clinical examination, routine investigation, including complete blood picture, and weight and size monitoring (m).

Sample preparation and biochemical analysis

Fasting in the morning, blood samples were obtained by venipuncture, and serum was separated by centrifugation. IL-1 (pg/mL), IL-6, CRP (mg/L), and TNF-alpha (pg/mL) levels in serum were measured using commercially available ELISA kits (ENZO Life sciences, New York).

Statistical analysis

The data was analyzed using "Statistical Product and Service Solutions" computer-based statistical tool, version 16.0. (SPSS) and mean and standard deviation was used to show all of the data. The independent samples "T-test and the Mann–Whitney Test" were employed in continuous variables with and without normal distribution and if there were any differences between malnourished and control (healthy) children in referenced weight gain. Because the inflammatory parameters were skewed, the data were log transformed in order to get data that was more evenly distributed throughout. The "Spearman Correlation Coefficient (SCC)" and the "Bivariate Correlation Coefficient (BCC)" were used to conduct the correlations.

Results

The present study observed that no significant variations in age or gender between the control and malnourished children. Data in Table 1 demonstrated that malnourished children had 'lower weight, Weight-for-Age Z score (WAZ), height, height-for-age Z score (HAZ), and hemoglobin than healthy children (control)'. Both male and female were initially evaluated for malnutrition; based on Body Mass Index (BMI) (weight (kg)/height^2 (m^2)) using World Health Organization (WHO) standards that specify typical levels for 5 to 12-year-olds as 15.26-17.53 for boys and 15.24-18.00 for females. When compared to the reference values, malnourished children had body mass index values that were two times lower than the standard deviation of those values [16]. Table 2 shows that malnourished children had significant increases in serum 'IL-1, IL-6, TNF-alpha', and CRP as compared to control value. Furthermore, among malnourished children, IL-1 revealed a strong negative connection with WAZ (r=0.298, p=0.005) and weight (r=0.354, p=0.005) in Table 3. IL-6, TNF-alpha, and CRP all revealed a significant negative connection with WAZ (r=0.247, p=0.005), (r=0.547, p=0.005) and (r=0.254, p=0.005), respectively, in the same table. It also revealed a strong positive association between CRP and IL-1 (r=0.265, p=0.05), IL-6 (r=0.198, p=0.05) and TNF alpha, (r=0.179, p=0.005), as well as IL-1,(r=0.265, p=0.005).

Table 1: Showing different demographic and anthropometric properties of control and malnourished children-Eastern Uttar Pradesh.

Demographic and anthropometric characteristics	Control (N=55)	Malnutrition Children (N=96)	P value
Sex M/F	24/21 (43%; 38%)	58/38 (60%; 39%)	
Age	8.32 ± 0.29	8.23 ± 0.13	0.956
Weight (Kg)	21.64 ± 4.15	16.4 ± 3.54	0.005
WAZ	-0.68 ± 0.7	-1 ± 0.58	0.000
Height (CM)	112.15 ± 10.2	101.58 ± 90.1	0.005
HAZ	-0.9 ± 0.85	-3.05 ± 0.7	0.005
BMI	14.66 ± 0.11	10.07 ± 0.15	0.001
Haemoglobin (gm/dL)	8.3 ± 15	11.2 ± 1.36	0.005

"Data are expressed as mean ± SD". "N: the number of subjects in each group (WAZ: Weight-for-age Z score, HAZ: Height-for-age Z score, BMI: Body mass index, BMIAZ: BMI-for-age Z score)".

*Significant difference- P<0.05.

Table 2: The levels of pro-inflammatory marker -IL-1β (pg/mL), IL-6, CRP (mg/L) and TNF-alpha (pg/ml) in the control and malnourished children of Eastern Uttar Pradesh.

Inflammatory Marker	Control (n=55)	Malnourished(n-96)	P-value	Cut off
IL-1β (pg/mL)	0.10 ± 0.89	0.05 ± 0.28	0.005	1.2-1.9
Interleukin$_6$ (pg/mL)	1.5 ± 0.3	1.8 ± 0.3*	0.005	1.3–1.7
CRP (mg/L)	0.7 ± 0.2	1.1 ± 0.2*	0.000	0.5–0.9
TNF-alpha (pg/mL)	1.6 ± 0.1	1.9 ± 0.1*	0.018	1.5–1.8

Analysed data were presented- Mean ± SD.

Table 3: Correlation between pro-inflammatory marker (log IL-1β, logIL-6, log TNF-alphaand log CRP) and measured parameters in malnourished children of Eastern Uttar Pradesh.

Parameter	log IL-1β (pg/mL)	LogIL-6 (pg/mL)	logTNF-alpha (pg/mL)	CRP (mg/L)

Age (years)	-0.197 (>0.05)	-0.189 (>0.05)	0.024 (>0.05)	-0.097 (>0.05)
Weight (kg)	-0.354 (<0.05)	-0.398 (<0.05)	-0.3114 (<0.05)	-0.147 (>0.05)
WAZ	-0.298 (<0.05)	-0.247 (<0.05)	-0.547 (<0.05)	-0.254 (<0.05)
Height (cm)	-0.337 (<0.05)	-0.331 (<0.05)	-0.356 (<0.05)	-0.195 (>0.05)
HAZ	-0.284 (<0.05)	-0.266 (<0.05)	-0.269 (<0.05)	-0.566 (0.00)
BMI (kg/m2)	-0.115 (>0.05)	-0.046 (>0.05)	-0.076 (>0.05)	-0.066 (>0.05)
BMIZ	-0.084 (>0.05)	-0.024 (>0.05)	0.041 (>0.05)	0.033 (>0.05)
CRP (mg/l)	0.265 (<0.05)	0.198 (<0.05)	--	--
TNF alpha (pg/ml)	0.319 (<0.05)	--	--	0.179 (<0.05)

"Significant correlations at P<0.05. (WAZ: Weight-for-age Z score, HAZ: Height for-age Z score, BMI: Body Mass Index, BMIAZ: BMI-for-age Z score, IL-1β and 6: Interleukin-6, Tumor necrosis factor-alpha (Tumor necrosis factor-alpha (TNF-alpha)): Tumor necrosis factor-alpha, CRP: C-reactive protein)"

Discussion

Pro-inflammatory cytokines (Interleukin-1(IL-1), interleukin-6 (IL-6), Tumor necrosis factor-alpha (Tumour necrosis factor-alpha (TNF-alpha), and CRP, sometimes known as inflammation markers, were found to be greater in malnourished children's serum. In a recent study level of pro-inflammatory marker in malnourished children, play important role in their development [11-13], it was discovered that malnourished children had significantly higher levels of Interleukin-1 (IL-1), interleukin-6(IL-6), Tumor necrosis factor-alpha (Tumor necrosis factor-alpha (TNF-alpha), and CRP than healthy control participants. Previously, it was revealed that undernourished children's mean plasma folate and vitamin levels were much lower than those of their controls [17-20]. The elevated concentrations of interleukin-1 (IL-1), interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-alpha), and CRP in malnourished children could be linked to the fact that micronutrient deficiencies in such children affect various immunity elements, including cell-mediated immune responses and cytokine production [7]. Long-term and excessive production of IL-1 and IL-6 in malnourished children slows linear growth by reducing circulating IGF-I, a hormone produced primarily by the liver that promotes growth hormone production [15]. TNF-alpha (tumor necrosis factor) (TNF-alpha) has also been proven to reduce circulating IGF-I levels by lowering its hepatic production [19]. The current study found a substantial negative correlation between IL-1, IL-6, Tumor necrosis factor-alpha (Tumor necrosis factor-alpha (TNF-alpha-alpha) and CRP with WAZ and BMI in malnourished children. Inadequate nutrient intake is likely to increase systemic inflammation, which has been shown to mediate malnutrition and promote systemic inflammation, eventually resulting in poor growth and development. Aside from these significant benefits, nutrients (minerals or micronutrients) have anti-inflammatory capabilities, where they can reduce the generation of pro-inflammatory cytokines and modulate the tissue-specific immune response [21]. Nutrient metabolism is mediated by binding to a high-affinity nutrients receptor, which functions as a ligand-activated transcription factor, controlling the expression of several cytokine genes to restrict TNF-a, interleukin-2, and interferon production, so lowering inflammation [22]. The current study found a strong negative connection between TNF-alpha and CRP levels in malnourished participants.

Conclusion

Our study revealed that malnourished children exhibited elevated or hyper serum levels of pro-inflammatory cytokines IL-1 beta, IL-6, TNF-alpha and CRP.

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Conflict of Interest

Authors declared no conflict of interest.

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