ORIGINAL ARTICLE

Evaluation of changes in pro-inflammatory cytokines in malnourished children: A Ghanaian case study

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Protein-energy malnutrition (PEM) is a public health problem and is associated with high morbidity and mortality. PEM is linked with changes in biochemical and immunological parameters. This study aimed at determining the level of pro-inflammatory cytokines among healthy (control) children and those with PEM as diagnostic indicators for PEM. The study was conducted between December 2009 and June 2010 comprising a total of 115 children (35 controls and 80 malnourished children) aged between 8 – 36 months attending the Maternal and Child Health Hospital (MCHH), Kumasi. Anthropometric parameters including weight, height and mean-upper arm circumference as well as immunological and biochemical parameters (interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), albumin, total protein) were assessed among the studied population and the control group. After the analysis, 67.5% had marasmus, 18.8% had marasmic kwashiorkor and 13.8% had kwashiorkor. There were no statistically significant differences (p>0.05) in the mean total protein concentration of the subjects before (66.3 ± 1.6 g L⁻¹) and after (69.6 ± 1.7 g L⁻¹) nutritional supplement when compared to that of the controls (68.37 ± 1.4 g L⁻¹). Serum albumin concentration in the control group (43.2 ± 0.9 g L⁻¹) was significantly higher than the concentration in the subject group before treatment (38.7 ± 0.9 g L⁻¹, p<0.0027). The mean concentration of IL-6 in the subjects at baseline (46.1 ± 7.5 pg mL⁻¹, p<0.0008) and after treatment (26.3 ± 5.2 pg mL⁻¹, p<0.0148) were significantly higher than that in the control group. A 43.8% decrease in the mean concentration of IL-6 was observed after treatment. TNF-α concentration before treatment (82.1 ± 6.0 pg mL⁻¹) was significantly higher when compared to the mean concentration in the control group (55.8 ± 2.2 pg mL⁻¹). The study observed increases in pro-inflammatory response in malnourished children with IL-6 concentration being a significant indicator of PEM in the subjects compared to TNF-α.

Keywords: Protein-energy malnutrition, children, kwashiorkor, haematology, Ghana

INTRODUCTION

Protein-energy malnutrition (PEM) is a problem of public health importance in many developing countries. It is a body depleting disorder that has been identified as an important underlying factor in about 50% of deaths of children <5 years of age in developing countries (Black et al., 2003). Children between the ages of 12 to 36 months who are susceptible to infections are particularly at risk (WHO, 2000). In Ghana, about 40% of all childhood (Under five) deaths are due to malnutrition. It is estimated that about 84% and 68% of children living in the rural and urban areas respectively are affected (GDHS, 2003; GDHS, 2008). Protein-energy malnutrition in surviving children is known to be associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A antibody concentrations, cytokine production and an
altered immune response as well as susceptibility to infection (Chandra, 1991; Pelletier et al., 1995).

Lack of food or presence of infections that increase the body’s nutrient requirements and losses are the main cause of PEM (WHO, 2000). It has been suggested that cytokines play an important role in the nutrition-infection complex. Protein-calorie malnutrition, deficiency of fatty acids, vitamins and trace elements impair cytokine production (Muñoz et al., 1995). On the other hand, infections increase pro-inflammatory cytokine production interfering with nutritional status by impairing metabolic activity and by inducing anorexia (Muñoz et al., 1995). The diagnosis of malnutrition in children has generally been based on measurements of nutritional status, which include assessments of oral intake, weight loss, anthropometric data, and determination of cell-mediated immunity, biochemical parameters, physical examination and body composition analysis (Hulst et al., 2004). The aim of the study is to evaluate the changes in pro-inflammatory cytokines in malnourished children, before and after nutritional intervention.

MATERIALS AND METHODS
This hospital-based case control study was conducted at the Maternal and Child Health Hospital (MCHH) in the Subin Sub-Metro in the Kumasi Metropolitan area of the Ashanti Region. All children between the ages of 8 to 36 months attending the child welfare clinic and the malnutrition rehabilitation center of MCHH during the period of December 2009 - June 2010 were recruited after fulfilling the inclusion criteria. Signed informed consent was obtained if parent or guardian demonstrated understanding of the study and was willing to enroll the child. The interview was conducted in Twi which is the local dialect in the region. The study was approved by the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi, Ghana.

A total of 80 children attending the malnutrition rehabilitation center of MCHH with anthropometric measurements of weight for age <70% (Z-scores) and weight for height <80% (Z-Scores) who were finally put on a starter (F-75) (for phase 1 treatment with duration of 2 – 7 days) and catch up (F-100) (for phase 2 treatment with duration of 1 – 3 days) formula diet regimen were included in this study. Children who were on either micronutrient supplementation or on other medications were excluded from the study. A total of 35 children attending the child welfare clinic for routine checkups with weight for age >90% (Z-scores) and weight for height >90% (Z-Scores) were recruited as controls.

Laboratory investigations
Three millilitres (3 ml) of blood sample was collected from both the malnourished and healthy subjects who fulfilled the inclusion criteria of which 2 ml was dispensed into vacutainer® plain tubes and allowed to clot. The clotted samples were centrifuged for 10 minutes at 1250 x g and serum stored at -80°C until analyzed. A portion of the sera was used to determine serum total protein and albumin using the Vitalab Flexor E (Vital Scientific NV Nederland) chemistry analyzer. The remaining portion of the serum was used for the analysis of IL-6 and Tumour Necrosis Factor-alpha (TNF-α) using Enzyme Linked Immunosorbent Assay (Enzyme Linked Immunosorbent Assay D System (Abingdon UK). The remaining 1 ml of the blood sample was dispensed into monovet® ethylene diamine tetraacetic acid (EDTA) tubes and used for the analysis of haemoglobin concentration (Hb) and total white blood cell count (WBC) using Sysmex 2000i xt (Sysmex Corporation, Kobe, Japan). Blood films were also prepared for malaria parasites. Because most of the children were admitted directly as out-patients and received their treatment on a weekly basis, follow up blood samples were taken between the 8th (for children who were able to complete phase 1 of F-75) to 16th (for children who completed phases 1, transition phase and phase 2, F-75 and F-100) days during the time of nutritional intervention. During this period, the children were stable, gained appetite and fluid and electrolyte imbalances were corrected (Reid et al., 2002).
Statistical analysis
Continuous data are expressed as mean ± SD whilst
categorical data are expressed as proportions. Statis-
tical comparisons were analyzed using one-
way ANO-
VA and corrected with Bonferroni’s Multiple Com-
parison test (post-hoc). Student’s t-test (paired) was
used to compare means in subjects before and after
treatment. The chi square test statistics was used to
compare the statistical significance of proportions. A
P value of less than 0.05 was considered significant.
All statistical analysis was performed using
GraphPad prism version 5.0 for windows.

RESULTS
Percentage changes in the concentration of haema-
tological parameters in the control group compared
to that of the subjects at baseline (before treatment)
and after treatment are presented in Table 1. The
mean haemoglobin concentration in the control
group (12.0 ± 0.2 g dL-1) was significantly higher
than that in the subjects before (8.1 ± 0.2 g dL-1;
p<0.0001) and after treatment (8.5 ± 0.2 g dL-1;
p<0.0001). The mean haemoglobin concentration
does not only increase by 3.2%, the proportion of
subjects with haemoglobin concentration <11.0 g dL-
-1 also decreased by -6.2% after treatment. Converse-
ly, the mean total white blood cell counts (TWBC)
of 12.4 ± 0.7 k µL-1 and 11.2 ± 0.6 k µL-1 in the sub-
jects before and after treatment respectively were
significantly higher than the mean TWBC of 8.8 ±
0.4 k µL-1 in the control group (p=0.0006 and
p=0.0153 respectively). A decrease in TWBC of -9.9%
and a -13.7% decrease in the proportion of
children with TWBC >12.0 k µL-1 was observed in
the subjects after treatment. The proportion of
children in the control group who tested positive
for malaria parasites was significantly higher when
compared to the subject group before (p=0.0080)
and after (p=0.0486) treatment (Table 1).

The mean concentration of total protein in the
control group (68.4 ± 1.4g L-1) compared to that in
the subjects before (66.3 ± 1.6 g L-1) and after
treatment (69.6 ± 1.7 g L-1) showed no statistically
significant differences (p>0.05) (Table 2). Howev-
er, a percentage increase of 5.8 was seen in the
mean concentration of total protein in the subjects
after treatment compared to the baseline concen-
tration. Serum albumin concentration in the control
group (43.2 ± 0.9 g L-1) was significantly higher
than the concentration in the subject group before
treatment (38.7 ± 0.9 g L-1) (p=0.0027). A 6.8%
increase in the mean concentration of serum albu-
mmin concentration was observed in the subjects
after treatment (Table 2). The proportion of chil-
dren in the subject group with a total protein con-
centration <60 g L-1 decreased by -16.3% after
treatment whilst the percentage proportional de-

Table 1: Changes in the concentration of the haematological parameters in the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>CONTROL</th>
<th>SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BEFORE</td>
<td>AFTER</td>
</tr>
<tr>
<td>N</td>
<td>35</td>
<td>80</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>12.0 ± 0.2</td>
<td>8.1 ± 0.2</td>
</tr>
<tr>
<td>&lt;11.0 g dL-1</td>
<td>5(14.3)</td>
<td>80(100.0)</td>
</tr>
<tr>
<td>TWBC</td>
<td>8.8 ± 0.4</td>
<td>12.4 ± 0.7</td>
</tr>
<tr>
<td>&lt;4.0k µL-1</td>
<td>0(0.0)</td>
<td>1(1.3)</td>
</tr>
<tr>
<td>&gt;12.0k µL-1</td>
<td>3(8.6)</td>
<td>36(45.0)</td>
</tr>
<tr>
<td>Malaria parasites</td>
<td>3(8.6)</td>
<td>0(0.0)</td>
</tr>
</tbody>
</table>

TWBC = total white blood cells, %Δ = percentage change, p = defines the level of significance when control was compared to subjects (before); p* = defines the level of significance when control was compared to subjects (after); p** = defines the level of significance when subjects (before) was compared to subjects (after)
Table 2: Changes in the concentration of biochemical parameters in the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>CONTROL</th>
<th>BEFORE</th>
<th>AFTER</th>
<th>%Δ</th>
<th>p</th>
<th>p*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>35</td>
<td>80</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein (g L⁻¹)</td>
<td>68.4 ± 1.4</td>
<td>66.3 ± 1.6</td>
<td>69.6 ± 1.7</td>
<td>5.8</td>
<td>0.4226</td>
<td>0.6615</td>
<td>0.1612</td>
</tr>
<tr>
<td>&lt;60g L⁻¹</td>
<td>3(8.6)</td>
<td>27(33.8)</td>
<td>14(17.5)</td>
<td>-16.3</td>
<td>0.0047</td>
<td>0.2145</td>
<td>0.0186</td>
</tr>
<tr>
<td>Albumin (g L⁻¹)</td>
<td>43.2 ± 0.9</td>
<td>38.7 ± 0.9</td>
<td>41.1 ± 0.9</td>
<td>6.8</td>
<td>0.0027</td>
<td>0.1476</td>
<td>0.0479</td>
</tr>
<tr>
<td>&lt;35g L⁻¹</td>
<td>4(11.4)</td>
<td>26(32.5)</td>
<td>13(16.3)</td>
<td>-16.2</td>
<td>0.0179</td>
<td>0.5027</td>
<td>0.0167</td>
</tr>
</tbody>
</table>

%Δ = percentage change, p = defines the level of significance when control was compared to subjects (before); p* = defines the level of significance when control was compared to subjects (after); p** = defines the level of significance when subjects (before) was compared to subjects (after).

crease in children with albumin concentration <35 g L⁻¹ was -16.2% (Table 2).

From table 3, the mean concentration of interleukin-6 (IL-6) in the subjects at baseline (46.1 ± 7.48 pg mL⁻¹) and after treatment (26.3 ± 5.2 pg mL⁻¹) were significantly higher than that in the control (7.0 ± 1.4 pg mL⁻¹) group (p=0.0008 and p=0.0148 respectively) with a -43.8% decrease in the mean concentration of IL-6 being observed after treatment. The proportion of children with IL-6 concentration >14 pg mL⁻¹ also decreased by 6.2% in the subject group after treatment. Tumour necrosis factor-alpha (TNF-α) concentration in the subject group before treatment (82.1 ± 6.0 pg mL⁻¹) was significantly higher when compared to the mean concentration (55.8 ± 2.2 pg mL⁻¹) in the control group but no statistically significant difference was observed in the TNF-α concentration in the subject

Table 3: Changes in the concentration of immunological analytes in the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>CONTROL</th>
<th>BEFORE</th>
<th>AFTER</th>
<th>%Δ</th>
<th>p</th>
<th>p*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>35</td>
<td>80</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokines</td>
<td></td>
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</tr>
<tr>
<td>IL-6 (pg mL⁻¹)</td>
<td>7.0 ± 1.4</td>
<td>46.1 ± 7.5</td>
<td>26.3 ± 5.2</td>
<td>-43.8</td>
<td>0.0008</td>
<td>0.0148</td>
<td>0.0320</td>
</tr>
<tr>
<td>IL-6 &gt;14pg mL⁻¹</td>
<td>5(14.3)</td>
<td>42(52.5)</td>
<td>37(46.3)</td>
<td>-6.2</td>
<td>0.0001</td>
<td>0.0011</td>
<td>0.4292</td>
</tr>
<tr>
<td>TNF-α (pg mL⁻¹)</td>
<td>55.8 ± 2.2</td>
<td>82.1 ± 6.0</td>
<td>72.5 ± 6.9</td>
<td>-11.4</td>
<td>0.0053</td>
<td>0.1110</td>
<td>0.2992</td>
</tr>
<tr>
<td>TNF-α &gt;8.1pg mL⁻¹</td>
<td>35(100.0)</td>
<td>80(100.0)</td>
<td>80(100.0)</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IL-6 = interleukin 6, TNF-α = Tumour necrosis factor-alpha, %Δ = percentage change, p = defines the level of significance when control was compared to subjects (before); p* = defines the level of significance when control was compared to subjects (after); p** = defines the level of significance when subjects (before) was compared to subjects (after).
group before and after (72.5 ± 6.9 pg mL−1) treatment. A percentage decrease of 11.4% was observed in the mean TNF-α concentration of the subjects after treatment (Table 3).

DISCUSSION
Changes in haematological and biochemical parameters are known to provide valuable information and act as sensitive indicators for overall management of PEM (Mishra et al., 2009). The alteration in the level of biochemical parameters were said to be related to food intake and biochemical metabolism mandatory during growth and development of children less than five years of age (Mishra et al., 2009).

The significant reduction in mean haemoglobin concentration (i.e. 100% anaemic) at baseline as well as the 6.2% decrease in the proportion with anaemia after intervention shows the ability of diet intervention to improve upon haemoglobin concentration and this finding compares well with that of Mishra et al., (2009). Gabay and Kushner, (1999) also reported on the effect of infections on erythropoiesis and the general lack of response to haematinics in the presence of active infection in children with PEM. A significant proportion of the subjects (45.0%) had elevated levels of total white blood cells (TWBC) when compared to the controls (8.6%) and this proportion decreased by about 13.7% after nutritional intervention. Bhan et al., (2003) attributed elevated TWBCs in children with severe PEM to asymptomatic infections and severe nutritional deficiency is imminent in the failure of the immune system to respond to chemotaxis, opsonization and phagocytosis of bacteria, viruses or fungi. Children with PEM in this study might therefore have asymptomatic infections as evidenced by the elevated TWBCs which could have had a negative impact on erythropoiesis hence the resultant decreases in haemoglobin concentration observed in the subjects at baseline.

Mishra et al., (2009) further showed a strong association of hypoproteinaemia in their PEM group compared to the control group with the risk of protein energy malnutrition being 3.7. Likewise, significantly higher decline in serum albumin level in the PEM group compared to the control group gave a relative risk of 5.2. A significant proportion of the subjects (33.8%) with PEM in this study developed hypoproteinaemia in comparison to the controls (8.6%) at baseline and this proportion decreased by about 16.3% after nutritional intervention. Also, 32.5% developed hypoalbuminaemia compared to 11.4% of the controls at baseline and this significant proportion decreased by 16.2% after nutritional intervention. These findings confirmed the contribution of hypoproteinaemia and hypoalbuminaemia in PEM and agree well with that of Mishra et al., (2009). Sullivan (2001) in his study on serum proteins related hypoalbuminaemia to increased vascular permeability to albumin probably mediated by cytokines (IL-6 and TNF-α). This study observed increased concentrations of IL-6 in the subjects at baseline which decreased by 6.2% after nutritional intervention and as such could have contributed to the significant decrease in serum albumin at baseline.

Different studies have produced varying reports on pro-inflammatory cytokines in the malnourished. Whilst Muñoz et al., (1994) and Abo-Shousha et al., (2005) indicate that pro-inflammatory cytokine levels in the malnourished are reduced, many researchers in this area have reported increases (Vaisman et al., 1989; Stenvinkel et al., 2002; Azevedo et al., 2005 and Cederholm et al., 1997) Morlese et al., (1996) suggested that increase in the pro-inflammatory cytokines could be due to stimulations either by the presence of endotoxin, bacterial exotoxin, fungi or viruses. This corroborate with a study conducted by Malave et al., (1998), who showed that CRP and IL-6 increased to approximately similar levels in sera from undernourished and control children with overt infections. These cytokines, during acute generalized infections initiate acute-phase reactions which include fever, malaise, myalgia, headaches, cellular hypermetabolism and multiple endocrine and enzyme responses (Beisel, 1995).

The acute-phase reaction and its cytokine-driven hypermetabolism have high nutritional costs (Beisel et al., 1977; Roubenoff et al., 1994; Constans et al., 1995). Cytokine-induced malnutrition is therefore
initiated by hypermetabolism (Beisel et al., 1977; Roubenoff et al., 1994) with its high basal metabolic rates. Body nitrogen and other elements are lost quickly, while body water and sodium are being retained (Beisel et al., 1977). Glucose and urea synthesis are both increased during cytokine-induced malnutrition, but ketone production is slowed (Beisel et al., 1977). Oxidation of branched-chain amino acids is increased and acute-phase plasma glycoproteins are created (Beisel et al., 1977) thereby activating the immune system. Opposite responses to such metabolic instances are typical of uncomplicated starvation (Beisel, 1995). Significantly increased concentration of IL-6 was observed in subjects (52.5%) in this study when compared to controls (14.6%) at baseline and because starvation is rarely uncomplicated in children, the resultant malnutrition observed in subjects in this study could be generally influenced by cytokine-induced (IL-6) components.

Tumour necrosis factor (TNF) plays essential role in the development of the metabolic and pathological consequences of the stress response (Fong et al., 1990). It has been detected in the serum of patients experiencing various diseases, such as parasitic or bacterial infections, tumour-bearing disease, burns and acute hepatic failure (Marano et al., 1990). Giovambattista et al., (2000) observed that basal TNF serum concentrations were significantly higher in malnourished children than in controls. In analyzing TNF-α concentration in the subjects and controls in this study however, no significant differences in TNF-α concentration was observed at baseline and after nutritional intervention. This finding is in agreement with that of Dulger et al., (2002), who reported no significant difference in the concentrations of TNF-α in children with PEM compared to controls in their study on pro-inflammatory cytokines in Turkish children with PEM.

CONCLUSION
This study observed increases in inflammatory response in children with PEM with IL-6 concentration being a significant diagnostic indicator of PEM in the subjects compared to TNF-α concentration. The impact of dietary intervention on haematological and biochemical indices assessed in this study shows the ability of nutritional intervention to achieve immunomodulation, promote growth, and improved immunity, general well-being and development of malnourished children less than five years of age.

ACKNOWLEDGEMENTS
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COMPETING INTERESTS
The authors declare that they have no competing interests.

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<td>Adjei-Frempong et al.,</td>
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