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**Infection and immunoglobulin levels in Sudanese children with severe protein-energy malnutrition**

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**ABSTRACT**

A hospital-based case control study was carried out to determine the pattern of infections and immunoglobulin levels in Sudanese children with severe protein energy malnutrition (PEM). The pre-dietary rehabilitation levels of the three major immunoglobulins (IgG, IgA and IgM) were compared with those of normal controls, and with the levels after dietary rehabilitation. Eighty one children were included in the study: 49 with severe PEM (23 with marasmus, 17 with marasmic – kwashiorkor and 9 with kwashiorkor), 13 with tuberculosis and 19 healthy children as controls. The study showed high incidence of infections, especially pneumonia and gastrointestinal infections in the malnourished children. Of special concern was the high incidence of urinary tract infection: 13 (26.5%) had significant pyuria and 9 of them had positive urine cultures, mainly *Escherichia coli*. Eight of the malnourished children also had pulmonary TB, and the ESR and Mantoux tests were not helpful in the diagnosis. The Mantoux test was negative in 88.8% of the malnourished group compared to 62.5% in those malnourished with TB. The malnourished groups had significantly higher plasma levels of the 3 immunoglobulins. While the marasmic group attained significantly higher levels of IgG and IgA compared to the marasmic – kwashiorkor and kwashiorkor groups, the 3 groups of PEM showed a uniformly higher level of the IgM. After 2 weeks of rehabilitation, the levels of the 3 immunoglobulins showed no significant changes, except for the IgA which significantly decreased in all malnourished and the oedematous groups, and the IgM which increased significantly in the oedematous group.

**Key words:** Immunoglobulins; Infections; Protein-energy malnutrition; Sudanese children; Acute respiratory infection; Tuberculosis; Diarrhoea; Urinary tract infection; *Escherichia coli*.

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INTRODUCTION

The negative effects of malnutrition on body function encompass physical activity, cognitive ability and the response to disease and infection [1]. In addition, there is increasing evidence that malnutrition in critical periods of development in fetal or early infancy may determine risk of chronic disease in later life, that points to child health as the basis of adult health [2,3]. It is well recognized that the interaction between malnutrition and infection is an intimate one, and it is often assumed that infection happens because of impaired immune function [4]. The interaction of nutrition and infection was first enunciated by Scrimshaw et al [5] in a classic article published in 1959. It was later refined and extended in the monograph series as interaction of nutrition and infection [6]. At its core is the notion of synergistic (mutually reinforcing) and antagonistic (mutually nullifying) influences of malnourished state and infectious conditions and vice versa [7,8]. Malnutrition is the primary cause of immunodeficiency worldwide and there is a strong relationship between malnutrition and infection and infant mortality [9]. Nutritionally damaged immune function and impaired disease resistance have their epidemiological consequences, as was shown by Ashworth [10] in 1982, who documented the fact that undernourished children have greater mortality from infectious diseases. Thereafter, a detailed epidemiological association has been described, with an estimated 53% of under-5-years-old deaths from childhood infections associated with underlying underweight [11]. The bulk of evidence on immunosuppression in PEM comes from work done on cellular immunity [12]. Malnutrition mostly reduces T cell numbers than B cells [13]. Malnutrition affects the humoral immune system in diverse fashions. B lymphocyte subpopulations, serum IgG, and IgA levels and immunoglobulin synthesis and metabolism are usually normal or increased [14]. The humoral immune response appears to be affected only in acute and severe PEM and is rapidly restored as the child recovers. As a consequence, even moderately malnourished children are still able to respond to routine immunization procedures [15]. Serum immunoglobulins are high in PEM [16] and adult levels may be reached by two years of age [17].

The aims of this case control prospective study were to determine the pattern of infections and immunoglobulin levels in children with severe protein energy malnutrition (PEM) and to evaluate their behavior during the recovery phase of PEM. Another goal was to examine the pattern of the different forms of severe PEM in Sudanese children admitted to two large hospitals in Khartoum.

MATERIAL AND METHODS

The study was conducted at the University Pediatric Wards, Khartoum Teaching Hospital (KTH) and Soba University Hospital (SUH), and the Pediatric Surgical Ward of the KTH, Khartoum, Sudan, during the period December 1992 to May 1993. The Pediatric Wards at KTH and SUH are general pediatric wards to which children with various medical problems are admitted, with 25-30 of the beds in each hospital assigned to children with severe forms of PEM.

A total of 81 children were included in the study. Forty-nine (60.5%) of these had various forms of severe PEM (marasmus 23 [46.9%], marasmic–kwashiorkor 17 [34.7%] and kwashiorkor 9 [18.4%]); including 8 children who also had pulmonary tuberculosis (TB). They were newly admitted with severe PEM classified according to the Welcome classification [18], using NCHS charts [19] and were aged between six months and five years. The remaining 32 comprised 13 children with various forms of TB, but without severe PEM, and 19 well nourished others (aged six months to five years) with minor surgical problems who served
as controls. The findings during detailed history and physical examination were entered in a comprehensive protocol sheet which was completed for each child. The patients and controls were weighed initially and again, two weeks later. The same weighing machine was used throughout and weight was recorded to the nearest 0.1 kg. The mid-arm circumference (MAC) was recorded to the nearest 0.1 cm using a fiberglass tape. Urinalysis, stool examination (for parasites), chest X-ray and Mantoux test were done on every child. Urine cultures and sensitivities were also done in those with significant pyuria (defined as pus cells in urine more than 5 per mm3 per high power field). Five ml of blood was obtained from each child into citrated tubes; 2.5 ml of this was sent for the estimation of haemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR) and white blood count (WBC) in the central laboratory of KTH. The other 2.5 ml was sent for plasma separation, in sterile cryotubes which were stored at -20°C and later transported in dry ice to Sweden for determination of the 3 major immunoglobulins.

Analysis of the plasma
Plasma samples were analyzed at the Central Laboratory, Uppsala University Hospital, Uppsala, Sweden. Each sample was analyzed for plasma albumin, using the dye binding (Bromocresol green) method and also for the 3 immunoglobulins (IgG, IgM and IgA). The various proteins were separated using the agarose gel electrophoresis. Then the level of each protein was measured using the rate nephelometric method (ArryTM protein system model 7571; Beckam Immunochemistry Systems CA, Palo Alto, USA). Follow up examination and blood sample collections were carried out, two weeks later, in 28 of the 49 children with PEM. These samples were obtained and analysed using the same methods that were used for the initial samples. During the intervening period, the children were fed with high calorie milk-based feeds, and any infection was treated with the appropriate antimicrobial drugs.

Statistical analysis
The data was analyzed by an IBM computer using Epi Info program and SPSS statistical package. The paired student t-test was used for comparison within the different study groups. A P-value <0.05 was considered significant.

RESULTS
Nine (11.1%) of the 81 were aged below 12 months, 60 (74.1%) were 13-36 months and 12 (14.8%) were older than 36 months. The groups were comparable with regard to age, except those with TB who were significantly older than the other groups (mean age 18.7 months; P < 0.001). The male: female ratio for the study population was 1.9:1; the ratio among the groups were 1.9:1 for marasmus, 1.1:1 for marasmic-kwashiorkor, 1.2 :1 for kwashiorkor, 2:1 for the controls and 5.5:1 for TB.

A significant number of the children were fully vaccinated , 89.5% of the control, 69.6% of the marasmus, 64.7% of the marasmic-kwashiorkor, 88.9% of the Kwashiorkor and 30.8% of the tuberculous group.

Excluding the tuberculous group, who were significantly older than the others, the weight, height and mid-arm- circumference (MAC) were significantly lower in the marasmic, marasmic-kwashiorkor and kwashiorkor groups compared with the controls. However, children with kwashiorkor showed less significant weight and height loss than the others.

The marasmic children showed significantly longer duration of illness compared to the oedematous group. The tuberculin response among the malnourished children revealed that 44 (89.8%) of the malnourished children had a negative Mantoux (tuberculin) test and 5 (62.5%) out of the 8 malnourished with TB had a
negative Mantoux test (Table 1).

Table 1- Demographic variables, anthropometric findings, illness duration and immunization status of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Marasmus</th>
<th>Marasmic-kwashiorkor</th>
<th>Kwashiorkor</th>
<th>Tuberculous</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.of children (%)</td>
<td>23 (28.4)</td>
<td>17 (21.0)</td>
<td>9 (11.1)</td>
<td>13 (16.0)</td>
<td>19 (23.5)</td>
</tr>
<tr>
<td>Mean age (range in months)</td>
<td>19.8 (6-36)</td>
<td>20.2 (98-36)</td>
<td>27.8 (16-60)</td>
<td>81.7 (13-120)</td>
<td>25.5 (11-36)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>1.9:1</td>
<td>1.1:1</td>
<td>1.2:1</td>
<td>5.5:1</td>
<td>2:1</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>5.7 (0.7)***</td>
<td>6 (1.2)***</td>
<td>9 (1.5)**</td>
<td>20.9 (9.6)**</td>
<td>11.7 (3.4)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>74.4 (5.7)***</td>
<td>72 (6.5)***</td>
<td>81.7(8)**</td>
<td>115.3(9.5)***</td>
<td>87.6(8.6)</td>
</tr>
<tr>
<td>MAC (cm)</td>
<td>9.9(0.9)***</td>
<td>9.7(1.1)***</td>
<td>10.9(1.7)***</td>
<td>14.7(1.9)*</td>
<td>15.7(1.7)</td>
</tr>
<tr>
<td>Immunization status: No (% fully vaccinated)</td>
<td>16 (69.6)**</td>
<td>11 (64.7)*</td>
<td>8 (88.9)*</td>
<td>4 (30.8)***</td>
<td>17(85.5)</td>
</tr>
<tr>
<td>Mean illness duration(weeks)</td>
<td>12.1(9.4)</td>
<td>5.17(2.6)**@</td>
<td>6.4 (5.5)**@</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tuberculin negative (%)</td>
<td>82</td>
<td>100</td>
<td>88.9</td>
<td>5.3</td>
<td>0</td>
</tr>
</tbody>
</table>

@ = compared to marasmus
M – male; MAC - mid-arm circumference F - female
* P > 0.05 (NS)
** P<0.05
*** P<0.001

Table 2 shows the haematological indices in the different malnourished groups compared to the control. The malnourished children had significantly lower levels of haemoglobin (Hb) and packed cell volume (PCV) compared to control, while they showed significantly higher ESR and white blood cell count (WBC). All groups of PEM had significantly low Hb compared to control, however the marasmic-kwashiorkor showed the lowest level. After two weeks of rehabilitation, these haematological indices including the ESR showed no significant changes (Table 5).
Thirty-two (78%) of 41 malnourished children without pulmonary TB, had acute respiratory tract infections (ARI); 18 (43.9%) had pneumonia, 8 (19.5%) had otitis media and 6 (14.6%) had other upper respiratory tract infections (URTI). The chest X-ray (CXR) was normal in 73.5% of the PEM group, while 13 (26.5%) had abnormal CXR. Nine CXR showed lobar consolidation (8 of them had tuberculosis, 3 showed patchy consolidation and one hilar lymphadenopathy). Forty-three (87.8%) of all the 49 malnourished children had diarrhoea, and 24 (49%) of these malnourished had parasites in the stool, mainly *Giardia lamblia* and *Entamoeba histolytica*. The urine was cultured in 13 malnourished children (28.5%) who had significant pyuria; nine had positive cultures of which 6 were E.coli, 2 were Proteus and one Klebsiella spp.

Table 3 showed the malnourished groups to have significantly higher plasma levels of the 3 immunoglobulins (IgG, IgA and IgM), compared to controls. While the marasmic group attained significantly higher levels of IgG and IgA compared to the marasmic-kwashiorkor and kwashiorkor groups, the 3 groups of PEM showed a uniformly significantly higher level of IgM.

<table>
<thead>
<tr>
<th>Haematological indices</th>
<th>All malnourished</th>
<th>Marasmus</th>
<th>Marasmic-kwashiorkor</th>
<th>Kwashiorkor</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR [mm/hr]</td>
<td>58.6(47)**</td>
<td>70.3(50)**</td>
<td>51.8(47)**</td>
<td>41.5(38)**</td>
<td>25(13.6)</td>
</tr>
<tr>
<td>Hb [g/L]</td>
<td>7.5 (1.8)**</td>
<td>7.8(1.7)**</td>
<td>6.9(2.1)***</td>
<td>7.5(1.7)**</td>
<td>9.3(1.8)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>23.1(7.1)**</td>
<td>24.7(5.9)**</td>
<td>20.1(8.2)**</td>
<td>24.8(6.1)**</td>
<td>28.1(4.8)</td>
</tr>
<tr>
<td>WBC [*/mm³]</td>
<td>5591(1744)*</td>
<td>5143(1280)*</td>
<td>5570(1856)*</td>
<td>6777(2951)*</td>
<td>4668(1225)</td>
</tr>
</tbody>
</table>

Values are given as mean (SD)
* P >0.05 (NS)
** P < 0.05
*** P<0.001

ESR – erythrocyte sedimentation rate, Hb - haemoglobin, PCV – packed cell volume, WBC – white blood cell count.

Table 2-Haematological indices according to types of malnutrition and in controls

<table>
<thead>
<tr>
<th>Immunoglobulins</th>
<th>All malnourished</th>
<th>Marasmus</th>
<th>Marasmic-kwashiorkor</th>
<th>Kwashiorkor</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG [g/L]</td>
<td>13.7(5.6)****</td>
<td>15(5)***</td>
<td>11.7(4.1)**</td>
<td>14.3(8.3)**</td>
<td>9.8(7.8)</td>
</tr>
<tr>
<td>IgA [g/L]</td>
<td>1.81(0.7)****</td>
<td>1.8(0.68)****</td>
<td>1.8(0.9)**</td>
<td>1.7(0.8)**</td>
<td>0.82(0.41)</td>
</tr>
<tr>
<td>IgM [g/L]</td>
<td>1.56(0.7)****</td>
<td>1.6(0.85)****</td>
<td>1.44(0.7)***</td>
<td>1.6(0.8)***</td>
<td>0.74(0.24)</td>
</tr>
</tbody>
</table>

Values are given as mean (SD)
* P >0.05 (NS)
** P < 0.05
*** P<0.005
**** < 0.001
Eight (16.3%) of the 49 malnourished children had pulmonary tuberculosis. No significant change in the ESR and other haematological indices was found in the malnourished with or without tuberculosis. Also no significant changes were observed in the 3 immunoglobulins (Table 4).

Table 4 - The haematological indices and the immunoglobulin levels in the malnourished children with tuberculosis compared to those without tuberculosis and controls

<table>
<thead>
<tr>
<th>Haematologilical index/Immunoglobulin</th>
<th>Malnourished with TB (no=8)</th>
<th>Malnourished without TB (no=41)</th>
<th>Control (no=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR [mm/hr]</td>
<td>75.9(49)</td>
<td>55.3(44.9)*</td>
<td>25.2(13.6)***</td>
</tr>
<tr>
<td>Hb [g/L]</td>
<td>7.4(1.6)</td>
<td>7.5(1.9)*</td>
<td>9.3(1.8)**</td>
</tr>
<tr>
<td>PVC [%]</td>
<td>22.5(5.4)</td>
<td>23.2(7.4)*</td>
<td>28.1(4.8)*</td>
</tr>
<tr>
<td>WBC [/mm³]</td>
<td>4750(1489)</td>
<td>5756(1758)*</td>
<td>4668(1225)*</td>
</tr>
<tr>
<td>IgG [g/L]</td>
<td>16.4(3.5)</td>
<td>13.2(5.8)*</td>
<td>9.86(2.56)***</td>
</tr>
<tr>
<td>IgA [g/L]</td>
<td>2.04(0.65)</td>
<td>1.77(0.8)*</td>
<td>0.82(0.43)***</td>
</tr>
<tr>
<td>IgM [g/L]</td>
<td>1.86(0.5)</td>
<td>1.49(0.8)*</td>
<td>0.74(0.24)****</td>
</tr>
</tbody>
</table>

Hb - haemoglobin, PCV - packed cell volume, TB – tuberculosis, WBC - white blood cell count.
Values are given as mean (SD)
* P > 0.05 (NS)
** P < 0.01
*** P < 0.001
**** P < 0.0001

After 2 weeks of rehabilitation, the haematological indices showed no significant change, except for the PVC which manifested a marginally significant increase in the oedematous group. Likewise, the levels of the 3 immunoglobulins showed no significant change except for the IgA which significantly decreased in all malnourished and the oedematous group, and the IgM which increased significantly in the oedematous group (Table 5).
The findings in this study show that marasmus is the prevalent form of PEM in these children, as in other countries in the Middle East [20,21].

This pattern was also shown by studies in Sudan [22,23]. The mean age incidence in this study is similar to those reported from Sudan; nevertheless, Taha [22] reported a much lower mean age for kwashiorkor in his study from Wad Medani in central Sudan. The mean age of...
kwashiorkor was 12.4 months compared to 27.8 months in our study. In the present study we found a late age of presentation of marasmus (mean age 19.8 months). This has been reported from Sudan [24] and differs from many other countries where marasmus has been regarded as a disease of early infancy [20,21], associated with bottle feeding since birth. In Sudan breast feeding is usual up to one year of age. In one study, Salih et al [25], found 92% of mothers were still breastfeeding at seven months and 65% continued to breastfeed at the end of the first year. Prolonged breast feeding is frequently associated with malnutrition in less developed countries, even after adjustment for socioeconomic confounders [26]. It is possible that prolonged breastfeeding impairs growth by depressing a toddler's appetite for non-breast milk foods [27]. In an urban-rural survey in Sudan, breast-fed children grew significantly slower in weight and length than did weaned children up to the age of 2 years, even after stratification for economic level and mother literacy [28].

The vaccination coverage of all the children in the present study was 69.2%, the defaulters were 12.5% whereas 18.5% were not immunized at all. The coverage for the control group was 89.5% which is comparable to the coverage in Khartoum state of 90% in the year 1992, while the coverage of the PEM group was 71.4% which is comparable to the national coverage of 62%. This may be explained by the fact that most of the malnourished children were from peri-urban areas for the displaced families. Nevertheless, this also indicates a great improvement in vaccination coverage compared to the figures given by Coulter et al in 1988 [23], who gave a coverage of 11% of the control group and only 1.2% for the PEM group.

In the present study most of the malnourished children were found to be anaemic, and the anaemia was more severe in the marasmic-kwashiorkor patients. This high incidences of anaemia has also been reported in studies from Sudan [22,24,29]. Also these studies reported very high incidence of megaloblastic anemia. Taha [22] found 71% of the cohort to have either pure megaloblastic anaemia or mixed iron deficiency and megaloblastic erythropoeisis, while Omer et al [24] found megaloblastic anaemia in 45% of their cases. The pathogenesis of anaemia in PEM is multifactorial. The extreme deficiency of nutrients is the primary cause but the role of repeated infections, especially of the gastrointestinal system, further aggravates the prevalence and severity of anaemia [30].

In the present study we found infections to be very common in the malnourished children and the commonest were the ARI, specially pneumonia, diarrhoeal diseases and intestinal parasites. These findings have been shown by many authors in hospital and field-based studies [6, 31-33]. Five infections diseases – pneumonia, diarrhoea, malaria, measles and AIDS – account for more than one-half of all deaths in children aged less than 5 years, most of whom are undernourished [9]. However, we also found urinary tract infection (UTI) to be common in the malnourished children. Thirteen (26.5%) of 49 malnourished children had significant pyuria and 9 of them had positive urine cultures, mainly Escherichia coli (E Coli). The same findings have been shown by many studies. Bagga et al [34] found significant bacteriuria in 15.2% of the malnourished children compared to 1.8% of the control, and they found that the risk of UTI increased significantly with the severity of the malnutrition and in patients with fever and diarrhea. In another 2 studies, the most common organism was the E coli, as has been the case in our study [35, 36]. This common association between infection and PEM may be explained by the immunodeficiency - well known in malnourished children - and the poor socioeconomic and unhygienic conditions in which these children usually live [23].

Not only acute infection but also chronic infections like TB, is common in PEM. Malnutrition is an important risk factor for TB, because cell-mediated
immunity (CMI), which is usually impaired in PEM, is the key host defense against TB [37]. In populations with substantial latent TB infection, the occurrence of malnutrition may be an important determinant of the incidence of TB [38]. In the present study, we found 8 (16.3%) of the malnourished children to have pulmonary TB. The levels of the ESR and the different immunoglobulins were not significantly different between the malnourished with or without TB. However, we found the CXR to be useful in the diagnosis of TB in malnourished children, and the CXR in the 8 patients with TB showed lobar consolidation. Also, the Mantoux test was not helpful in the diagnosis, as 88.8% of the malnourished children had a negative response to the Mantoux test and 62.5% of the 8 malnourished with TB had a negative test, as well. The poor response of malnourished children to the tuberculin test has also previously been reported [39,40]. Chandra and Newbern [13] demonstrated that delayed-type hypersensitivity (DTH) skin test response to tuberculin and to numerous other antigens is reduced in PEM. Satyanarayana et al [41] showed that milder grades of malnutrition did not affect the skin test response to purified protein derivative (PPD) 6 months after immunization with BCG, but that children with kwashiorkor were skin test negative. Moreover, among tuberculosis patients, PPD skin test reactivity was directly proportional to serum transferrin level, a sensitive indicator of protein malnutrition [13].

The high levels of the 3 immunoglobulins (IgG, IgA and IgM) in the present study was reported by many other authors [42,43,17] and also in a study from Sudan [24]. Our finding of higher levels of IgG and IgA in the marasmic children is in complete agreement with the findings of McMurry et al [44]. However we found the level of IgM to be uniformly high in the three PEM subtypes which is unlike their finding of higher IgM level in the marasmic and marasmic-kwashiorkor compared to kwashiorkor. The high levels of immunoglobulins in PEM may be explained by the recurrent infections and the increased permeability of the gastrointestinal tract of malnourished children to food antigens. Alvarado et al [45] found that during specific infections, immunoglobulin levels become further elevated [45]. An exception to elevated immunoglobulins in malnutrition may occur in infants less than one year of age. These infants often had low levels of immunoglobulins which remained depressed even after nutritional correction [46] After two weeks of dietary and antimicrobial treatment, we found significant decrease in the level of IgA in all malnourished and the oedematous groups and a significant increase in the level of IgM in the oedematous group, while no change was observed in the IgG level. These findings are in partial agreement with those of McMurry et al [44].

In conclusion, the findings in this study suggest that marasmus is the predominant type of PEM in Sudan and that it has a late presentation. Anaemia is common in the malnourished children with studies from Sudan emphasizing the importance of megaloblastic change as a common cause and that the routine use of folic acid is entirely rational. Infections are common in the malnourished children, mainly pneumonia and gastrointestinal infections. Nevertheless, we also recommend especial concern for UTI and TB. The study also showed that the ESR and the Mantoux tests are not helpful in the diagnosis of TB associated with PEM. The study also support the findings of other studies by showing high levels of the 3 major immunoglobulins (IgG, IgA and IgM) in children with severe PEM. However, we did not find any striking change in these levels after a short period of dietary rehabilitation.

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