Children with severe acute malnutrition
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Citation for published version (APA):
Chapter 3

Both exocrine pancreatic insufficiency and signs of pancreatic inflammation are highly prevalent in children with complicated severe acute malnutrition: an observational study

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ABSTRACT

**Objectives:** To assess whether pancreatic function: 1) is impaired in children with severe acute malnutrition (SAM), 2) is different between edematous versus non-edematous malnutrition, and 3) improves by nutritional rehabilitation.

**Study design:** We followed 89 children with SAM admitted to Queen Elizabeth Central Hospital in Blantyre, Malawi. Stool and blood samples were taken on admission and three days after initial stabilization to determine exocrine pancreatic function via fecal elastase-1 (FE-1) and serum trypsinogen and amylase levels.

**Results:** 33 children (37.1%) had non-edematous SAM whereas 56 (62.9%) had edematous SAM. On admission, 92% of patients showed evidence of pancreatic insufficiency as measured by FE-1 < 200 μg/g of stool. Patients with edematous SAM were more likely to have low FE-1 (98 vs. 82.8%, p=0.026). FE-1 levels remained low in these individuals throughout the assessment period. Serum trypsinogen was elevated (>57 ng/ml) in 28% and amylase in 21% (>110 U/l) of children, suggesting pancreatic inflammation.

**Conclusions:** Exocrine pancreatic insufficiency is highly prevalent in children with SAM and especially in children with edematous SAM. In addition, biochemical signs suggestive of pancreatitis are common in children with SAM. These results have implications for standard rehabilitation treatment of children with SAM who may benefit from pancreatic enzyme replacement therapy.
INTRODUCTION

Despite a decrease over the last decade, mortality rates in children remain high with up to forty-seven percent of deaths in children under five years occurring in Sub-Saharan Africa (1,2). An estimated forty-five percent of deaths worldwide are attributable to under-nutrition defined as a weight for height (W/H) less than or equal to -2 standard deviations (SD) from the norm (3,4) despite protocolized WHO treatment(5–7). Diarrhea is commonly found in children with severe acute malnutrition (SAM) and is associated with increased risk of death (8–10). The broad etiologies of diarrhea in SAM include enteropathy related to malabsorption leading to osmotic diarrhea and infectious secretory diarrhea (11). Extra-intestinal factors such as changes in bile acid secretion or exocrine pancreatic function have not been well studied in the etiology of diarrhea in SAM.

Previous studies have suggested that severely malnourished children may suffer from exocrine pancreatic insufficiency (EPI) (12–23). EPI is defined as a lack of digestive enzyme production; this in turn leads to impaired weight gain and growth due to protein and lipid malabsorption (24). EPI is a common complication in diseases such as Cystic Fibrosis (CF) (25), Shwachman-Diamond syndrome (26), and HIV (27). In children with CF, pancreatic function is an important predictor of long-term survival (28).

Nearly all studies on pancreatic (dys-) function in malnourished children were done in the 1940s-1980s in small groups of children (12–14). These studies showed that malnourished children had reduced pancreatic enzymatic output compared to reference ranges (15,16,19) with evidence that pancreatic function recovered after refeeding (13,15,16,29). Autopsy studies on children with malnutrition describe a combination of pancreatic atrophy and loss of zymogen-secreting pancreatic acinar cells in SAM (16–19,30–32). Since these studies, the assessment of pancreatic function has advanced and warrants reassessment.

The current diagnosis of EPI relies on ‘direct’ or ‘indirect tests’ of exocrine pancreatic function (33). Direct tests are expensive and invasive, which limits their use in children and in low-resource settings or routine clinical practice (34). More feasible tests are indirect test via the measurement of pancreatic enzymes in serum (trypsinogen, amylase), in stool (fecal elastase-1, fecal chymotrypsin), or the detection of C13-mixed-triglyceride in a breath test (33). Fecal elastase-1 (FE-1) is a clinically validated marker with good specificity and sensitivity to diagnose severe EPI and is currently recommended as a screening tool of EPI (33,35,36). The role of trypsinogen in detecting pancreatic insufficiency is valuable in the assessment of pancreatic function in patients with CF (37,38). Both serum trypsinogen and amylase levels are released by damaged pancreatic cells and are therefore used as a marker of pancreatitis (39,40). Their use in diagnosing EPI is limited by their low sensitivity and specificity (33).
The aim of this study was to assess pancreatic function children with SAM. We hypothesized that pancreatic function in children with SAM as assessed by FE-1 and serum trypsinogen and amylase levels is: 1) impaired, 2) correlates to clinical outcomes of duration of hospital stay and number of days from admission to clinical stabilization, 3) differs between edematous versus non-edematous malnutrition, and 4) improves during nutritional rehabilitation.

METHODS

Study design and population

This observational study was completed within the framework of a nutrient prospective intervention trial (ISRCTN 13916953). This “TranSAM Study” was conducted at the MOYO Nutritional Rehabilitation Unit (NRU) of the Pediatric Department at Queen Elizabeth Central Hospital in Blantyre, Malawi. Sample size calculations were originally based on numbers needed to assess the primary outcome of the TranSAM study (carbohydrate malabsorption). The TranSAM study aimed to determine whether the use of transition phase diets with different carbohydrate contents affected fecal pH, length of stay and other clinical outcomes in severely malnourished children. For the TranSAM study, children were randomly assigned to treatment with either F75 + RUTF (Ready-to-use therapeutic foods), RUTF only or F100 after ‘clinical stabilization’ (absence of acute life-threatening conditions, return of appetite, improvement of gastrointestinal losses and edema, and absence of WHO ‘danger signs’). Accounting for contingencies, the study aimed to recruit a total of 108 patients to detect a 20% difference in primary outcome with α=0.05 and 80% power based on previous findings (41). The study was approved by the Malawi College of Medicine Research and Ethics Committee and carried out according to Good Clinical Practice guidelines which are based on the Declaration of Helsinki (42).

Children with SAM admitted to the NRU between January 2013 and July 2013 (n=509) were screened for recruitment. Informed consent was obtained from parents or guardians by verbal and printed explanations in Chichewa, the main local language in Malawi, or English with witnessed consent by signature or by thumbprint for those unable to write.

Inclusion criteria were: children aged 6 – 60 months admitted with a diagnosis of severe acute malnutrition as defined by WHO by a weight-for-height (W/H) of less than – 3 SD and/or a mid-upper arm circumference (MUAC) of less than 115 mm (non-edematous malnutrition) and/or presence of bilateral edema (edematous malnutrition) (43). Both HIV positive and negative children were included. We excluded children who were previously admitted to the NRU within the year or presented with severe hemodynamic
instability, a hematocrit level of ≤15%, or severe neurological symptoms. After admission children were treated according to WHO guidelines (5).

Clinical data and sample collection
All children admitted to MOYO NRU had a thick blood film examined for parasitemia (malaria) and a hematocrit count done. HIV antibody test was offered with appropriate pre- and post-counseling. After initial anthropometry, the following clinical data were collected daily: weight, stool frequency and consistency, number of days until clinical stabilization, and duration of hospital stay. Blood was collected at admission and stool collected on admission and three days after initial clinical stabilization. Upon collection, stool samples were immediately homogenized and frozen at -80°C until further analysis. Diarrhea was defined according to WHO standards (3 or more loose or watery stools in the past 24 hours) (44). Severe diarrhea was defined as 10 or more loose or watery stools in the past 24 hours. Information about stool frequency, consistency and (severe) diarrhea was obtained from the mother or guardian through verbal recall.

Laboratory Analysis
FE-1 levels were determined using enzyme-linked immune assay (ELISA) at the clinical laboratory of the University Medical Center Groningen, the Netherlands. The same procedures were applied to watery and non-watery stool. In accordance with standard practice, exocrine pancreatic insufficiency was defined as FE-1 levels below 200 μg/g of stool and severe exocrine pancreatic insufficiency as FE-1 levels below 100 μg/g of stool (35,45).
Serum trypsinogen concentrations were determined in a random subset of patients (N=39) by the clinical laboratory of the Hospital for Sick Children, Toronto, Canada using a radioimmunoassay as described previously (46). For this study, reference values for normal trypsinogen levels were 10 - 57 ng/ml (Hospital for Sick Children, Toronto, Canada). Serum pancreatic amylase concentrations were determined in 80 patients by ELISA (Abcam, Cambridge, UK). The upper limit of normal was set at 110 U/l.

Data and Statistical Analyses
Data were collected on standardized forms and analyzed with IBM® SPSS® Statistics Version 22.0.0.0 Software (47) and with R statistical software (Version 3.2.2). Descriptive statistics were used to show baseline characteristics of study participants. Median differences in levels of serum trypsinogen, serum amylase, and fecal FE-1 were tested by Wilcoxon rank sum test. Fisher’s Exact test was used to analyze differences in patient numbers between clinical groups. Mixed effects logistic regression models were used to compare FE-1 levels of patients at admission and three days post stabilization to account for within-patient measures; i.e. patient set as a random factor and accounting for miss-
ing values. Non-parametric Spearman’s correlation was used to relate FE-1, trypsinogen, and amylase. All tests were conducted at 95% level of significance.

RESULTS

Baseline characteristics

In total, 89 children with SAM were included in this study; clinical characteristics are detailed in Table 1. In this cohort, most children were diagnosed with edematous SAM as opposed to the non-edematous form. Thirty-three patients were HIV-reactive of whom twenty-seven were newly diagnosed and four already on antiretroviral therapy at admission; two patients had missing information on HIV treatment status. Mortality amongst the study cohort was 15.7% (n=14) and was significantly higher in the non-edematous group compared to the edematous group (27.3 vs. 8.9%, \( p=0.03 \)). No significant differences in mortality were found in HIV non-reactive versus HIV-reactive patients (12.5% vs. 21.2%, \( p=0.4 \)).

Table 1. Patient characteristics

<table>
<thead>
<tr>
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<th>N=89</th>
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<tbody>
<tr>
<td>Edematous, n (%)</td>
<td>56 (62.9%)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>39 (44.3%)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>21 (16-27)</td>
</tr>
<tr>
<td>HIV reactive, n (%)</td>
<td>33 (37.1%)</td>
</tr>
<tr>
<td>Weight on admission (kg)</td>
<td>7.3 (5.5-8.7)</td>
</tr>
<tr>
<td>Weight for Height (SD) &lt;= -3, n (%)</td>
<td>51 (58.0%)</td>
</tr>
<tr>
<td>MUAC (cm) &lt; 11.5, n (%)</td>
<td>45 (50.6%)</td>
</tr>
<tr>
<td>Duration of illness before admission (days)</td>
<td>14 (7-28)</td>
</tr>
<tr>
<td>Duration of hospital stay (days)</td>
<td>9 (8-12)</td>
</tr>
<tr>
<td>Discharged alive, n (%)</td>
<td>71 (79.8%)</td>
</tr>
<tr>
<td>Died, n (%)</td>
<td>14 (15.7%)</td>
</tr>
<tr>
<td>Absconded, n (%)</td>
<td>4 (4.5%)</td>
</tr>
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\( ^{\text{a}} \) Median (inter quartile range); missing values sex (1, 1.1%), Weight for Height (1, 1.1%), duration of illness (4, 4.5%), MUAC is mid upper arm circumference.

Pancreatic insufficiency in patients with SAM

On admission, overall levels of FE-1 were markedly reduced, specifically in the edematous group (Table 2). Evidence of pancreatic insufficiency (FE-1 < 200 μg/g of stool) was seen in 92.2% of patients, while prevalence of severe pancreatic insufficiency (FE-1 < 100 μg/g of stool) was 76.6% on admission. Edematous SAM patients had significantly
lower FE-1 levels on admission as compared to patients without edema (median 22 μg/g of stool, IQR 15 – 57.5 vs. median 80 μg/g of stool, IQR 19-150, \( p=0.009 \); Figure). Severe pancreatic insufficiency was significantly higher in the edematous group compared to the non-edematous group (n=42, 87.5% vs. n=17, 58.6%, \( p=0.006 \)). During hospital admission, FE-1 levels increased only modestly across all patients from 35 μg/g of stool (IQR 15-90) on admission to 69 μg/g of stool (IQR 15-160) three days after clinical stabilization (\( p=0.03 \)). FE-1 levels remained abnormally low in the majority of children (82.9%).

FE-1 levels can appear misleadingly low in patients with watery diarrhea (33), therefore we conducted an analysis in which they were excluded (n=17). FE-1 was significantly lower (\( p=0.004 \)) in patients with watery stools than in those with semi-formed or normal stools (median of 15 μg/g stool, IQR 15 – 20 vs. 43 μg/g stool, IQR 16 – 115, \( p=0.004 \)); however, 57 children (90.5%) without watery stools had low FE-1 levels and 46 (73.0%) showed severe pancreatic insufficiency (FE-1 < 100 μg/g of stool). This study was not powered to test differences in mortality, and no differences were found between pancreatic sufficient versus insufficient patient groups. We did not find any relation between FE-1 levels and sex, age, HIV status, length of hospital stay, or number of days until clinical stabilization.

### Table 2: SAM patients with abnormal measurements of markers of pancreatic insufficiency and pancreatic inflammation

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<thead>
<tr>
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<th>Abnormal, n (%)</th>
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<tbody>
<tr>
<td></td>
<td>n= 39</td>
</tr>
<tr>
<td>Trypsinogen (ng/mL)</td>
<td></td>
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<tr>
<td>Amylase U/l</td>
<td></td>
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<tr>
<td>Fecal Elastase-1 (μg/g)</td>
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<table>
<thead>
<tr>
<th></th>
<th>n= 70</th>
<th>Clinical Cut-off</th>
<th>All patients</th>
<th>Non-edematous</th>
<th>Edematous</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal Elastase-1 (μg/g)</td>
<td></td>
<td>&lt; 200 μg/g</td>
<td>58/70 (83%)</td>
<td>20/24 (83%)</td>
<td>38/46 (83%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 100 μg/g</td>
<td>42/70 (60%)</td>
<td>14/24 (58%)</td>
<td>28/46 (61%)</td>
<td>1</td>
</tr>
</tbody>
</table>

Number of patients out of total patients, with or without edema, that present with abnormal pancreatic markers. Clinical cut-off for fecal elastase of <200 μg/g represents pancreatic insufficiency, <100 μg/g is deemed severe pancreatic insufficiency. \( p \)-values obtained with Fisher’s exact test; significance code: * \( p<0.05 \), ** \( p<0.01 \).

### Pancreatic inflammation in patients with SAM

We measured levels of serum trypsinogen in a random subset of children (n=39). High levels (>57 ng/ml) were found in 11 patients (28.2%); this group had median serum trypsinogen values of 130.7 ng/ml (IQR; 70.8 – 239.2). The number of patients showing high
trypsinogen levels differed between edematous and non-edematous groups (10.5%, n=2 vs. 45%, n=9, \( p=0.03 \)). Trypsinogen levels did not correlate with FE-1 (\( P=0.15, \ p=0.4 \)). No significant difference in mortality was found between those with normal vs. abnormal trypsinogen levels at admission. We found no relation between serum trypsinogen levels and sex, age, HIV status, length of hospital stay, or duration in days until clinical stabilization.

Serum pancreatic amylase levels were determined in 80 patients. Levels above 110 U/l were found in 21% of children (n=17) with a median level of 148.4 U/l (IQR; 119.5 – 202.3). Serum amylase levels did not significantly differ between non-edematous and edematous patients (\( p=0.4 \)). A significant correlation was found between amylase and trypsinogen levels (\( P=0.36, \ p=0.03 \)). Serum amylase levels did not differ with sex, age, HIV status, length of hospital stay, days until clinical stabilization, or mortality.

**DISCUSSION**

To our knowledge, this study is the largest to investigate pancreatic function in children with severe acute malnutrition and is the first to assess their pancreatic function using clinically validated tests. The results strongly support our hypothesis that pancreatic
function is impaired in children with SAM. Our data suggest that both exocrine pancreatic insufficiency and pancreatic inflammation are common in children with SAM.

Pancreatic function can be tested both direct and indirectly. Direct tests are expensive, invasive, not well standardized for children and unavailable in most low resource settings like Malawi. Therefore, we chose to use indirect clinically validated tests. These can be divided into three categories; those that: 1) assess urine or serum for markers of pancreatic enzyme processing; 2) analyze unabsorbed or undigested fat in feces or, 3) use breath tests and measures of pancreatic enzymes in stool or serum (36). FE-1 is the most sensitive indirect test and the most widely used clinically to evaluate EPI in high-income settings (33). Immunoreactive trypsinogen measured in serum is currently used for newborn screening of CF and is elevated when the pancreas is inflamed (33). However, the reliability of trypsinogen as a marker for EPI below the age of seven has been questioned (36,48). Serum amylase concentrations are used to diagnose pancreatic inflammation, i.e. pancreatitis, but only have a supporting role in the diagnosis of EPI in conjunction with additional clinical and biochemical information (33,49). Increased concentrations of amylase can also be found with intestinal obstruction or reduced renal clearance (50).

As compared to clinical reference values, our data indicate that the majority of children with SAM have EPI. Significant differences in the degree of EPI were found between children with and without edematous SAM, as both moderate and severe EPI were more prevalent in the edematous group. In addition, our data suggest that pancreatic insufficiency persists beyond initial clinical stabilization. The high prevalence of EPI seen in our cohort of children with SAM is consistent with previous studies that revealed pancreatic insufficiency via duodenal aspirates and post-mortem investigations (12-22); however, these studies lack clear reference values and are mostly published between 1940 – 1980 with varying sample sizes. These studies do not offer information on the differences in the prevalence of EPI between edematous and non-edematous SAM.

In 2005, a small study by el-Hodhod et al. reported exocrine pancreatic dysfunction in SAM patients by relating pancreatic size determined by ultrasound to changes in serum amylase and lipase levels (23). The elevated trypsinogen and amylase concentrations found in a significant proportion of children with SAM suggest that in addition to EPI, pancreatic inflammation is also present. Pancreatic inflammation has not been reported in children with SAM, but it has been reported in case studies of patients with eating disorders leading to malnutrition (51). The presence of pancreatic inflammation may be related to the presence of a systemic pro-inflammatory state in children with SAM due to increased pro-inflammatory cytokines (52) perhaps in combination with decreased anti-oxidant concentrations (53,54). For this study, we chose an amylase concentration above 110 U/l as abnormal, which has been used before. However, this cut-off value is
conservative, as our measures of pancreatic amylase are consistently lower than those found in healthy children (55).

The differences in amylase and trypsinogen levels in edematous vs. non-edematous SAM phenotypes suggest the possibility of differences in the pathophysiology leading to pancreatic damage in these children. Brooks et al. reviewed histological sections of pancreas and liver from 65 children who died of malnutrition-related causes and found a high rate of pancreatic atrophy (20); it is possible that this pancreatic atrophy and fibrosis seen after longstanding malnutrition in children with edematous SAM can result in very low levels of trypsinogen production (18).

Our study did not find relations between the degree of pancreatic insufficiency or inflammation and clinical outcomes of duration of hospital stay or length to reach stabilization. This is likely due to the small number with normal FE-1 levels (>200ug/ml) (n=6). In addition, our study was not designed to capture clinical symptoms normally associated with pancreatitis such as vomiting, anorexia, or epigastric abdominal pain.

One of the limitations of our study was the lack of a control group of healthy Malawian children. However, previous research has shown that EPI is significantly more prevalent in children with SAM versus population controls (13,15,21). A second limitation is the reliability of FE-1 in watery stools, which can be low due to dilution rather than decreased production (33). However, when evaluating FE-1 levels in the subset of children without diarrhea, the prevalence of EPI was only marginally lower than those with diarrhea; this suggests that FE-1 reveals EPI in most children with SAM. A third limitation is the short time that passed between stool sample collection for FE-1 analysis. To study the long-term influence of nutritional rehabilitation on pancreatic function, it would have been interesting to repeat our measures after a longer period of time. A fourth limitation was that the trypsinogen testing could only be conducted in 39 patients out of the 89 as the quantity of samples obtained were insufficient to complete all assays in all patients. Finally, we did not have enough biological specimens to determine other markers of pancreatic function or inflammation, such as serum lipase concentrations. Lipase is known to be a more sensitive marker of pancreatic inflammation than amylase in infants and toddlers (56).

In conclusion, our study shows that EPI is highly prevalent in children with SAM and more common in children with edematous malnutrition. Furthermore, our study is the first to document signs of pancreatic inflammation in these children. We additionally showed that short-term nutritional rehabilitation improves but does not normalize exocrine pancreatic function. Future research is needed to investigate whether this patient population would benefit from the addition of treatments addressing EPI such as pancreatic exocrine replacement therapy (PERT) or the use of a diet adjusted to account for an altered digestion secondary to pancreatitis.
ACKNOWLEDGEMENTS

We would like to thank all study participants and their guardians for their participation and the nursing staff and clinical officers of the MOYO NRU at Queen Elizabeth Central Hospital in Blantyre, Malawi, for their hard work in conducting high-quality patient care and research.
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