Developmental Screening and Nutritional Intervention of Severe Acute Malnourished Children in Southern Punjab, Pakistan

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A thesis submitted to the University of the Punjab in accordance with the requirements of the degree of Doctor of Philosophy in Public Health

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of University of Punjab and all the work in this thesis apart from the help and advice acknowledged unless indicated is entirely my own contribution and is a record of work performed by me in the department Public Health. This thesis has not been presented to any other University for examination either in the Pakistan or overseas. No portion of the work referred to in this research project has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

Signed …………………………………….. Date ……………………………………..

I certify that the work reported in this thesis has been performed by Dr Javeria Saleem and during the period of study she has fulfilled the conditions of the ordinances and regulations governing the Degree of Doctor of Philosophy, University of Punjab.

Signed …………………………………….. Date ……………………………………..
ABSTRACT

**Background:** Malnutrition in the early years of child life can cause long-lasting deleterious effects which may prevent behavioural, motor, cognitive development, educational achievement and reproductive health. Children with severe acute malnutrition (SAM), which is associated with delayed growth and development, often have multiple micronutrient deficiencies, including vitamin D deficiency. According to UNICEF and WHO joint malnutrition estimates for 2016 in Pakistan, 10.5% of children are wasted, 45% are stunted and 31.6% are underweight. If untreated, severe under-nutrition can progress to irreversible effects, with delay in development thereby declining upcoming productivity of these children and worsen the economic burden of country. Therefore, it is important to find predictors for malnutrition to properly address this problem. There are insufficient national statistics on the developmental outcome of severe acute malnutrition (SAM) among children in Pakistan as well as randomized control trials of vitamin D supplementation in growth along with development of SAM children are lacking. So we have tried to explore in this study whether supplementation of vitamin D₃ (cholecalciferol), in combination with “ready-to-use therapeutic food (RUTF)”, would increase child growth along with developmental status during the rehabilitation phase of SAM. Clinical trials in SAM with supplementation of vitamin D have not carried out in this population before.

**Methods:** This study was designed in to two phases. First phase was cross-sectional with the aim to reveal the impact of malnutrition on development quotient of children and to explore the dietary and socio demographic factors responsible for severe acute malnutrition and developmental quotient of children. In second phase of study we carried out a “randomised, placebo-controlled, trial of vitamin D₃ supplementation” in 185 children.
between 6-59 months of age with uncomplicated severe acute malnutrition, in southern Punjab, Pakistan. Children were randomly allocated to receive either two oral doses of 200,000 IU vitamin D3, or placebo, along with RUTF, at 2 and 4 weeks. Participants and study staff were unacquainted of treatment assignment. The primary outcome was the proportion with weight gain >15% of baseline and the secondary outcome were mean weight-for-height/length z-score and global developmental status. Developmental quotient of children (Assessed with the Denver Development Screening Tool II) were done at start of study and at end of 2 months. Structured sociodemographic and nutritional questionnaire were used to collect information for predictors on same trial population. “This study is registered with ClinicalTrials.gov, number NCT03170479”.

**Findings:** Out of 194 kids initially randomly enrolled in the study, 185 kids completed follow-up and data records of these 185 kids were included in the analysis. So out of 185 children, 69 (37.3%) have normal developmental, 108 (58.4%) had suspected delayed development and 8 (4.3%) had untestable profile in overall developmental score. Random allocation of children were done in vitamin D₃ group (n=93) or placebo group (n=92). Vitamin D₃ did not influence the proportion of SAM kids gaining >15% weight from baseline (relative risk [RR] 1.04, 95% CI 0.94-1.15, p=0.47) but it did increase weight-for-height/length z-score (adjusted mean difference 1.07, 95% CI 0.49-1.65, p<0.001) and reduce the proportion of participants with delayed global development (adjusted RR [aRR] 0.49, 95% CI 0.31-0.77, p=0.002), delayed gross motor development (aRR 0.29, 95% CI 0.13-0.64, p=0.002), delayed fine motor development (aRR 0.59, 95% CI 0.38-0.91, p=0.018) and delayed language development (aRR 0.57, 95% CI 0.34-0.96, p=0.036). In sociodemographic and nutritional questionnaire results indicate that weight for height is
strongly associated with the family income $\beta = -0.16$ with $95\% \text{ CI} (-0.89 \text{ to } -0.04) \ p=0.03$ and weaning practices $\beta = -0.21$ $95\% \text{ CI} (-1.14 \text{ to } 0.19) \ p=0.01$. In length/height for age (stunting) z-score the significant factors are, family monthly income $\beta = -0.16$ $95\% \text{ CI} (0.26 \text{ to } 1.08) \ p=0.04$ mother knowledge of complimentary diet $\beta = 0.15$ $95\% \text{ CI} (0.25 \text{ to } 0.96) \ p=0.03$ household food security $\beta = 0.16$ $95\% \text{ CI} (0.11 \text{ to } 1.48) \ p=0.02$ and exclusive breast feeding practices, $\beta = -0.22$ $95\% \text{ CI} (-1.47 \text{ to } -0.30) \ p=0.00$.

**Conclusion:** There was not any significant difference among two groups in the primary outcome, however high-dose vitamin D3 supplementation increased mean weight gain and the developmental status of children receiving standard therapy for uncomplicated SAM in Pakistan. Further researches are required to determine whether positive outcomes can be replicated in other settings. Moreover, developmental screening ought to be vital for primary healthcare system, specifically in high risk malnourished children and policy makers considering for betterment in children nutritional status should promote health-seeking practices and knowledge of families in this regard in Pakistan.

**Keywords:** Vitamin D supplementation, Severe Acute Malnutrition, Predictors of malnutrition, Child Development delay, Pakistan.
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LIST OF ABBREVIATIONS

25(OH) D: 25-hydroxyvitamin D

CMAM: Community management of acute malnutrition

CTC: Community Based Therapeutic Care

DDST II: Denver Development Screening Tool II.

HAZ: Height for Age

IRMNCH: Integrated Reproductive Maternal & New born Child Health

IU: International units

MAM: Moderate acute malnutrition

MUAC: Mid upper arm circumference

NNS: National Nutritional Survey

NRU: Nutrition Rehabilitation Unit

OTP: Outpatient therapeutic programme

Re So Mal: Rehydration solution for Malnourished Children.

RUTF: Ready- to- use therapeutic food

SAM: Severe acute malnutrition

SC: Stabilization center

SFP: Out-patient supplementary feeding program
SPE: Solid-Phase Extraction

TFC: Therapeutic Feeding Centre

UNICEF: United Nations Children Fund

WAZ: Weight for Age

WHO: World Health Organisation

WHZ: Weight for Height
THESIS ARRANGEMENT

This thesis is written and divided into six main chapters

**Chapter One:** states brief introduction of the problem and background info to comprehend, the children theory of malnutrition. Particularly, the narrative will investigate the accessible data in broad-spectrum for understanding the universal impact of malnutrition in children and severe acute malnutrition (SAM) specifically. Literature will also narrate that, how malnutrition impacts on child existence and development. Moreover, child growth and development with developmental screening by Denver developmental screening tools II were briefed. Furthermore, Vitamin D, mechanism in body, its deficiency globally and at national level, with manifestation of child vitamin D deficiency on children and particularly on SAM have been discussed. The management of SAM, applying a community treatment based model, with vitamin D intervention, which is the main field of study, is too illustrated. By utilizing previous data and literature, the requisite of this study with implementation for community-management based model and the therapeutic doses of vitamin D in management of children with SAM is rationalized. Research objectives with specific research questions are summarized in the concluding section of 1st chapter.

**Chapter Two:** represents data investigated from the review of literature after applying a systematic approach with narrative evince methodology. This chapter is divided into four parts, 1st part describes the developmental screening with its different tools and impact of malnutrition and other socio-demographic factors responsible for delay in child development. 2nd part explains different Vitamin D trials to understand the manifestations of its deficiencies and to improve child health at global level by intervention of vitamin D in
different diseases in the world. In 3rd part of the chapter the enormity of the evidence that occur in effect of community-management based model programs for SAM, with interventions done in this programmer at global level were narrated. Last 4th part briefed the different socio-demographic and dietary factors responsible for severity of malnutrition in children at national and global level. The aim of literature review was to identify the breaches that can be focused in this current research.

Chapter Three: defines the methodology followed in this research for assessment of developmental screening, anthropometry, identification of SAM and methods used to collect and analyze data, with recognition of risk factors for malnutrition and development delay with all complete procedure of conducting trial in the community based malnutrition treatment programmer in Punjab, Pakistan.

Chapter Four: explains the three components of the research that were conducted comprising the findings of these studies and the main results of these three components, i.e. results of developmental screening, main trial finding and predictors for severity of acute malnutrition as well as an interpretation of these results.

Chapter Five: Discussed study findings, integration with previous literature cited and summarizes the main findings of the different study parts, as well as an explanation of how this study results were incorporated to stipulate an explanation of what worked, how and why intervention should be done in the community based programs. Study limitations and strengths are also presented in this section.

Chapter Six: Comprises of brief summary of the thesis followed by conclusion of subject. Also few strategies implemented in Pakistan for child health, growth along with
development are discussed as well as few recommendations for making policies with practices are stated in the concluding section of 6th chapter. Important related documents and testimonials are attached in appendix.
CHAPTER ONE

1. INTRODUCTION

Nearly 20 million children suffer from severe acute malnutrition (SAM) worldwide: the majority of cases arise in low- and middle-income countries in Asia and Africa. SAM affects an estimated 1.4 million children in Pakistan, where it has been reported to carry a 6.2% case fatality rate (WHO, 2013). Malnutrition is the most important key public health issue in all over the developing countries of the world. If untreated, SAM can exhibit deleterious consequences on child health along with development and also increases susceptibility to infectious diseases (Duggan, 2003). Severe malnutrition is responsible approximately deaths of two million, under 5 years aged children per annum and is accountable for almost 22% of disability-adjusted life years globally in less than 5 years of age children (Schubl, 2010; Black et al., 2013). According to UNICEF and WHO joint malnutrition estimates for 2016 in Pakistan, 10.5% of children are wasted, 45% are stunted and 31.6% are underweight (UNICEF, 2016). Children who survive an acute episode of SAM are at increased risk of experiencing long-term adversative impacts on their physical fitness along with mental vigour (Lelijveld et al., 2016; Galler et al., 2012) which may also compromise their economic productivity as adults. (Galler et al., 2012).

First United Nations Millennium Development Goal (MDGs) aims at eradication of extreme poverty and hunger and its objectives include fifty percent reduction in the population facing hunger for the years 1990 to 2015. Despite its global precedence, hunger still prevails in lower and middle-income countries all over the globe (Travis et al., 2004). Presently 1.02 billion people throughout the world, mainly in underdeveloped countries,
suffer from hunger. Hunger is mostly described with reference to under nutrition. This word comprises various situations, from slight under-nutrition to episodes of severe acute malnutrition (SAM). Under nourished individuals are slower in physical and mental vigor. Their immune system is also weak, that makes them prone to infections and illnesses (Black et al., 2013). Famished of the satisfactory nutrition, persons will die from diseases like measles or diarrhea.

Globally severe acute malnutrition is the most important reason accountable for the morbidity and mortality of children under the age of five years. SAM is a lethal ailment, if prompt treatment is not provided; then many cases of SAM will go on to develop complications like diarrhea, anemia, edema, anorexia, acute respiratory infection, septicemia, shock and death. Emaciation or wasting is a severe form of SAM characterized by outcome of decline in energy intake, collective with an inequity in the consumption of carbohydrates, lipids and proteins, and micronutrients deficiencies (WHO, 2013). The term “Marasmus” is frequently applied to this kind of malnutrition. It is mostly the consequences of starvation or illnesses (or collectively both of them). Severe under-nutrition can progress to irreversible effects, resulting in slowing up the metabolism in effort to preserve energy and a slowing of protein turnover. The body then becomes incapable of temperature regulation. Neuromuscular functions weaken and immune system becomes ineffectual. This also affects the vital organs and may cause death by cardiac overload, hypoglycemia, or hypothermia or by an infection (WHO, 2009). Hunger and malnutrition are among the greatest threats for the health of growing children, especially in developing countries like Pakistan (Poverty, 2015).
The following parameters are utilized to identify severe acute malnutrition “a very low weight-for-height (below -3 SD of the median WHO growth standards) visible severe wasting, the presence of bilateral pitting edema known as edematous malnutrition (WHO, 1999) and a mid-upper arm circumference lower than 11.5 cm in children under five years of age” (UNHCR, 1999). Chronic malnutrition also known as stunted growth is described by utilizing the indicator of height-for-age. Moreover, a complex type of malnutrition together stunting with wasting is identified by the classification of weight-for-age.

Descriptions for classifications of nutritional status in 0-59 month old children are (WHO, 1995):

a. “Wasting (acute malnutrition) is defined as a weight for height (WHZ) of < -2 whereas severe wasting is considered if WHZ was < -3 OR if mid upper arm circumference (MUAC) < 11.5 cm”.

b. “Stunting (chronic malnutrition) is defined as a height for age (HAZ) of < -2 whereas severe stunting is considered if HAZ was < -3”.

c. “Underweight (mixed acute and chronic malnutrition) is defined as a weight for age WAZ of < -2 whereas severe underweight is considered if WAZ was < -3”.

d. “MUAC criteria for diagnosis: severe acute malnutrition is < 11.5 and moderate acute malnutrition is 11.5 to 12.5 (WHO, 1983)”.

1.1 Background of the study

In recent years, Pakistan has faced natural calamities like floods, famine/drought and earthquake. The most alarming consequence of these disasters is increasing malnutrition in children. National Nutrition Survey (2011) disclosed that approximately sixty percent of
people in Pakistan confront food insecurity and out of them while fifty percent of these children and women are suffering from malnutrition. Other critical finding of the survey (NNS, 2011) exhibit that in Pakistan stunting in addition to wasting and micronutrient deficiencies are prevalent. More than half of the children in Pakistan are unable to grow to their full physical and mental potential owing to malnutrition.

Acute Malnutrition is a problem which requires a comprehensive integrated public health approach to address it. Acute malnutrition is a result of food consumption deficiency or poor health causing both moderate-acute-malnutrition (MAM) as well as severe-acute malnutrition (SAM). The assessment of nutritional outcome of under-five aged children particular into countryside area is important determinant of child survival and to be also considered as one of the most important indicator of a household's living standard. The reasons are basically poverty, illiteracy, poor public health strategies and social segregation. From them many cases can be averted by implementing planned public-health policies to enhance only dietary quantity and quality, without any requisite of medical input and economic development. The management of SAM comprises a unique stance among clinical treatment and public health and requires specialized medication and prevention interventions. Effective interventions for the management of SAM could prevent millions of child deaths each year and contribute to achieve MDGs, for reduction of child morbidity and mortality.

On the basis of experience, Community based approach of CMAM has been found to be successful and cost effective solution to the problem. Community-based-therapeutic approach employs ready-to-use therapeutic food as a vital nutritional element for the prevention and treatment of malnutrition. This strategy comprises both assessment and
timely identification of children with SAM and house care of those without complications. The children with complications will still need facility based treatment. Home based care therapy for severe malnutrition in children is doing well in different ways for the last five 5 years (Manary, Ndkeha, Ashorn, Maleta & Briend, 2004).

1.2 Malnutrition: Global Public health implication

Malnutrition in its acute form is an important public health concern. Globally 26% aged below five year’s children are suffering from moderate or severe malnutrition. Approximately 9% of children in Africa sub-Saharan region and roughly 15% children in territory of south Asia have diagnosed with moderate acute malnutrition (Onis, 2003) and around 2% of children in developing world have in distress because of SAM. Lancet (2008) highlighted that Indo Pak region experience the maximum proportion of child population with severe wasting. However, seventy-eight percent of global children with wasting belong to India, Pakistan and Bangladesh. Whereas 2·8 percent below five years of age children in India facing severe wasting. SAM is the main reason of paediatric hospital admissions in several poverty-stricken countries like Malawi. These figures exclude children mortality because of oedematous malnutrition also known as kwashiorkor, underrating the proportion of child mortality related to acute malnutrition. This will be also the one of the major factor involved in delayed development of children in all four domain of development including “Gross Motor, Fine Motor, Language and personal social contact” affecting also intelligent quotient of children.
1.3 Malnutrition: Public Health significance in Pakistan

The prevalence of underweight, stunted and wasted children is higher in South-eastern Asia as compared to other regions of the world and prevalence of malnutrition also increases in Pakistan. According to the national nutritional 2011 survey, 31.1% of children less than 5 years are underweight, 43.7% children below five have stunting in 2011 in contrast to 41.6% in 2001. Likewise, 15.1% children are wasted in 2011 against 14.3% in 2001. In Pakistan females and children are also highly deficits in essential micronutrients (NNS, 2011). The national nutritional 2011 survey, also revealed the bio chemical values of Vitamin D3 inadequacy 1st time on a big scale for that vitamin D concentrations were examined on the samples obtained from the mothers and children. At national level vitamin D3 insufficiency prevalence was 41.1% between index children. Vitamin D3 deficiency is from the main factors involved in delayed growth as well as in child development. To comprehend the health profile of under 5 year children, mortality rate of children under the age of five years are deemed to be the crucial marker for health evaluation in this age group population. The under 5 mortality Rate in Pakistan is 84/1000 live births (World Bank, 2012). This glimpse of health profile depicts that Pakistan is still far away from attaining the health associated Millennium Development goals targets. Pakistan is signatory of the Millennium declaration intent to reduce maternal and child mortality.

1.4 Community Management of Acute Malnutrition

Community management of acute malnutrition (CMAM) is the treatment of acute malnutrition in the community by giving all treatment services at the doorstep of the patient’s residence. Conventionally, SAM children are cured in “centre-based care:
paediatric ward, therapeutic feeding centre (TFC), nutrition rehabilitation unit (NRU), other inpatient care places”. This concept terribly reduces coverage and impact. The centre based treatment approach practise the World Health Organization (WHO) protocol for SAM treatment. In 2001 CTC (Community Based Therapeutic Care) model was presented in emergency conditions and showed spectacular expand of program coverage and figure of effectively treated children. Community based malnutrition management were developed from the concept of Community–Based-Therapeutic Care (CTC).

The community-based management of malnutrition concept is a public-health paradigm, were planned for delivering effectual treatment for severely malnourished children as outpatient programme, by means of community mobilisation to involve families of concerned children to provide them maximum attention and treatment plan (WHO, 2007; Collins et al., 2006). This model is based on: timely case finding and management of the identified children having severe acute malnutrition with no medical complications through ready-to-use therapeutic foods and with regular prescription (Collins et al., 2006).

Investigators with health workers comprising Collins along with others explored that majority of almost > 90 percent children diagnosed with malnutrition without any medical complications come to hospital for treatment and will require specific hospital treatment from the time when they are capable to take regular energy dense diets in their home (Collins, 2001).

Investigators, explore that domestic approach to treat these children can minimize the risk of cross and hospital acquired infections and in turn reducing the mortality because of severe acute malnutrition and as well as decreasing the burden of pediatrics ward admissions in developing countries. This model admits children right away into
community-based management programs having age between 6-59 months without medical complications. Children's having medical complications like bilateral pitting grade three edema, convulsions, unconsciousness, severe anemia, hypoglycemia, hypothermia < 35 c, loss of appetite, intractable vomiting, lethargy not alert, severe dehydration with electrolytes imbalance, lower respiratory-tract infections and high-grade fever >39c are managed in stabilization centers as an in-patient care by following the WHO guidelines till they are sufficiently well for shifting in community-based programs (WHO, 2009). The model is created on the rule that each child diagnosed with any form of malnutrition should get proper attention and support on time without delay, irrespective of each child geographical site (Collins et al., 2006). It also realizes the social and financial conditions of people concerned for their child who are suffering from malnutrition, along with hurdles to gain access to look after in hospital (Briend, 2001). Therapeutic-programs are extended by utilizing respective model for decreasing terrestrial hurdles and involve concentrated community approach with community mobilization to enhance acknowledgment and contribution (Briend, 2001; Briend et al., 2006; Collins et al, 2006).
**Figure 1: Classification of acute malnutrition**

Source: “Pakistan National Guidelines for the Community Based Management of Acute Malnutrition 2009”.

A brief sum-up of basic elements of the community management-based model are (Collins et al., 2006):

1: Community mobilization for Sensitisation of local community members for identification of acute malnutrition to support early presentation.

2: Out-patient therapeutic Programme (OTP) meant for children between 6-59 months old diagnosed with SAM.

3: Stabilization Centre (SC) or in patient therapeutic program meant for children between 0-59 months aged with diagnosis of severe acute malnutrition.
4. Out-patient supplementary feeding program (SFP) aimed for children between 6-59 months old having moderate acute malnutrition for prevention of their progression to SAM.

CMAM basic principles are

1. “Maximum access & Coverage
2. Timeliness
3. Appropriate Medical Care
4. Nutrition Rehabilitation Care as long as it is needed”.

![Target Groups of CMAM Components and transfer routs](image)

*Figure 2: Target groups of CMAM components and transfer routs*

Source: “Pakistan National Guidelines for the Community Based Management of Acute Malnutrition 2014”.
Well the working of community based approach in Pakistan is in collaboration between government and non-government organization and follow the following structure. Lady health workers and visitors are playing key role.

**Figure 3: Different CMAM modalities in Pakistan**

Source: “Pakistan National Guidelines for the Community Based Management of Acute Malnutrition 2014”.

### 1.5 RUTF - A Therapeutic approach for treatment of SAM

For rehabilitation of malnourished children in hospital former treatment of SAM children depends on F-100, a water-based therapeutic nutritional regime prepared with milk
powder and sugar supplemented with micronutrients (WHO, 1999). Even though this ready to use therapeutic diet has been effectual for boosting speedy nutritional healing in mal-nourished children, it has many confronts like, its preparation requires safe hygienic environment to prevent bacterial contamination. Uncontaminated water is prerequisite for preparing this liquid and after preparation this liquid should be drunk in few hours. Remaining should be put for storage in fridge, if not must be throw away (Briend, 2001; Collins et al., 2006). So, the usage of F-100 diet is limited in hospitals only where children should live till their revival, or should arrange hygienic storage means. These prerequisites limit utilization of F-100 in community based approach for recovering children as numerous countryside villages in the under-developing countries are not capable for these simple preparation and storage precondition because shortage of nontoxic drinking water, electricity and suitable hygiene environment (UNICEF, 2006).

Central to outpatient care is the innovation of “Ready- to-use therapeutic food (RUTF), it is lipid-based nutrient-dense solid diet, with similar nutrient profile but greater energy and nutrient density than F-100 and mineral and vitamins enriched food specially designed to treat SAM. It is prepared from a mixture of peanut butter, powdered milk, powdered sugar, vegetable oil supplemented with micronutrients” (Briend et al., 1999). As Ready- to-use-therapeutic food (RUTF) is an oil based paste, having minimal water content, with shelf life of three to five months when tears and have no risk of bacterial contamination even when unintentionally adulterated (Briend, 1997; Briend, 2001). It did not require any preparation before eating due to very low water content, it resists significant bacterial growth. Hence, it can be utilized securely at home with no need of refrigeration and without any optimum hygienic situations and also do not need any specific expertise to give them
to their child (MSF, 2008). They are design in a way that child can eat by their own without help, only young infants require help to eat. Ultimately, the making of RUTF is simple and the items utilized are made by local community by easy processing. The ultimate invention before eating need no cooking or heating (Nutriset, 2000).

Plumpy-nut is a specimen of normally used lipid-based RUTF. It contains high amount of protein and energy with a peanut based paste wrapped in foil. It comprises a balance of all macronutrients and micronutrients. It is a very high energy dense food contains around 5.5 kcal/g comprising weight of 92 grams (Collins, 2001). Plumpy nut effectiveness has been verified in many states such as India, Malawi, Niger, Ethiopia, and Sudan. So the intervention of (RUTF) had mostly solved the difficulties in offering appropriate high nutrient energy dense diet and are secure to utilize in community based approach for cure of SAM children. The Ashworth appraisal specifies that the new development of RUTF has significantly reduced the complexities linked with giving an appropriate high-energy, nutrient-dense diet that is secure for consumption in outpatient programs. RUTF have same nutrient formulation but high energy and nutrient density in comparison with F100, the therapeutic diet advised by the WHO in the recovery period of SAM management (Briend at al., 1999). As RUTF is consumed without any prior cooking, heat-labile vitamins are remaining intact in preparation and the labor, energy and water needs on poor families are minimized. The manufacturing procedure is easy and RUTF can be produced from native crops with simple technology that is easily accessible in developing countries (Manary, 2006).
RUTF is utilized for children between 6-59 months of age for 6 to 8 weeks (Brined, 1999) and provide 200 kcal/kg of corporal weight/day until the targeted weight/height ratio is reached or 2 sachets/days for children weighting between 5 and 6.9 kg, 3 sachets/days for children weighting between 7 and 9.9 kg and 4 sachets/days for children weighting more than 10 kg. The innovation of RUTF was invented by a nutrition scientist Dr. Andre Briend as a substitute of F100 food and leads to the success of community-based approach to cure SAM children. The composition of RUTF are presented in table 1.
Table 1: Nutritional components for Ready-To-Use Therapeutic food (RUTF).


<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount per 100g RUTF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>545 kcal</td>
</tr>
<tr>
<td>Protein</td>
<td>13.6 g</td>
</tr>
<tr>
<td>Lipids</td>
<td>35.7 g</td>
</tr>
<tr>
<td>Calcium</td>
<td>300 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>300 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,111 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>92 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>14 mg</td>
</tr>
<tr>
<td>Copper</td>
<td>1.8 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>11.5 mg</td>
</tr>
<tr>
<td>Iodine</td>
<td>100 µg</td>
</tr>
<tr>
<td>Selenium</td>
<td>30 µg</td>
</tr>
<tr>
<td>Sodium</td>
<td>&lt;290 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>910 µg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>16 µg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>20 mg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>53 mg</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>1.8 mg</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>1.8 µg</td>
</tr>
<tr>
<td>Vitamin</td>
<td>21 µg</td>
</tr>
<tr>
<td>Biotin</td>
<td>65 µg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>210 µg</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>3.1 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>5.3 mg</td>
</tr>
</tbody>
</table>
1.6 Defining Growth and Development

1.6.1 Growth

Growth is defined as the “a measure of physical maturation, signifies an increase in size of body and its various organs, it can be measured in terms of centimeters and kilograms”. It can be also defined as the “growth represents the summation of all the processes that convert fetus through childhood to a sexually mature adult by increase in cell number, increase in cell size and increase in inter-cellular matrix in prenatal and postnatal period”. Growth is mainly due to multiplication of cells and increase in intracellular substances. Growth is a systematic phenomenon, proceeding in orderly fashion. Rates with different patterns of growth are particular with specific parts of the body. Broad individualistic differences occur in growth rates (Karlberg & Albertsson, 1995) like postnatal phase comprises of three distinct phases infancy, childhood and puberty (ICP model).

According to David Sinclair (2006) “Growth never lasts from conception to death it goes in to stages like early embryonic (everything is growth and there is no function) growth and function lasts up to maturity, functional activity and replacement at its lowest level”. Growth is sensitive indicator of health and nutritional status during childhood (WHO, 1995). Divergence in growth particularly in reduced growth are linked with an added risk of illnesses mutually in short as well as in long term. Growth monitoring is a valuable tool in examine the health and fitness of children specifically in countries where other diagnostic tools are sparse. It is also an essential parameter in advance clinical setting but mostly it is neglected in to the determinant of more advanced assessment. Early growth is linked with long term development and health. Wide individual differences exist in growth rates because of different causes.
Growth and development are influenced by multiple factors, like nutritional, socio-economic, environmental and seasonal factors, chronic illness, genetic factors, prenatal and intrauterine, emotional, health, exercise, sleep, hormonal and growth potential factors.

Growth spurts or acceleration of growth consist of three periods. Accelerated growth velocity is growth spurts and includes different periods and patterns.

### 1.6.2 Periods of growth spurts

- Infantile growth spurts 0 – 1 years
- Mid growth spurts 6 – 8 years
- Adolescent growth spurts 10 – 14 years

### 1.6.3 Different pattern of growth

- Somatic growth

  (“Weight, height, mid arm circumference, head circumference, Chest circumference, body mass index and skin fold thickness”).

- Neural growth
- Lymphatic growth
- Reproductive growth

### 1.6.4 Development

Development is defined as “acquisition of qualitative and quantitative skills and competencies in a social milieu”. It depends on maturation and myelination of brain. It is a continuous process. “Child development refers to how a child becomes able to do more complex things as they get older”. Development process is different from growth phenomenon; growth only indicates that the child is becoming bigger in size both interrelated impossible to separate. Development process advances from simple to
multiplex and from general to peculiar. Growth and development are influenced by multiple factors.

<table>
<thead>
<tr>
<th>Prenatal Factors</th>
<th>Neonatal Factors</th>
<th>Post Neonatal Factors</th>
<th>Social Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic Maternal</td>
<td>Low birth weight</td>
<td>Nutritional factors</td>
<td>Parenting</td>
</tr>
<tr>
<td>Neonatal Seizures</td>
<td></td>
<td>Acquired insult to Brain</td>
<td>Poverty</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endocrine factors</td>
<td>Lack of stimulation</td>
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<tr>
<td></td>
<td></td>
<td>Associated factors</td>
<td>Violence and abuse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Environmental factors</td>
<td></td>
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</tbody>
</table>

**Figure 5: Factors effecting development**

Source: GHAI Essential Pediatrics.

1.6.5 Normal development

Normal development is a complicated process and has a variety of aspects still it is easy to understand and evaluate development under the following areas.

1.6.5.1 Gross motor Development

Gross motor development refers to control of child over his body and is observed in ventral suspension, supine, prone, sitting and standings positions utilizing large groups of muscles for sitting, standing, walking and running, for maintaining balance and changing positions.
1.6.5.2 Fine motor skill Development

Fine motor skill development refers to good coordination of eyes, hand-eye, hand-mouth and skills for manipulation with hands like utilizing hands for eating, drawing, dressing, playing and writing as well as for doing various other things.

1.6.5.3 Language Development

Language development refer to hearing sounds, understanding, true speech and speaking by applying body language with gestures, communication and understanding with others.

1.6.5.4 Personal and Social Development

Personal and social development refer to interpersonal and social skills like social smile, mimicry, waving bye-bye and interaction with others. It also comprises family relationships, dealing with friends and teachers, collaborating and reacting with others emotions.

1.6.6 Developmental Milestones

“Developmental milestones are a set of functional skills or age-specific tasks that most children can do at a certain age range” (Anderson, 1998). Mostly pediatrician applies milestones to examine child development. Though every single milestone has a specific age level, the definite age when a normal developing child attains that milestone can differ a bit. As each child is exceptional the developmental milestones impart a broad indication of the expected changes as child grows older, but it is not alarming if child development proceeds in a marginally different direction. Every individual child proceeds at her own rate, so, if child shows possibly developmental delay signs for particular age limit they should be kept under supervision for screening of developmental delay for that specific age group.
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 2: Important developmental milestones at a glance</strong></td>
<td></td>
</tr>
<tr>
<td>Source: Adopted from (Onis, 2006).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Social</th>
<th>4 to 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head holding</td>
<td>3 months</td>
</tr>
<tr>
<td>Sits with support</td>
<td>6 months</td>
</tr>
<tr>
<td>Sits without support</td>
<td>7 months</td>
</tr>
<tr>
<td>Reaches out for a bright object and gets it</td>
<td>5 to 6 months</td>
</tr>
<tr>
<td>Transfers object from one hand to the other</td>
<td>6 to 7 months</td>
</tr>
<tr>
<td>Starts imitating a cough</td>
<td>6 to 7 months</td>
</tr>
<tr>
<td>Crawls</td>
<td>8 to 10 months</td>
</tr>
<tr>
<td>Creeps</td>
<td>10 to 11 months</td>
</tr>
<tr>
<td>Stands holding furniture</td>
<td>9 months</td>
</tr>
<tr>
<td>Walks holding furniture</td>
<td>12 months</td>
</tr>
<tr>
<td>Stands without support</td>
<td>10 to 11 months</td>
</tr>
<tr>
<td>Says one word with meaning</td>
<td>12 months</td>
</tr>
<tr>
<td>Says 3 words without meaning</td>
<td>13 months</td>
</tr>
<tr>
<td>Joins 2 or 3 words into sentence</td>
<td>15 to 18 months</td>
</tr>
<tr>
<td>Feeds self with spoon</td>
<td>24 months</td>
</tr>
<tr>
<td>Climbs stairs</td>
<td>24 months</td>
</tr>
<tr>
<td>Takes some clothes off</td>
<td>2 years</td>
</tr>
<tr>
<td>Dry by day</td>
<td>3 years</td>
</tr>
<tr>
<td>Dry by night</td>
<td>3 to 4 years</td>
</tr>
<tr>
<td>Dresses self fully</td>
<td>3 years</td>
</tr>
<tr>
<td>Knows full name and sex</td>
<td>3 years</td>
</tr>
<tr>
<td>Rides tricycle</td>
<td>3 years</td>
</tr>
</tbody>
</table>
1.6.7 Evaluation of Development

Developmental delay or severe developmental disorders are possible to recognize in early infancy. It includes certain steps like, comprehensive history has a key role for development assessment and clinical examination especially physical growth, physical assessment for genetic disorders, screen for vision, hearing and neurological assessment. Developmental delays and other problems in children because of development can be reduced to a major level by timely recognition and early intervention through periodic screening meant for recognition of developmental delays throughout the early pre-school years (Branson, Vigil and Bingham, 2008). Assessment of development is vital step in timely recognition of divergence in child developmental pattern. It is an easy and time efficient tool to endorse satisfactory surveillance for developmental advancement. Parameters evaluated are cognitive, fine and gross motor, hearing and language, behavioral with personal social and adaptive milestones.

1.7 Development Screening

Screening is defined as a “brief assessment procedure designed to identify children who should receive more intensive diagnosis and assessment”. Such an evaluation assists in early intervention assistances, succeeding a positive effect on development, behaviour and following school performance (Committee on Children with Disabilities, 2001). It also provides a chance for early detection of diseases and developmental incapacities. Preferably all children should regularly have screened but at least those with perinatal risk factors should be screened. So developmental screening is concise testing process designed to detect child who should have essentially more intensive diagnosis or
evaluation for identification of abnormal developmental delays. Different test is available for screening of developmental delays.

1.7.1 Test for Screening of Developmental Delays

- Denver Developmental Screening Test 11 DDST (infancy & preschool years)
- Bayley’s development scale
- Gesell’s criteria
- Wood side DST
- Developmental profile (DP-11)
- Early Language milestones scale (ELM)

1.7.2 Denver Developmental Screening Test II

The generally applied screening test for identifying developmental delay in infants and preschool age group is known as “Denver Developmental Screening Test II” (DDST). The DDST Items was formed in 1967’s as tool to recognize the early problems revealed in the development of children. It was utilized by the people of different sectors (health professionals, educators, and social service providers). DDST was modified and progressed in language along with articulation area after several years and were practiced and observed on 2,000 children (Frankenburg, Dodds, Archer, Shapiro & Bresnick, 1992). They had made interpretation in term of expanding the concepts with the separation of models for subgroups that were revealing clinical substantial discrepancies for development and admit them to modify for specific inhabitants.
The “Denver Developmental Screening Test- II” was formed “at the university of Colorado center in Denver” with the aim to develop a standardized tool to quickly screen the children in comfy and simple way for performing and inferring the test results corresponding to the strengths and debilities of the children. It is practiced to recognize child development problem in early age and help to plan an early intervention (Frankenberg & Meriitt, 2007).

1.7.3 Purpose of DDST II

The purpose of DDSTII depends on the child age as follows “New born child: to detect if there is a neurological problem like cerebral palsy. Infants: to identify nature of the possible problems for the early intervention. Children: to delineate academic and social problems in order to give an early intervention” (Medterm, 2007).

The Denver II is not an intelligent quotient (IQ) assessment test, this test is beneficial for screening of asymptomatic children to detect their possible developmental problems, moreover it is not generated to make diagnostic labels and it must not be substituted for a physical assessment or diagnostic estimation. It is the most commonly utilized screening test from birth to till 6 years aged children and need 20-30 minutes for completion of the test tasks. Normed on sample of miscellaneous population and on many diverse languages, it comprises of total multiple 125 tasks which are assembled in the DDST form in four different segments for screening the functions of following areas. “Personal – Social: getting along with people and caring for personal needs. Fine Motor Skills: eye-hand coordination, tearing the papers and problem solving. Language: hearing, understanding, saying words. Gross Motor: sitting, walking, jumping and others use of large muscles”.

It also includes five “Test Behavior” test items to detect child on task completion of 25%, 75% and 90% and after that “scored as concern if child completing task in shaded area (75-90%) scored as failure if not completed by time (90% complete)”. Referrals are justified for one failure or maximum two concerns. Prematurity are corrected till the chronological age of 2 years.

1.7.4 Advantage and disadvantage of DDST- II

It is a standardized tool that has been tested on a different population. It can be applied in a quick and easy way by the skilled professional and para-professional workers. The DDST had been critiqued for lack of sensitivity in children screening who might be problematic for later developmental outcome or in school performance. It can be directed and score as needed, but mostly cultural problems effect the results (Barlow, 2007). The strength of DDST- II aimed for testing the development of wide variety of diverse proficiencies in less time. It was not planned to test for single, or a few theoretical hypotheses like intelligence level, motor functioning and social-skills.

This tool was standardized on 2,000 children representative of Colorado before its validity (Denver, 2007). The inter rater and 7 to 10 day’s test retesting on same assessor and same viewer consistency was verified for all 141 possible items. Out of all, 125 tasks chosen in favor of final version, 107 ensured superb inter-rater consistency, 63 held outstanding and remaining 25 exhibited moderate to good testing, retesting consistency (Denver, 2007). But in few cases DDST-II worker should be familiar with cultural aspects of the area such as difference in urban and the village life that can influence the assessment results such as in certain areas numerous children were taught to eat by using
right hand because of cultural and religious aspects without using fork and spoon. Moreover, there are not any other easy as well as quick developmental screening tool for recognition of range of disorder as “intelligence, language, mental health, and motor and self-help skills adapted to the developmental ranges of norms”. This test skilled the administrator to recognize immediately the children strengths and weaknesses. It provides chances for additional inspection in particular area of concern (Franckenburge & Merrit, 2007).

1.8 Malnutrition impact on child Growth and Development

UNICEF report that rudest impact of malnutrition ensues before birth, when fetus cannot grow properly as well as in children initial life years, when child physical or mental development is delayed. If not prevented or cured, malnutrition can cause harmful effects on child development, growth and overall health. Kauffman, Jones and Kluger (1986) observed that malnutrition may possibly reduce child capability to react against trauma, and child become more prone towards infectious diseases. Golden (2000) reveals that one of the major risk factor for child high morbidity and mortality is severe malnutrition and further brief that if child is severely wasted his immunity against infections is reduced and child become more susceptible to diseases like diarrhea, respiratory tract infections and other infectious ailments and child mortality rate enhanced.

Kaufmann and coworkers also explained that consequences of malnutrition lead to alteration in the immune system response that causes weakened lymphocyte response, compromised phagocytosis consequential to reduced complement of specific cytokines and also diminished secretory immunoglobulin. These alterations prone children to
infections both acute and chronic (Kaufmann et al., 1986). Pipes explained further that chronic infections because of a compromised immune system leads towards low nutritional status in children, causing loss of appetite, reduced nutrient absorption, enhanced metabolic demands and more nutrient loss (Pipes & Trahms,1993). WHO (2005), state if child is malnourished before their 2nd birthday, they undergo irreparable physical and cognitive impairment, which can affect their health and development later on in life. Black with their associates brief that severely malnourished children who live in this state mostly are stunted in their parenthood and in turn may deliver low-birth weight or small children (Black et al., 2008; Bennett, 2009).

Malnutrition have social, behavioral and psychological impact, children with malnutrition reveal apparent behavioral transforms in the critical stages (Graham et al., 1983). They noticed that child with diagnosis of malnutrition is more lethargic, irritable and less energetic in comparison with the well-nourished children. It also depicts that such children in comparison with normally nourished counterparts have poor intelligent quotient and have delay in achievement of developmental milestone. Tomkins (1993), add-on that when these children confront iron and other micronutrient deficiencies, they incline to have learning difficulties in later life. Black et al. (2008) approximated that malnutrition single handedly were responsible around twenty-two % of disability-adjusted life years at global level for under five aged children.

1.9 Child Development in: Developing Countries

Child development represents community as well economic development, as efficient children become the basis of an affluent and maintainable society. In developing world
children less than five years are in danger to several risks, comprising poverty, undernutrition with ill health and discouraging home atmosphere which damages their development in cognitive, motor and social-emotional area. These deprived children probably have bad school performance and because of that reason suffer from high fertility rate, poor income and deliver inadequate care to their offspring's therefore influencing for the inter-generational spread of poverty (Chattopadhyay & Saumitra, 2016).

1.10 Vitamin D Overview

Vitamin D is an essential micronutrient also known a prohormone or sunlight hormone. Vitamin D is acknowledged as fat-soluble vitamin and it crucial function is regulation of bone mineralization (Laaksi et al., 2007). Vitamin D role is significant in skeletal development, cellular functions, calcium homeostasis and for promotion of calcium absorption in intestine (Ward, Gaboury, Ladhani & Zlotkin, 2007). Its key role is in the growth and preservation of bones (Cantorna, 2000; Rockett et al., 1998). Now a day's vitamin D role in linking Toll-Like receptors activation with antimicrobial responses for innate immunity is also proven (Laaksi et al., 2007).

The major resources for vitamin D in human being are foods and supplements and the endogenous production (Huh & Gordon, 2008). Vitamin D key source is endogenous and it is mostly generated in the skin after ultraviolet B rays’ exposure, if because of any reason sun exposure is inadequate, it can be provided through the exogenous sources by eating vitamin D enriched foods and supplements (Ward et al., 2007).

Fischer, Thacher and Pettifor (2008) stated that vitamin D is attainable mostly from oily fish with the limit excess in eggs and liver. Breast milk also is not a good supplier of vitamin
D. Another dietetic suppliers of vitamin D comprise diets enriched with vitamin D2 or vitamin D3. “Vitamin D2 is (ergocalciferol generated through the ultraviolet irradiation of ergosterol by using yeast) and Vitamin D3 is (cholecalciferol generated through the ultraviolet irradiation of 7-dehydrocholesterol using lanolin)” for examples cereals, dairy foodstuffs, infant formula milk having fortification with vitamin D (Huh & Gordon, 2008; Fischer et al., 2008). More than ninety percent of vitamin D is obtaining by endogenous synthesis in humans and rest of amount almost lower than ten percent is gained by mean of food resources (Misra, Pacaud, Petryk, Collett & Kappy, 2008).

There are numerous stages in endogenous production of vitamin D and the most essential segment is assimilation of “Ultraviolet B radiations (wave lengths 290-310 nm) through 7-dehydro-cholesterol 27 in the skin to procedure pro-vitamin D3”. There is quick conversion of “pro-vitamin D3 to vitamin D3” (Fischer et al., 2008). Ultraviolet rays activate production in the skin, but in people having dark skin this process is lowered (Holick, 1995; Clemens, Henderson, Adams & Holick, 1982). Mostly in winters when there is inadequate sun exposure and Vitamin D3 is produced in the body naturally in low amount then it is liberated by body fat store to carry out essential functions in the body (Fischer et al., 2008).

Vitamin D2 or D3 once generated in the body will have to pass by the liver for hydroxylation to generate 25-hydroxyvitamin D. The next step is to promote hydroxylation in kidneys to generate “1, 25 di-hydorxy vitamin D, which is the active metabolite of vitamin D”. Generally circulatory amount for “intermediate metabolite of vitamin D (25 hydorxyvitamin D)” is utilized like good marker for vitamin D level in the body (Pettifor, 2004; Kochupillai, 2008; Thacher, Fischer, Strand & Pettifor, 2006).
Factors responsible for reduction of absorption of the ultraviolet radiations in skin are mostly winter climate, dark skin pigmentation, by application of sunscreen, high altitude (northern latitude), atmospheric air pollution, limit to indoors activities, skin wrapped with clothes and skin infections like ichthyosis (Huh & Gordon, 2008). The ultra violet light needed in winter season is decreases by distance from the equator to 0 at high altitude above 50° (Thacher et al., 2006). Hepatic ailments and in malabsorption illness like cystic fibrosis, intestinal absorption of vitamin D is decreased (Fischer et al., 2008). By considering the difference in people skin pigmentation along with the intensity of sunlight exposure in different altitude, sunlight exposure required in big child are around five to thirty minutes, minimum two times a week in noontime for arms and legs. In infants, also two times exposure of head and shoulder in a week are perhaps adequate for accelerating satisfactory production for vitamin D3 (Fischer et al., 2008).

Moreover, the intense outcome of vitamin D on human immunity, working as a modulator of immune system has been gradually recognized, inhibiting unnecessary expression of “inflammatory cytokines and enhancing the oxidative burst potential of macrophages” thus enhancing the killing of bacteria (Otsuji et al., 2013).

Current data, points the finger at vitamin D role in adaptive immunity, for its effect on the differentiation of T cells among the regulatory along with pro-inflammatory subset. The known outcomes of vitamin D is linked with calcium absorption and osteoblastic activity. Furthermore, vitamin D has a significant role, in maintenance of calcium homeostasis and also enhance the calcium with phosphorus, intestinal absorption. The process of absorption occurs in small intestine by “binding of (1, 25-Dihydroxyvitamin D) to vitamin D receptors to promote trans-cellular absorption of calcium and phosphorus” (Kochupillai,
2008). Broad observation is that deficiency of calcium enhances the child demand for vitamin D, as well as also put child at risk to minor insufficiency of vitamin D (Fischer et al., 2008). It has been proven in different researches that low ingestion of calcium increases the catabolism of 25(0H) vitamin D by the stimulation of 24-hydorxylase as an outcome of raised 125-Dihydroxy vitamin D absorption (Pettifor, 2007).

Vitamin D has an important part in mineralization and development of skeleton (Kochupillai, 2008). Production of vitamin D is also altered by genetic factors in the body. Its key function has been explained as polymorphism of enzyme “7-dehydrocholestrol reductase in the skin, cytochrome p450 25-hyroxlase in the liver and vitamin D binding protein in the circulation”. These aspects can alter functions of vitamin D, moreover by intruding with up taking of 25(0H) D in target cells, or by altering effectiveness of hydroxylation to generate the active metabolite of vitamin D{125(0H) 2D} (Thacher & Clarke, 2011). It is documented that non-calcemic role of vitamin D in the body involve every tissue and each cell comprising immune cells, brain cells, breast cells, colon, prostate and several more with vitamin D receptors (Holick, 2010). It was discovered by many researches that upwards of two thousands genes are controlled by 1,25(OH)2D by direct or indirect way (Nagpal & Rathnachalam, 2005).
1.11 Global Prevalence for Vitamin D deficiency

Taylor (2008) described deficiency state of vitamin D in the body as 25-hydroxy vitamin D concentration "<20 ng/mL". Approximately in general one billion individuals is suffering from vitamin D deficiency (Taylor, 2008). In children nutritional rickets is the key indication of Vitamin D deficiency (Basile, Taylor, Wagner, Quinones & Hollis, 2007) and rickets still
is a widespread problem round the world (Huh & Gordon, 2008). The occurrence of rickets in children because of vitamin D deficiency, is most frequent diagnosis in several emerging states, deficiency ranges in children aged under five years between 5 to 45% even though in the presence of sufficient sunlight: Turkey (Ozgür, Sümer & Koçoğlu, 1996) Saudi Arabia (Elidrissy, Sedrani & Lawson, 1984) India, (Ghai & Koul, 1991), Iran (Salimpour, 1975), China (Zhao, 1991) Mongolia, Algeria (Maroof, 2011) and Nigeria (Akpede, Omotara & Ambe, 1999).

Rickets persists an endemic disease in several emerging countries and has relapsed in various developed countries as well (Pettifor, 2008). The common risk factors for rickets involved in infancy are exclusive breastfeeding without vitamin D supplementations, dark skin pigmentation, reduced sunlight exposure, winter climates, high altitudes and deficiency of vitamin D in mothers (Balasubramanian, Shivbalan & Kumar, 2006; Huh & Gordon, 2008). Approximately more than 1 billion population on the earth is suffering from inadequate circulatory vitamin D status. Dietetic calcium inadequacy is more in emerging states. Around half population of North American kids are vitamin D deficient and kids of the European states are also in danger in spite of constant supplementation (Fischer et al., 2008). In neonates deficiency of vitamin D because of inadequate concentration of vitamin D in mothers during pregnancy is not unusual. Comparison with cow milk, breast milk has low vitamin D level, so continued breastfeeding in many areas has been linked with vitamin D insufficiency (Fischer et al., 2008).

Concentration of 25(OH) vitamin D in new born babies, are almost two third of the maternal level and the “half-life of 25(OH) D” is about for three weeks, for that reason newly born child will require vitamin D from exogenous source after some weeks of birth.
(Pettifor, 2004). Recommendation is that by increasing breast milk level of vitamin D by vitamin D supplementation in lactating mothers, in high dose is enough for prevention of rickets in breast fed infants (Fischer et al., 2008). Deficiency of vitamin D is documented more in emerging countries like United Arab Emirates, Pakistan, China and vitamin D insufficiency (serum 250HD<10 ng/ml) prevalence in exclusively breastfed infants have been stated eighty-two percent (n=78), fifty-five percent (n=62), and twenty percent (n=42) in that order. (Balasubramanian et al., 2006).

![Figure 7: Vitamin D Status in Children and Adolescents (<18 years)](source)

Source: Adopted from International Osteoporosis Foundation (2012).
1.12 Vitamin D deficiency: Prevalence in Pakistan

Pakistan the nation-state is 70 years old with the increase in population from 34 million in 1947 to an approximated population of 207.8 million in 2017 census. Mother and Child mortality and morbidity were main confronts till now. Pakistan also signed the Millennium declaration and showed an affirmed intention to decrease mother and child death rate. According to national nutritional survey conducted in Pakistan 2011, with collaboration of government of Pakistan and UNICEF Pakistan, found high prevalence of essential vitamins and minerals deficiencies in Pakistani women and children (NNS, 2011). For the 1<sup>st</sup> time in Pakistan national nutritional survey 2011, revealed the bio chemical status of vitamin D deficiency on large extend. Levels of vitamin D were assessed on the samples obtained from the mothers (both in pregnant and in non-pregnant) and in children. It was observed that deficiency of vitamin D is prevalent in Pakistan. Higher prevalence of vitamin D deficiency around 45.9% were noticed in urban areas. Considerable disparities were observed in children among the provinces, varying from 28.9% in Khyber Pakhtunkhwa (KP) to 43.4% in Baluchistan. The nationwide frequency of vitamin D deficiency in index children was 40.0 % (NNS, 2011).
The National Nutritional Survey 2011, also noticed elevated frequency of vitamin D deficiency in non-pregnant females, which is 66.8% ("72.5% in urban areas and 64.3% in rural areas"). Deficiency of vitamin D in non-pregnant females, province wise in Pakistan was: “in Punjab 66.4%, in Sindh 71.2%, in KP 61.0%, in Baluchistan 54.6%, in AJK 73.3% and in Gilgit Baltistan 80.9%”. The NNI assessed vitamin D deficiency among pregnant women, showing 68.9% deficiency overall ("73.5% in urban areas and 67.2% in rural areas"). Statistics in provinces disclosed vitamin D deficiency in pregnant women: “in Punjab was 71.1%, in Sindh 66.9%, in KP 63.8%, in Baluchistan 43.6%, in AJK 73.4% and in Gilgit Baltistan 76.1%” (NNS, 2011).
Table 3: Prevalence of Vitamin D Deficiency in Pregnant and Non Pregnant Mothers in Pakistan


<table>
<thead>
<tr>
<th>Vitamin D Deficiency - Non Pregnant Mothers</th>
<th>Total</th>
<th>Residence</th>
<th>Province / Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urban</td>
<td>Rural</td>
</tr>
<tr>
<td>Severe Deficiency (&lt;8.0 ng/mL)</td>
<td>25.3</td>
<td>46.3</td>
<td>18.4</td>
</tr>
<tr>
<td>Deficiency (8.0 - 20.0 ng/mL)</td>
<td>40.9</td>
<td>30.5</td>
<td>44.4</td>
</tr>
<tr>
<td>Desirable (&gt;20.0 - 30.0 ng/mL)</td>
<td>18.8</td>
<td>11.2</td>
<td>21.4</td>
</tr>
<tr>
<td>Sufficient (&gt;30.0 ng/mL)</td>
<td>14.9</td>
<td>12.1</td>
<td>15.8</td>
</tr>
<tr>
<td>N</td>
<td>5402</td>
<td>1965</td>
<td>3437</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamin D Deficiency - Pregnant Mothers</th>
<th></th>
<th>Residence</th>
<th>Province / Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urban</td>
<td>Rural</td>
</tr>
<tr>
<td>Severe Deficiency (&lt;8.0 ng/mL)</td>
<td>26.5</td>
<td>50.7</td>
<td>20.2</td>
</tr>
<tr>
<td>Deficiency (8.0 - 20.0 ng/mL)</td>
<td>42</td>
<td>27.7</td>
<td>45.7</td>
</tr>
<tr>
<td>Desirable (&gt;20.0 - 30.0 ng/mL)</td>
<td>17.6</td>
<td>7.9</td>
<td>20.1</td>
</tr>
<tr>
<td>Sufficient (&gt;30.0 ng/mL)</td>
<td>13.9</td>
<td>13.7</td>
<td>13.9</td>
</tr>
<tr>
<td>N</td>
<td>699</td>
<td>222</td>
<td>477</td>
</tr>
</tbody>
</table>

In Pakistan, dietetic intake of vitamin D is low and only a small percentage of inhabitants with good socioeconomic conditions can pay for quality food rich in vitamins and minerals. Thus, foods enrich with vitamin D, like fish and liver, are not in the range of many people. Fortified formulas with vitamin D and fortified milk is costly. Other dietetic resources of vitamin D, such as eggs are generally available and used by large number of peoples. Covering of infants with clothes that decreases exposure to sunlight is also common (Manaseki et al., 2008). Thus young children have more chances of suffering from deficiency of vitamin D.
UNICEF stated that vitamin D deficiency rickets, in high-risk groups is prevalent in most Middle Eastern countries, in a geographic region extending from Morocco to Pakistan and can appear also in south as far as in Ethiopia. It is also usual in many areas of Eastern Europe. Insufficiency of exposure to sun combining with low dietary intake of vitamin D and high intake of Pythic acid (found in bread) can be the reason of causing rickets. Residents of desert regions where atmospheric dust works as a filter for ultra-violet light are at risk to vitamin D deficiency, specifically when people stay indoor to stay away from the heat of the day and wear massive clothes. Peoples who are compelled to stay indoor due to bombing or fighting are also at risk.

1.13 Classification Vitamin D Levels in the body

Misra et al. (2008) a latest review of literatures categorizes the vitamin D deficiency level for children established on status of serum 25(0H) D in ng/mL.

1 Severe deficiency: 25(0H) D less than 5 ng/mL
2 Deficiency: 25(0H) D between 6-14 ng/mL
3 Insufficiency: 25(0H) D 15-20 ng/mL
4 Sufficiency: 25(0H) D 21-100 ng/mL (50-250nmol/L)
5 Excess: > 1 00 ng/mL
6 Intoxication :> 150ng/mL (>250nmol/L)
<table>
<thead>
<tr>
<th>Deficient</th>
<th>Insufficient</th>
<th>Sufficient</th>
<th>Optimal</th>
<th>Treat Cancer and Heart problem</th>
<th>Excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 ng/ml</td>
<td>20-30 ng/ml</td>
<td>&gt;30 ng/ml</td>
<td>50-70 ng/ml</td>
<td>70-100 ng/ml</td>
<td>&gt;100 ng/ml</td>
</tr>
</tbody>
</table>

**Figure 9: Vitamin D (Calcidiol) Level**


### 1.14 Vitamin D deficiency manifestations: in children

Deficiency of vitamin D is the key factor, involved for child delayed growth and development. Rickets because of vitamin D deficiency, is one of the main reason for delayed gross motor milestones in malnourished children. Vitamin D, 25(OH) D level less than 5ng/ml (12.5nmol/L) is severe deficiency state. It is outcome of reduced bone mineralization in child, mainly because of insufficient calcium and phosphorus in the growth palate (Thacher et al., 2006). Rickets defined by walker “the infant with rickets has often received sufficient calories and may appear well-nourished but is restless, fretful, pale with flabby muscles, prone to respiratory and gastro-intestinal infections. Development is delayed and teeth often erupt late along with failure to sit, stand, crawl and walk at the normal ages”. The bony changes are the characteristic signs of rickets (Walker, Colledge, Ralston & Penman, 2013).

Nutritional rickets occurs in children because of insufficient calcium, even after having sufficient vitamin D level (Thacher et al., 2006). Metabolites of vitamin D cross the placenta
that’s why infants are usually defended from rickets in the early months of life. Children between the ages of nine to eighteen months are more susceptible to rickets (Pettifor, 2004). Rickets because of nutritional origin is more common in developing countries but it is not properly addressed and reported there. Particularly in Muslim countries because of traditional practices, females cover herself properly by wearing heavy clothing which reduces sunlight exposure to females and their kids (Balasubramanian & Ganesh, 2008).

A research conducted in Saudi Arabia, in one of an urban hospital discovered that fifty-nine percent of females were (n= 100) giving birth and out of them seventy percent of their new born babies were deficient in vitamin D with level < 25 nmol/L (Thacher et al., 2006).

Deficiency of vitamin D is also linked with delayed milestones in children and there is high probability that the minor cases will be overlooked. Like a flabby baby at the end of the 1st year, is incapable to pull itself up, fretful, irritable, showed delayed dentition and predisposed to profuse sweating and should always be expected to have rickets (Mann & Truswell, 2012). Other epidemiological researches strongly recommend that deficiency of vitamin D inclines towards viral respiratory tract and mycobacterial infections, as increased levels of calcitriol in serum have been demonstrated in patients with both tuberculosis and hypocalcaemia (Chocano & Bedoya, 2009). Manifestations of vitamin D inadequacy in different infectious etiology comprises, inadequate vitamin D uptake or metabolism and manifest an essential role in the progression of many diseases involving the central nervous system (CNS), skeleton with various organs where metabolic disturbances might play a part in the advancement of tumors (Roukos, 2011). To sum up in children a link concerning nutritional rickets with respiratory compromise has been established from long
time (Walker & Modlin, 2009). Latest epidemiological findings prove the association among vitamin D inadequacy and the increase prevalence of respiratory diseases with the effect of vitamin D in the host defense reaction to infection. In vitamin D insufficiency infants and children are more prone to viral instead of bacterial infections. The link among vitamin D, infections and role of immune system in children indicate vitamin D supplementation for possible interventions and adjuvant treatments (Walker & Modlin, 2009).

Figure 10: Impact of vitamin D deficiency
Source: Adopted from Karlic and Varga (2011).

1.15 Possible side effects: for vitamin D supplementation

Because of vitamin D hypercalcemic impacts, it may produce acute as well as chronic intoxication primarily. "Acute intoxication is linked with nausea, vomiting, dehydration, anorexia, apathy, polyuria, polydipsia, hypertonia, constipation, corneal clouding and
hypercalcemia” (Cranney, Weiler, Donnell & Puil, 2008; Orbak et al., 2006). Continued vitamin D intoxication have an effect on kidneys causing nephron calcinosis, renal colic, renal failure and neurological illnesses (Vieth, 2007; Chiricone, Santo & Cirillo, 2003). Research paying attention on evaluation of vitamin D, side effects are still deficient. Problems of acute along with chronic intoxication of vitamin D in adults as well as in children were recorded by many register cases from various states (Ko, Liberman & Salzmann, 1991). A review of twenty one different clinical experiments, associating the possibility of vitamin D toxicity from a low dosage on a daily basis (3800 IU/day) to a very high dosage on a daily basis (100,000 IU/day) in adults, accomplished that persisted consumption of (10,000 IU/day) as upper limit of vitamin D has slightest possibility to enhance the probability of side effects in the public, though the ability of circulating vitamin D binding-protein might affect the safety regarding vitamin D supplementation (Hathcock, Shao, Vieth & Heaney, 2007).

1.16 Intervention of Vitamin D with Ready to use therapeutic food

“Ready-to-use therapeutic food (RUTF)” is an energy dense micronutrient-enriched paste represents the mainstay for community treatment of uncomplicated SAM (i.e. where children are clinically well and alert, with good appetite). The World Health Organisation (WHO) has highlighted the need for research to identify adjunctive therapies that may improve response to RUTF, including administration of broad-spectrum antibiotics and high-dose vitamin A (WHO, 2013). The potential for adjunctive vitamin D to improve weight gain and developmental outcomes in children with SAM has been overlooked. However this is surprising because rickets and deficiency of vitamin D are common among SAM
children (Raghuramulu & Reddy, 1980; Berkley et al., 2016; Ejaz & Latif, 2010). Vitamin D deficiency associates with severe wasting in malnourished children (Jones et al., 2017) and vitamin D supplementation has been proven to increase weight gain in low birthweight infants (Kumar et al., 2011). Vitamin D have also been indicated to produce favourable outcomes on function of skeletal muscle, (Hazell, DeGuire & Weiler, 2012) neurodevelopment (Eyles et al., 2009) as well as immune function (Martineau et al., 2017; Coussens et al., 2012) its anti-inflammatory and antimicrobial actions might enhance response to standard therapy for SAM, a condition in which both increased systemic inflammation and infections associate with adverse outcome (Attia et al., 2016).

Although RUTF contains modest amounts of vitamin D (15 micrograms [600 IU] per sachet) we questioned whether intake from this source would be sufficient to elevate “circulating levels of 25-hydroxyvitamin D (25[OH]D)” into the optimal range in children with SAM in Pakistan, given the particularly low baseline levels reported locally (Raghuramulu & Reddy, 1980: Berkley et al., 2016) with the presence of a systemic inflammatory response that possibly will dysregulate metabolism of vitamin D and increase vitamin D requirements (Mangin, Sinha & Fincher, 2014).

Plumpy nut effectiveness has been demonstrated in many countries such as India, Malawi, Niger, Ethiopia, and Sudan. No sufficient data available on its effect on development of children as compared to growth of children. As well as no data available as intervention of vitamin D in therapeutic with RUTF to accelerate growth and development of children. Although, vitamin D role in growth and development of children is already proven.
1.17 Statement of the Problem

In Pakistan, floods of 2010 hit gravely in the month of July & August affecting millions of people economically and socially in Pakistan and recent flood in 2014 worsen this situation. In 2010 unfortunately, the flood affected districts in Punjab were those where indicators of maternal, new born and child health were not good even before they became flood-hit. The children are severely suffered from malnutrition in the said areas causing their delayed growth and development. In Pakistan 90% population are suffering from deficiency of vitamin D and this deficiency does not only affect the growing bone of children but also presents with others varying signs and symptoms inclining the children towards various others illnesses and skeletal deformities. These children are more prone to respiratory and gastro intestinal infections playing a major role in vicious circle of malnutrition. Because of this development of children is delayed, tooth usually erupt late and there is failure in sitting, standing, crawling and walking at the normal ages. Affected children are also retarded in growth.

As the Ministry of Health, Government of Pakistan plan to incorporate “Community management of acute malnutrition (CMAM)” in to existing regular health services for children under 5 years and to expand “community management of acute malnutrition” all over the country. The knowledge obtained from this study may guide policy makers in future decisions regarding the inclusion of therapeutic doses of vitamin D with RUTF because of significant improvement in development quotient of child with growth, depict in this study. Development screening of child before and after RUTF therapy also highlighted the RUTF effect on it with or without vitamin D intervention.
1.18 Research in Context

1.18.1 Evidence before this study

Rickets and deficiency of vitamin D have long been recognized to be existed in children with severe acute malnutrition in both Asia and Africa, and deficiency of vitamin D has been proven to associate with severe wasting in malnourished children. “Ready-to use therapeutic food (RUTF)”, the mainstay of therapy for SAM, contains relatively modest quantity of vitamin D that are insufficient to elevate “circulating levels of 25-hydroxyvitamin D (25[OH] D)” into the optimal physiological range. High-dose supplementation of vitamin D has been proven to have anti-inflammatory and antimicrobial actions that could enhance response to standard therapy for SAM, a condition in which both increased systemic inflammation and infections associate with adverse outcome. However, randomized controlled trials evaluating effects of high dose supplementation of vitamin D in children with SAM have not previously been done.

1.18.2 Added value of this study

This is the first randomized trial to assess the outcomes of high-dose vitamin D supplementation in children with SAM. In a cohort of children aged 6-58 months in Pakistan receiving RUTF for the treatment of uncomplicated SAM, we report that administration of adjunctive high-dose vitamin D affected in a large increase for mean weight-for-height/length z-score at 2 months. It also significantly reduced the proportion of participants with delayed global, motor and language development at 2 months.
1.18.3 Implications of all the available evidence

Vitamin D content of “Ready-to-use therapeutic food” may not be optimal to support weight gain along with development in children with SAM. Further trials are required to conclude whether high-dose vitamin D can improve weight gain along with developmental indices in children with SAM in other settings.
1.19 Objectives of the Study

- To examine the impact of malnutrition on development quotient of children
- To determine the effectiveness of ready to use therapeutic food (RUTF) in improving the development quotient of severe acute malnourished children under five year of age.
- To investigate the outcome of Vitamin D therapeutic doses intervention with RUTF rehabilitation on growth and development of malnourished children.
- To explore the dietary and socio demographic factors responsible for severe acute malnutrition and delayed development in children.

1.20 Research Questions

i. Is there any impact of malnutrition on development quotient of the children?

ii. Is there any improvement in development quotient of children after treating malnutrition with high caloric RUTF?

iii. Is RUTF rehabilitation with vitamin D therapeutic doses more effective to accelerate growth and development quotient of children?

iv. Are the dietary and socio demographic factors responsible for severe acute malnutrition and delayed development in children?
CHAPTER TWO

2. LITERATURE REVIEW

This chapterbriefs data investigated from the literature review after applying a systemic approach with narrative evince methodology. This chapter is divided in to four parts, 1st part describes the developmental screening with its different tools with impact of malnutrition and other socio-demographic factors responsible for delay in child development. 2nd part explains different vitamin D trials to understand the manifestations of its deficiencies and to improve child health at global level by intervention of vitamin D in different diseases in the world. In 3rd part of the chapter the enormity of the evidence that occur because of effect of “community based management treatment programs” for severe acute malnutrition and interventions done in this program at global level were narrated. Last 4th part briefed the different socio-demographic and dietary factors responsible for severity of malnutrition in children at national and global level. The aim of literature review was to identify the breaches that can be focused in this current research.

2.1 Developmental Screening, Predictors and Intervention

Studies of factors associated with developmental screening in Pakistan are scant. However similar studies elsewhere in the world shows these following results.

Ozkan, Senel, Arslan and Karacan (2012) planned a study to identify the socio-economic and biological risk factors associated with developmental delay in socioeconomically disadvantaged (3 months–5 years) old children in Ankara, Turkey. The consequences of biological and, socio-economic risk factors on developmental delay were studied in 692
children by utilizing the Denver II screening test. Results depict that low level mother education as well as low-level of father education, low family income and more than 3 children in the family were strongly linked with abnormal Denver II test results. Based on univariate analysis in biological risk factors, comprising birth weight with gestational age at birth, and maternal age less than 20 years at birth, were associated with suspected delay on Denver II test results. Low level of mother education, premature birth: 32–36 weeks of gestation have strong association with abnormal results on Denver II screening test. On multivariate analysis with low father education, low family income, premature birth with low weight on birth and mother age at birth <20 years were also strongly related with suspected delay on Denver II screening test results. It was concluded that socio-economic risk factors were considered as significant as biological risk factors in the development of 3 months to 5 years old children.

Simon, Pastor, Avila and Blumberg (2013) studied the relationship among socio-economic disadvantage and developmental delay in US children. For this to accomplish they had categorized all children from 18 months to 5 years old in to 3 groups on the probability of development delay from “2007 National Survey of Children’s Health” by utilizing the revised survey report of the “Parents’ Evaluation of Developmental Status questionnaire”. On applying bivariate and multivariate multinomial logistic regressions it was suggested that older children had increased chances of probable delay in comparison with unlikely delay, other important factors were, low birth weight ethnicity of non-Hispanic black or Hispanic in a non-English-speaking people vs non-Hispanic white with low family income. To wrap up four features older, male, low birth weight and Hispanic living in a non-English-speaking household respectively were related to more odds of possible delay compared
with unlikely delay. Conclusion were made that demographic features and markers of social disadvantage, differentiate children with possible developmental delay from them unlikely to have developmental delay.

Mangani et al. (2013) investigated that whether addition of nutrient supplements which is lipid based (LNS) in the dietary regime of infants and young child were still effective after achieving of their particular developmental milestones. For this purpose, total (840) healthy infants aged 6 months were registered for a randomised trial. No supplementation was given to control participants but for 12 month’s study arm were supplemented with milk-containing lipid based nutrient supplements, soy-containing nutrient supplements which is lipid based (LNS) or corn-soy blend (CSB). Outcomes of study were the specific age for attaining of an important milestone like, motor: standing, walking alone or with the help of assistance, running etc., social: includes that one is drinking by using cup as well as eating by own, language: by waving goodbye and by uttering particular single understandable words. This study concluded that no effect was observed in young offspring of rural Malawi after giving tested formulations and with micronutrient fortified with LNS or CSB dosages after achieving of their particular developmental milestones.

Paiva, Lima and Eickmann (2010) performed a study on population belongs to low socioeconomic conditions to explore various poverty settings and to determine their impact on the neuro psychomotor development of infants. They had suggested that child development is associated negatively with various risk elements related to poverty and highlighted the significance of the population with low socioeconomic conditions. They have selected 136 infants in Recife, Brazil between 9 to 12 months of age from four different units dealing with family health. Socio-economic class was evaluated by means
of specific index along with child development by using the "Bayley III screening test". This study disclosed that the maximum number of tots having receptive communication alleged delay belongs to families of lowest socio-economic class. Unemployment of both mother and father have negative effect on the receptive communication along with cognition. The families without mobile handset represent the lowest socioeconomic level and is related to poor cognitive and gross motor function. While the male infant’s revealed high incidence of suspected delay in receptive communication. This study has suggested to initiate the development surveillance and intervention programs for the target subgroup to deliver the infants an increased probability of becoming fruitful nationals in the coming years.

Afarwuah et al. (2007) in Ghana piloted a randomized trial to evaluate three kinds of micronutrient supplements as a complementary food which is home based for assessing its effect on child growth along with motor development. They had examined that whether adding multiple micronutrients to complementary diets which are made at home would improve growth along with good impact on motor development and whether that result would enhance with addition of extra energy from fat. For that 313 Ghanaian infants aged between 6 to 12 months have given random allocation to get on daily basis correspondingly potential low cast solution to complementary diets with sprinkle powder, crushable nutri-tabs, or fat base high energy (108Kcal/d) nutri- butter comprising of six, sixteen, and nineteen vitamins with minerals. Anthropometric measurements were evaluated at six, nine, and twelve months, micronutrient profile at six and twelve months, development of motor milestones at twelve months and any morbidity on weekly basis. Total 96 Infants who were not chosen for the intervention randomly (non-intervention) were also evaluated at twelve months. They had made conclusion that all three supplements
had effected positively on achieving motor developmental milestone at twelve months in comparison with infants received no intervention, but out of these supplements only fat base nutri butter (NB) have been proven for affecting growth positively.

Nahar et al. (2009) designed time-lagged controlled research in children between six to twenty-four months of age suffering from severe malnutrition and admitted in Nutritional Rehabilitation Unit of Dhaka hospital. The purpose of research is to integrate stimulation in to the standard treatment protocol of children diagnosed as having severe malnutrition in nutrition unit with assessment of its outcome on child growth along with development. The control group of forty-three children was investigated firstly, pursued by group of fifty-four children getting intervention and all children were also receiving routine nutritional care. The involved mothers and children took part in study for two weeks in hospital for daily group meetings with exclusive play gatherings and were also followed for six months at home. Child growth was evaluated and “Bayley Scales of Infant Development” were applied for determination of development. In both groups primarily children had same developmental achieves and anthropometry but six months later, the group received intervention had recovered better in comparison with the controls group with an average of weight-for-age z scores. In finale psychosocial stimulation incorporated in hospital for management of severely malnourished children with six months monitoring at home visit, were successful in increasing child growth along with development and recommendation were given for inclusion of this as an essential part in their management.

Yousafzai, Rasheed, Rizvi, Armstrong and Bhutta (2014) investigated the outcome of integrated responsive stimulation and nutrition intervention to boost child health outcomes, growth along with development in Pakistan. To execute this study, they have designed a
“cluster-randomized factorial effectiveness trial” which are community based into the program of Lady Health Worker of rural Sindh. They did random allocation of 80 clusters of children (1489 registered mother and infant) to obtain regular health along with nutrition services and nutrition education with multiple micro-nutrient powders, responsive stimulation or combining both interventions in 1:20 allocation ratio with no indications of any serious impairments. Interventions given to the families having child till the age of 24 months by the Lady Health workers in regular per month group meetings and at home visits. The main outcome of study was assessment of child development by the “Bayley Scales of Infant and Toddler Development at 12 and 24 months of age” along with growth assessment at age of 24 months. They have revealed that kids who had obtained responsive stimulation have considerably high developmental attains at age of twelve and twenty- four months on their cognitive outcome, language milestones along with motor scales and at age of twelve months on the achievements of social-emotional development in comparison with those who had not received intervention. Moreover, children who have taken increased nutrition had notably high developmental attains on the cognitive outcome, language milestones and achievements of social-emotional development at age of twelve months in comparison with control group but language scores were still notably high at 24 months of age. But additional benefits of combining responsive stimulation with nutrition interventions were not recorded. In term of growth children getting enhanced nutrition had considerably improved height-for-age z scores at 6 months and 18 months than control group. Conclusion were made that the responsive stimulation intervention was delivered successfully by LHWs with positive developmental outcomes.
Kar, Rao and Chandramouli (2008) did a study on children in India to assess the impact of “chronic protein energy malnutrition on cognitive development”. By applying neuropsychological measures, they have observed the impact of stunted growth on the cognitive process of developmental quotient of kids. They have enrolled 20 malnourished and 20 well-nourished children among the age category of five to seven and eight to ten years in the study. They have used the methodology of NIMHANS neuropsychological battery based on trials of “motor speed, attention, visuospatial ability, executive functions, comprehension, learning and memory for children” susceptible of the consequences for brain dysfunction along with age associated productivity. It has been evaluated that cognitive development seemed to be controlled together by age plus nutritional status. Children having malnutrition shows poor performance in all aspects of attention, visuospatial ability, working memory, learning and memory and only excluding the trial of coordination with motor speed. To wrap up it was evaluated that children having malnutrition performed poorly in comparison with the functioning of children who are well nourished. So inference depict that chronic protein-energy malnutrition also known as (stunting) alters the continued developmental process of advance cognitive functions in early years of kids to certain extent that simply indicate a generalized cognitive loss. Stunting also slows down the age linked performance in a few but not in all high class cognitive functions and end up in longstanding cognitive damage.

Manno et al. (2012) planned a randomised double blinded control trial to judge the hypothesis that plentiful fortification of micronutrients in diets produced at local level did not improve mental as well as motor development in Zambian children. They have assessed the outcome for the rich fortification of micronutrient and porridge fortified with
basal on mental along with psychomotor development in children of Zambia. Total six months old 743 kids were randomized and took the rich micronutrient fortified or the diet fortified with basal and monitored till the age of 18 months. Each infant was assessed on a monthly basis for attainment of different developmental milestones in order. The “Bayley scales of infant development II” have been applied on a group of 502 children at six, twelve and eighteen months. They have judged that rich fortification with micronutrient had not any considerable effect upon the subsequent parameters like attainment of different developmental milestones, age of walking without any support and speaking 3 or 4 clear words “with mental development index (MDI) and psychomotor development index (PDI) of the Bayley scales”. To sum up the findings did not prove the hypothesis that Zambian children mental along with motor development were improved with plentiful micronutrient fortification.

Phuka et al. (2012) piloted a trial on Malawian children aged 18 months with the main objective to assess their improvement in development quotient after one-year consumption of complementary feeding enriched with nutrient supplements which are lipid-based or micronutrient fortified corn-soy flour porridge. The other purpose of this study were to reveal the socioeconomic aspects linked by developmental outcomes in the similar inhabitants. They had registered 163 rural Malawian 6 months old children in a randomized control trial in which the control children’s took corn-soy flour around 71 gram having 282 kcal [“Likuni Phala (LP)”] supplements on daily basis and children’s of the intervention block took lipid-based nutrient supplement around 50 gram having 264 kcal calories on daily basis (FS50) or 25 grams of lipid-based nutrient supplement of 130 kcal (FS25). They applied “Griffiths developmental scores at 0–2 years” to determine main
outcome measures. Analysis of variance were used for independent comparison of study
groups and "mean raw scores, quotients, or mental ages" has been evaluated. Correlation
for development results with risk factors were researched by applying multiple regression.
At the age of 18 months, the mental ages in the “LP, FS50 and FS25” groups were
significant (P > 0.99). At 18 months' length/age z-score improvement throughout the study
duration and maternal literacy were linked with results in development quotient. Wrapping
up, rural Malawian infants getting the nutrient diets which are lipid based or fortified corn-
soy flour as a 12 month’s supplements on daily basis have comparable development
outcomes at age of 18 months.

Chattopadhyay and Saumitra (2016) did a review on developmental outcome in
malnourished children. They have searched for related literature on different relevant
websites. They have presented in their results the effect of nutrition on the developing
brain and showed that malnutrition especially in fetal and initial couple of years of postnatal
life, is a main threat for weak neurodevelopment, indicating delay in motor milestone,
speech and cognitive development with the behavioral problems along with learning
debilities. “*Macro and micro-nutrients like proteins, Iron, Iodine, Zinc, Vitamins-B, C and
D, choline and essential fatty acids*” are important for brain good development.
Supplementation of several micro-nutrients specifically “Iron, Iodine, vitamins B12 with
Folate and choline” among susceptible population most likely in pregnant and lactating
mothers, infants and in toddlers group had shown favorable outcomes. They have
concluded that adding dietary supplements for lactating and expecting mothers, infants
and tots beside with a pleasant socioemotional environment combined with cognitive
stimulation starting an initial years of life can show long term effect on high risk child for attaining his developmental potential.

Larson and Yousafzai (2017) work out on meta-analysis of nutrition interventions in lower and middle-income states on mental development of under two years aged children. They have planned this meta-analysis with the fact in mind that these nutrition interventions can enhance nutritional outcomes of young offspring’s in lower and middle income states and also may add up in the improvement of their mental along with motor development. This current meta-analysis answered two important queries regarding that did pre-natal and post-natal nutritional efforts progress mental development and dose these outcomes of mental development linked with both hypothetically attention-grabbing mediators known as physical growth plus motor development?. They have evaluated that out of ten pre-natal and twenty-three post-natal nutrition interventions, the commonly utilized were “zinc, iron, folic acid and vitamin A or multiple micronutrients, with a few tested macronutrients”. So it was answered that motor development, without growth status, were considerably linked through mental development in post-natal interventions. In short nutritional interventions had minor impacts on mental development. Upcoming researches may show more impact if they concentrate on macro-nutrient insufficiencies and combining child stimulation, hygiene in conjunction with sanitation interventions.

In this section we have discussed the development screening of children along with its predictors in many studies from different areas of the world by using different developmental screening test according to study and local requirement. We have also found that different attempts are made to enhance child development by using different
therapies like nutritional and stimulation therapies and its effect were measured on development. Impact of malnutrition on development quotient of children were also checked by different countries in many studies. We have also made an attempt to screen the severe acute malnutrition children for their development quotient as in our country there are limited statistical data available for developmental profile of under nutrition children. We have also tried to find socio-demographic factors responsible for delay along with malnutrition for making policies to overcome this problem and give society a healthy wealthy children. As these type of studies were not conducted in our study area before, so results of this study will be helpful for policy makers in this context.
Table 4: Summary of the studies on Development screening of children

<table>
<thead>
<tr>
<th>Author</th>
<th>City, Country</th>
<th>sample size</th>
<th>Age group studied</th>
<th>Objective of study</th>
<th>Study Design</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozkan et al. (2012)</td>
<td>Ankara, Turkey.</td>
<td>692</td>
<td>3 months to 5 years children.</td>
<td>To recognize the socio-economic and biological predictors linked with developmental delay in young children.</td>
<td>Descriptive study</td>
<td>Low level of mother education, premature birth before (32–36) weeks with low father education, low family income, low birth weight and mother age at birth &lt;20 years were associated with suspected delay on Denver II screening test results.</td>
</tr>
<tr>
<td>Simon et al. (2013)</td>
<td>United states</td>
<td>Children from 2007 National Survey.</td>
<td>18 months to 5 years.</td>
<td>To detect socio-demographic factors linked with dubious, likely and possible delays in development of US preschool children.</td>
<td>Descriptive study</td>
<td>Being older, male, low birth weight and Hispanic living in a non-English-speaking household were linked with high probability of possible delay in comparison with unlikely delay.</td>
</tr>
<tr>
<td>Author</td>
<td>Country</td>
<td>Number</td>
<td>Age Range</td>
<td>Purpose</td>
<td>Study Type</td>
<td>Results</td>
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<tr>
<td>Wijedasa (2012)</td>
<td>Sri Lanka</td>
<td>4251</td>
<td>0-80 months old</td>
<td>To set up DDST-2 standards in Sri Lanka.</td>
<td>Cross-Sectional Study</td>
<td>It was concluded that Screening tests for development must be normalized for the target populace before application.</td>
</tr>
<tr>
<td>Manganie et al. (2013)</td>
<td>In Malawi</td>
<td>840</td>
<td>6 months old healthy infants.</td>
<td>Assessed that is addition of LNS in the dietary regime of young children were still effective after achieving of their developmental milestones.</td>
<td>Randomized trial</td>
<td>No effect was observed in young offspring's after giving tested formulations with micronutrient fortified LNS or CSB dosages after achieving of their developmental milestones.</td>
</tr>
<tr>
<td>Paiva, Lima and Eickmann (2010)</td>
<td>Recife Brazil</td>
<td>136</td>
<td>9-12 months</td>
<td>To determine the impact of poverty on neuro psychomotor development of infants.</td>
<td>Cross-sectional study</td>
<td>They concluded that child development is associated negatively with various risk elements related to poverty.</td>
</tr>
<tr>
<td>Shin, kwon and Lim (2005)</td>
<td>Korea</td>
<td>113</td>
<td>6 months to 5 years</td>
<td>To assess the validity of Korea Denver 11 developmental screening</td>
<td>Hospital based cross-sectional study</td>
<td>Korea Denver 2 is valid and has good sensitivity and moderate</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Sample Size</td>
<td>Age</td>
<td>Objective</td>
<td>Methodology</td>
<td>Findings</td>
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<tr>
<td>Kyung (2008)</td>
<td>Korea</td>
<td>153 children</td>
<td>Under 2 year age</td>
<td>To investigate biological and environmental risk factors of developmental screening of children.</td>
<td>A questionnaire and “home observation for measurement of the environment inventory (HOME)” were used to collect data.</td>
<td>HOME score is useful to identify children for developmental delay and interventions will be more useful if mothers are provided with more appropriate social environmental.</td>
</tr>
<tr>
<td>Yousafzai et al. (2014)</td>
<td>Rural Sindh, Pakistan</td>
<td>1489</td>
<td>2.5 months old</td>
<td>To investigate the outcome of “integrated responsive stimulation” along with nutrition interventions on child health status, growth parameters and development.</td>
<td>community-based “cluster-randomized effectiveness trial”</td>
<td>Children with responsive stimulation had high scores in their cognitive and language along with motor developmental scales and at twelve months on the scales of socio-emotional. In term of growth enhanced nutrition had improved height-for-age Z scores at</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Participants</td>
<td>Age Range</td>
<td>Study Details</td>
<td>Methodology</td>
<td>Findings</td>
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<tr>
<td>Afarwuah et al. (2007)</td>
<td>Ghana</td>
<td>313 infants</td>
<td>6-12 months</td>
<td>To compare three types of micro nutrient supplement for home-fortification of complementary food with their effect on growth and motor developments.</td>
<td>Randomized control trial</td>
<td>All the supplements had affirmative impacts on motor milestones at twelve months but only NB had effect on growth.</td>
</tr>
<tr>
<td>Koruk, Simsek, Tekin, Doni and Gürses (2010)</td>
<td>Anatolia Turkey</td>
<td>168 children</td>
<td>6-59 months</td>
<td>To measure the nutritional status of children and to find out the prevalence of intestinal parasites anemia and delay in psychomotor development.</td>
<td>Cross-sectional study using probability sampling</td>
<td>3.81% children were stunted underweight were 20.8% and wasted were 5.4%. Out of total 17.8% had general psychomotor developmental delay.</td>
</tr>
<tr>
<td>Nahar et al. (2009)</td>
<td>Dhaka Bangladesh</td>
<td>97 children</td>
<td>6-24 months</td>
<td>To incorporate stimulation into the regular treatment of SAM children and to assess their effect on growth and development.</td>
<td>Time-lagged control study</td>
<td>Psychosocial stimulation into treatment of SAM children was successful in increasing growth and developmental of children.</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Participants</td>
<td>Ages</td>
<td>Study Design</td>
<td>Summary</td>
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<tr>
<td>Kar et al. (2008)</td>
<td>India</td>
<td>40 children</td>
<td>5-7 years 8-10 years</td>
<td>Cross-sectional</td>
<td>To assess the impact of &quot;chronic protein energy malnutrition on cognitive development&quot;.</td>
<td></td>
</tr>
<tr>
<td>Oelofse et al. (2003)</td>
<td>Western Cape, South Africa</td>
<td>30</td>
<td>6 to 12 months</td>
<td>Randomized control</td>
<td>To evaluate the efficacy of a multiple &quot;micronutrient-fortified complementary food on the micronutrient status, linear growth and psychomotor development&quot; of six to twelve months old babies.</td>
<td></td>
</tr>
<tr>
<td>Manno et al. (2012)</td>
<td>Zambia</td>
<td>743</td>
<td>6-18 months</td>
<td>Randomised double-blind control trial</td>
<td>To judge the hypothesis that plentiful fortification of micronutrients in diets produced at local level did not improve mental as well as motor development in Zambian children.</td>
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</table>

Stunting alters the continuing development for high cognitive functions in infantile years and result in long term cognitive damage.

Not any difference noted in linear growth and no change was detected in psychomotor developmental scores between the two groups, study and control at the end of six months of intervention.

They have judged that rich fortification with micronutrient had not any considerable effect upon the subsequent parameters like attainment of different developmental milestones, age of
<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Sample Size</th>
<th>Age Range</th>
<th>Objective of the Study</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckett, Durnin, Aitchison, and Pollitt (2000)</td>
<td>Indonesia</td>
<td>23</td>
<td>12 and 18 months</td>
<td>To compare development quotient of poorly nourished children at age of 12 to 18 months who had taken high or low-energy products of milk along with micronutrients.</td>
<td>Randomized trail</td>
<td>18 months old who took the high energy food for one year had high performance on mental test established on the “Bayley Scales of infant Development” in comparison with their colleague’s children who took the low energy food.</td>
</tr>
<tr>
<td>Phuka et al. (2012)</td>
<td>Malawi</td>
<td>163</td>
<td>6-18 months old</td>
<td>To assess their improvement in development quotient after one year consumption of complementary feeding</td>
<td>Randomized controlled trial</td>
<td>Developmental scores did not show any significance. At age of 18 months Length/age z-score</td>
</tr>
</tbody>
</table>
which are lipid-based or micronutrient fortified corn-soy flour porridge.

improvement in the study duration and mother literacy were linked with outcomes in developmental quotient.
2.2 Vitamin D deficiency and Supplementation

In this section we have described different vitamin D trails on child health (infectious and noninfectious) along with growth and development at global level.

Kumar et al. (2011) designed a randomized control trial in India on term infants who are low birth weight till the age 6 months to explore the impact of supplementation of vitamin D on weekly basis on infant’s mortality and morbidity along with growth. Around 2079 participants “low birthweight infants born at term: >37 weeks’ gestation” were enrolled from a big hospital operated by government in New Delhi with aim to find primary outcome of hospital admissions and deaths in the initial six months of life and growth were the secondary outcome to follow. They have intervened with weekly vitamin D supplementation up to six months at a dose of 35 μg/week and one recommended nutrient intake on daily basis. Infants were followed on weekly basis at home to witness supplementation and were carried to the treatment center every month for medical evaluations and anthropometric assessments. They have made assessment from this trial that in respect of death or hospital admissions or transfer to the out-patient center for moderate morbidity among groups no significant difference was noted but vitamin D administration would have showed improved vitamin D levels among groups at six months as measured by the status of plasma calcidiol in the body. Moreover, supplementation of vitamin D considerably improved z-scores at six months for weight combined with length and mid arm circumference as well as reduced the percentage of kids with stunted growth. So it was determined that vitamin D doses on a weekly basis were good enough to enhance vitamin D levels but not succeeded to reduce the frequency of mortality or acute morbidity between term young infants with low birth weight.
Manaseki et al. (2010) experimented a “double-blinded individually randomized placebo controlled trial” in Kabul to explore the outcome of supplementation for vitamin D (100,000 IU) in total 453 children with diagnosis of pneumonia. Data were collected from outpatient clinics and inpatient department in an inner-city Kabul hospital, Afghanistan. Inclusion criteria were children aged from one to thirty-six months, resident of known high vitamin D deficiency area and clinically detected with pneumonia severe or non-severe. The rationale for this trail is to assess the role of vitamin D in regulating immune function as well as also to evaluate its deficiency as a probability for incidence of pneumonia in children. The authors explored that supplementation of “100 000 IU of vitamin D3” with antibiotics decreases the time of disease in children diagnosed with pneumonia and also vitamin D3 supplementation decreases the risk of recurrence of disease. Results depict that for treatment of pneumonia one high dose vitamin D3 oral supplementation in child with antibiotic combination does not alter length of pneumonia, but decrease the risk of relapse of pneumonia episodes.

Brehm et al. (2010) explored potential benefits of vitamin D supplementation for children with asthma. Researchers got data from 103 asthma patients and 102 normal controls. All samples were assembled in the winter and in early spring season in Denver, Colorado. The authors discovered that serum vitamin D concentration in asthmatics were not different from general population. Almost 50% of study subjects in both groups had serum concentration of vitamin D in the deficiency range (<20ng/ml). At the same time the results of this study reveal higher prevalence for vitamin D inadequacy in adults then in children and prevalence for deficiency of vitamin D in children older than 12 years was proved to be same as adults. Since in this study all samples were gathered during the season when skin production of vitamin D is minimum, the results highlight the significance of nutrition
and life style aspects that influence high serum vitamin D status in children. The author's judgments recommend that vitamin D administrations in asthmatic children may improve corticosteroid response, control atopy and could increase asthma control.

Bergman et al. (2012) with his colleagues “at Karolinska institute and Karolinska University hospital” planned a study in patients who were prone to infections to evaluate vitamin D effect for avoidance of respiratory tract infections in them. Giving high doses of vitamin D in infection-prone patients for a period of twelve months declines their odds of acquiring infections of respiratory tract and subsequently their needs for antibiotics. They argued that, their study showed a significant outcome in patients suffering from recurrent infections, with low immunity along with lack of antibodies and also proved to be helpful in prevention of increase antibiotics resistance due to their misuse. Contrary to, in favor of notion that vitamin D3 is beneficial for healthful people having normal respiratory tract with acute infections did not prove anything worth noted. It was also established that low levels for vitamin D can enhance the possibility of infections and sufficient level of vitamin D can also activate the immune system. So they conclude that supplementation with vitamin D3 may decrease disease burden in ill peoples having recurrent infections of respiratory tract.

Kutluk, Çetinkaya and Basak (2002) studied that vitamin D, oral supplementation of 100,000 IU for 3 months have evidenced to be secure and effectual for relieving insufficiency of vitamin D in high-risk healthy kids or in kids diagnosed with rickets. For this a randomized controlled experimental hospital base study were conducted in Istanbul, Turkey to evaluate the outcome of combine form, intake of calcium orally along with vitamin D in high dose for cure of nutritional rickets in forty-two children between the age of six to thirty months. It was discovered from this study that an only one, high dose for
vitamin D in intramuscular form (300,000 IU) administration in combination with oral calcium were secure and effectual regime for rickets in children.

Zeghoud, Mekhbi, Djeghri and Garabedian (1994), in Paris by randomized control trial asses the “subclinical vitamin D deficiency in neonates” and examine their reaction against the vitamin D supplementation. Thirty neonates were allocated only one dose of 5mg (200 000 IU) cholecalciferol at birth or to 2.5mg cholecalciferol at birth, three months and six months after birth. Samples from venous blood were taken from each child after each dose and for several times to assess serum vitamin 25(0H) D. It was revealed that both regimes give same protection against vitamin D insufficiency without any risk of vitamin D toxicity.

Manaseki et al. (2012) carry out research in Afghanistan, the neighboring country of Pakistan in winter during the year of 2005 to prove, that the population of Kabul district have high occurrence for vitamin D insufficiency. The “serum 25-hydroxyl vitamin D concentration” was examined and found “5ng/ml (range 2-25ng/ml”) in 108 kids having age between six to forty-eight months. Out of all, 104 (96.2%) had level lower then 15ng/ml, the lowest value measured to be adequate, and 79 (73%) had level lower then 8ng/ml, a value believed to be considerably inadequate.

Pawley and Bishop (2004) state that “prenatal vitamin D status appears to affect postnatal mineral homeostasis” and can also alter growth process. Post-natal levels of vitamin D influence process of growth, additionally important for mineral homeostasis and consequently might be also effecting bone mass. Undoubtedly further information on functional outcomes in infants and children, perhaps extension up to the adolescence, are desirable for determining the impacts of supplementation of diverse amounts of vitamin D in condition of pregnancy. Currently it is not feasible to recommend a single general advice
for supplementing vitamin D in pregnancy state, though supplementing with “400-1000 IU/d” in the last trimester in those having more odds of suffering from deficiency, might be secure and beneficial.

Brehm et al. (2010) piloted a research to evaluate the association amid serum vitamin D levels and consequential acute asthma exacerbations. Data collected from 1024 participants to assess “25-hydroxyvitamin D levels” in sera assembled with mild-to-moderate unremitting asthma on the time of registration in a multicenter clinical trial of participants randomized to take budesonide, nedocromil, or placebo in the “Childhood Asthma Management Program”. Applying multivariate modeling, they analyzed the association among baseline vitamin D levels and the probabilities of any hospitalization or admission in emergency throughout the period of around four years of trial they have found that 35% participants were vitamin D insufficient. Mean vitamin D levels remained low in African American citizens and high in white citizens. Subsequently modifying for age, gender, BMI, income and treatment group, inadequate vitamin D concentration were linked with a high chance for any hospitalization or admission in emergency. So they infer that in children of North America deficiency of vitamin D is widespread along with mild to moderate asthma which is persistent in nature and also linked with high chances of severe acute exacerbations around the time of four years.

Trilok et al. (2015) conducted a randomized control trial follow-up “to assess the effects of vitamin D supplementation in infancy on growth, bone parameters, body composition and gross motor development at age 3–6 years”. The Delhi Infant Vitamin D Supplementation “(DIVIDS-2) study” tracked the “DIVIDS children”, those at that time grows up to age of three to six years, to reveal long-lasting impacts of vitamin D supplementation. Thy have followed, 446 kids among the group of vitamin D and 466 kids between the placebo group,
from DVID children. They have collected data about anthropometric measurements, blood pressure, gross motor milestones, bone structure along with bone strength by using “quantitative ultrasound” and draw samples from blood for estimating vitamin D levels, and deuterium dilution scan for body composition on a subgroup of 229 children. They have concluded that body mass index z-scores was low in the vitamin D group in comparison with the placebo group because of marginally low gain in weight and a little increase in height. The arm of vitamin D also showed low “thigh circumference” including “arm muscle area” and marginal low “mid-upper arm circumference” (MUAC). Not any significant arm difference was found in “body fat percentage”, bone structure and strength by using quantitative ultrasound or by measuring blood pressure but a slight difference is observed in development of motor milestones. They have made conclusion that vitamin D administration in low birth-weight babies during infant-hood period ensuing children thinner at the age of 3–6 years without any differences being observed in functional outcomes.

Rejnmark (2011) did a review from randomized-controlled trials to assess “the effects of vitamin D on muscle function and performance”. It was proven in the meta-analyses of RCT’s that there was, low probability of falls in elder person who were supplemented with vitamin D, possibly will because of improvement in “neuromuscular function” after having sufficient vitamin D levels. In many researches which are observational in nature, vitamin D levels in the body have positive relation with “muscle strength and postural stability” and also physical activation is linked by way of vitamin D levels and muscle strength, assessed in different randomized control trial. In systematic approach of 16 randomized controlled trials “on the effects of treatment with vitamin D on muscle function” were found and out of them only one study was done on participants above 50 years of age. In seven studies
from these studies, a progressive outcome of treating with vitamin D was recorded for “muscle strength of the lower legs and physical performance”. To sum up, data from randomized controlled trials (RCTs) supported the results of supplementing with vitamin D for muscle strength along with function in old persons, but dilemma is that many published reports depicts no useful effects than researches proving beneficiary outcomes. Still data is deficit for potential outcomes in young person’s so more trials required in this context.

Walli, Munubhi, Aboud & Manji (2017) conducted a cross-sectional study to estimate the vitamin D levels in under 5 years malnourished children admitted in a Tertiary Care Center at Tanzania. For this they have measured serum vitamin D status, alkaline phosphatase and X-ray wrist were done on 134 children. They have evaluated that vitamin D deficiency (VDD) were present in 41 (30.6%) children with the mean vitamin D value of 74.8 nmol/l. The mean alkaline phosphatase measure was 176.6U/l. Severe stunting were diagnosed in 64 (48%) children out of them 20 (31.2%) were vitamin D deficient. Moreover, they have revealed that marasmic child had high odds of vitamin D deficiency with comparison of other types of malnutrition. They have suggested that malnourished children have higher pervasiveness of vitamin D deficiency and highlights the importance for effective surveillance and aggressive management.

Saad et al. (2016) designed a double-blinded, randomized control clinical trial (RCT) for vitamin D administration in kids having “autism spectrum disorder” (ASD). The present trial enrolled 109 kids diagnosed with “autism spectrum disorder” among three to ten years of age (85 boys and 24 girls). The objective of the trial was to evaluate the outcomes, for vitamin D administration in the kids for autism signs around four month’s period. The
serum status for vitamin D were evaluated at trial start and at end. Childhood Autism Rating Scale (CARS), were used for assessing the acuteness of autism. The ASD kids, social maturity was evaluated through “Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS) and the Autism Treatment Evaluation Checklist (ATEC)”. They have concluded that administration of vitamin D daily doses (“300 IU vitamin D3/kg/day, not to exceed 5,000 IU/day”) was tolerated satisfactorily by the autism spectrum disorder kids. The autism core symptoms of the children were recovered considerably, in 4 months of vitamin D3 administrations compared with, placebo arm. This trail proves the effectiveness and acceptability of high doses for vitamin D3 in ASD kids based upon parameters evaluated in this trail. It was revealed that, vitamin D administration in oral form can securely recover signs along with symptoms for ASD and may perhaps suggested for ASD kids.

In this section we have described different vitamin D trails on child health (infectious and noninfectious) along with growth and development. We have seen that these trials were conducted according to different functions of vitamin D on the body. But trials on vitamin D role in undernutrition is still lacking and need consideration in this context. As we searched that the nutritional composition of RUTF and requirements of children with SAM are largely based on expert opinion rather than trials with actual functional outcomes. The mechanistic rationale for this trial of vitamin D with using a placebo control is an attempt to give a more scientific view. We have tried in our present trail to fulfill this gap to some extent.
<table>
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<th>Authors</th>
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<tbody>
<tr>
<td>Kumar et al. (2011)</td>
<td>New Delhi, India</td>
<td>2079</td>
<td>Birth to 6 months</td>
<td>To explore the impact of supplementation of vitamin D on weekly basis on infant’s mortality and morbidity along with growth till the age of 6 months.</td>
<td>Randomized control trial</td>
<td>Vitamin D doses on weekly basis increases, vitamin D levels and anthropometry but didn’t reduce the incidence of death or acute morbidity between infants with low birth weight.</td>
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<td>Manaseki et al. (2010)</td>
<td>Kabul Afghanistan</td>
<td>453</td>
<td>1-36 months old children</td>
<td>To explore the outcome of supplementation for vitamin D with diagnosis of pneumonia.</td>
<td>“Double-blind individually randomized placebo controlled trial”</td>
<td>Supplementation of “100 000 IU of vitamin D3” with antibiotics decreases the length of disease in children diagnosed with pneumonia and also vitamin D3 supplementation decreases the risk of recurrence of disease.</td>
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<tr>
<td>Brehm et al. (2010)</td>
<td>Denver, Colorado</td>
<td>205</td>
<td>Under 12 year children</td>
<td>To explore potential advantages of vitamin D supplementation for children with asthma.</td>
<td>Multicenter randomized control trial</td>
<td>Vitamin D supplementation in asthmatic children may improve corticosteroid responses, control atopy and could there by increase asthma control.</td>
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<tr>
<td>Study</td>
<td>Location</td>
<td>Sample Size (n)</td>
<td>Age Range</td>
<td>Outcome</td>
<td>Study Type</td>
<td>Description</td>
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<td>Kutluk et al. (2002)</td>
<td>Istanbul, Turkey</td>
<td>42</td>
<td>6 to 30 months</td>
<td>To evaluate the outcome of combine form, intake of calcium orally along with vitamin D in high dose for cure of nutritional rickets.</td>
<td>Randomized controlled experiment base study</td>
<td>It was discovered that an only one, high dose for vitamin D in intramuscular form (300,000 IU) administration in combination with oral calcium were secure and effectual regime for rickets in children.</td>
</tr>
<tr>
<td>Zeghoud et al. (1994)</td>
<td>Paris, France</td>
<td>30</td>
<td>Neonates</td>
<td>To assess the &quot;subclinical vitamin D deficiency in neonates&quot; and examine their reaction against the vitamin D supplementation.</td>
<td>Randomized controlled trial</td>
<td>Both used regimes give same protection against vitamin D insufficiency without any possibility of vitamin D toxicity.</td>
</tr>
<tr>
<td>Manaseki et al. (2012)</td>
<td>Kabul, Afghanistan</td>
<td>108</td>
<td>6 to 48 months old children</td>
<td>To prove that the population of Kabul district have high prevalence of vitamin D insufficiency.</td>
<td>Descriptive study</td>
<td>Out of all, (96.2%) had level lower then 15ng/ml, the lowest value measured to be adequate, and (73%) had level lower then 8ng/ml, a value believed to be considerably insufficient.</td>
</tr>
<tr>
<td>Gilbert et al. (2010)</td>
<td>Bogota, Colombia</td>
<td>479</td>
<td>5-12 year children</td>
<td>To find out the associations among vitamin D sero-status and alterations in “body mass index, skinfold-thickness ratio, waist circumference, and height” in a children.</td>
<td>Longitudinal study</td>
<td>Vitamin D sero-status were inversely related with the development of adiposity in kids.</td>
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<tr>
<td>Study</td>
<td>Country</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Study Design</td>
<td>Findings</td>
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<tr>
<td>Lan et al. (2010)</td>
<td>Vietnam</td>
<td>385</td>
<td>Adults Men and women</td>
<td>To assess the association among vitamin D levels, “parathyroid hormone” and the chance of TB in a Vietnamese residents.</td>
<td>Matched case-control study These results accomplish that vitamin D inadequacy was linked with tuberculosis in males, but not in females. However, it need further determination that this link is a causal correlation.</td>
<td></td>
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<tr>
<td>Karim, Nusrat and Aziz (2011)</td>
<td>Karachi, Pakistan</td>
<td>50</td>
<td>Women</td>
<td>To assess vitamin D deficiency prevalence in pregnant females and to “correlate maternal and cord blood vitamin D deficiency”. To measure potential factors responsible for deficiency of vitamin D.</td>
<td>“Observational, analytical, cross-sectional study” “Vitamin D sufficiency in (22%), insufficiency in (32%), and deficiency in (46%) out of the 50 females”. But sufficiency as well as deficiency, were observed in (12%) and (88%) of the newly-born child. A progressive relationship among the vitamin D status in maternal along with cord blood were noted. Maternal levels of vitamin D were considerably influenced by exposure to sunlight and dietary quality.</td>
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<tr>
<td>Marwa et al. (2005)</td>
<td>Delhi, India</td>
<td>5137</td>
<td>10–18 years</td>
<td>To assess the “calcium, vitamin D-parathyroid hormone axis” in healthy kids from two diverse socio-economic settings.</td>
<td>Cohort Study Higher prevalence of clinical and bio-chemical hypo-vitaminosis D found in apparently well school children.</td>
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<tr>
<td>Authors</td>
<td>Location</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Objective</td>
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<td>Trilok et al. (2015)</td>
<td>New Delhi, India</td>
<td>912 from two different arms</td>
<td>3-6 years</td>
<td>“To assess the effects of vitamin D supplementation in infancy on growth, bone parameters, body composition and gross motor development at age 3–6 years”.</td>
<td>Randomized control trial</td>
<td>Vitamin D administration of low birth-weight infants during infancy ensuing children thinner at the age of 3–6 years without any differences being observed in functional outcomes.</td>
</tr>
<tr>
<td>Walli et al. (2017)</td>
<td>Dares Salaam, Tanzania</td>
<td>134</td>
<td>Under 5 years</td>
<td>To estimate the vitamin D levels in under 5 years malnourished children.</td>
<td>Cross-sectional study</td>
<td>Vitamin D deficiency were present in 41 (30.6%) children with the mean of 74.8 nmol/l. The mean alkaline phosphatase measure were 176.6U/l. Severe stunting were diagnosed in 64 (48%) children out of them 20 (31.2%) were vitamin D deficient.</td>
</tr>
<tr>
<td>Saad et al. (2016)</td>
<td>Egypt</td>
<td>109</td>
<td>Aged 3-10 years</td>
<td>To evaluate the outcomes, for vitamin D administration in the kids for autism signs around four months period.</td>
<td></td>
<td>Vitamin D daily doses (“300 IU vita D3/kg/day, not to exceed 5,000 IU/day”) was tolerated satisfactorily by the autism spectrum disorder kids. The autism core symptoms of the children were recovered in 4 months of vitamin D3 administrations compared with, placebo arm.</td>
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2.3 CMAM / Ready to use Therapeutic Food (RUTF)

In this section we have searched the literature and existing data for treating “children aged 6-59 months with severe acute malnutrition” by using CMAM model with RUTF effect, on global level.

Akparibo, Harris, Blank, Campbell and Holdsworth (2017) piloted a study in Ghana to explore the effectiveness of “community-based-management of severe acute malnutrition programme” in under five years aged children in nonemergency standard community health-care setting. They have designed a “retrospective cohort study” on 488 kids among 6–59 months of age who are getting treatment under CMAM programme. They have collected data from enrolment cards of 56 out-patient centres for recovery rate, default and mortality rates. It was observed that satisfactory recovery rates of children were 71.8% with seven time greater odds of recovery in children who were entered with high MUAC of >11.5 in comparison with children of having low MUAC of < 11.5. Moreover, children without diagnosis of malaria at baseline have odd ratio of recovery, OR = 30, p < .001, more in comparison with children diagnosed with malaria. The “average weight gain was $4.7 \, \text{g}^{-1} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$”, which were affected by MUAC value at entry with diagnosis of malaria and total duration of stay in programme. Furthermore, the default rate was high and mortality rate was low than international standards references by Sphere. So they have concluded that “community based management of severe acute malnutrition” have same success rate when given in routine nonemergency settings.

Kerac et al. (2009) designed a “double blinded, randomised, placebo-controlled efficacy trial in 795 Malawian” children from the age range of 5-168 months to measure the “clinical and nutritional effectiveness of a probiotic and prebiotic functional food for treating severe acute malnutrition” in HIV dominant area. After initial stabilization phase of giving milk
feeds, random allocation of children was done for “ready-to-use therapeutic food” (RUTF) by giving symbiotic or without symbiotic. The primary outcome of study included were weight-for-height >80% for two sequential visits in outpatient clinic. Secondary outcomes comprised mortality, gain in weight, time taken for treatment, along with clinical symptoms. The results show nutritional cure were same in symbiotic and control groups. Secondary outcomes were also same in both groups. HIV positive children showed worse outcomes on the whole, however did not change or perplex the adverse outcome. Subgroup evaluations points out potential developments to decreased out-patient death rate in the symbiotic group. So it is concluded that symbiotic did not affects severe acute malnutrition outcomes.

Irena et al. (2013) planned a “non-blinded, parallel group, cluster randomized, controlled, equivalence experimental study” to evaluate the efficacy of a “milk-free soy-maize-sorghum-based, ready-to-use therapeutic food to standard RUTF” food with twenty five percent milk for nutritional treatment of severe acute malnourished kids in Zambia. An analyst randomly allocated health centers either one to the “soy-maize-sorghum-based ready-to-use therapeutic food (SMS-RUTF) (n = 12; 824 enrolled) or peanut-based RUTF (P-RUTF) (n = 12; 1103 enrolled)”. All severe acute malnourished children who were entered for admission at the health centers, were registered for study. Result were analyzed on individualistic grounds. The primary outcome for the study were recovery rate and gain in weight. “The recovery rates for milk-free soy-maize-sorghum-based ready-to-use therapeutic food and P-RUTF were 53.3% and 60.8% for the intention-to-treat analysis and 77.9% and 81.8% for per protocol assays”, correspondingly. This study did not succeed to prove the theory of equivalence between “milk-free soy-maize-sorghum-based
ready-to-use therapeutic foods to standard RUTF” food with twenty-five percent milk for nutritional management of severe acute malnourished children.

Bhutta et al. (2008) studied interventions in Aga khan university Karachi that influence maternal and child undernutrition and nutrition-related problems. These intercessions involve support of breastfeeding, approaches to enhance complementary feeding practices, with or without food supplement, micronutrient interventions and tactics to enhance family and community nutrition with decrease in disease load. They proved that policies in support of breastfeeding had great outcome upon survival, but for stunting they have shown minimum effect. WHO instructions “for management of severe acute malnutrition” decreased case-fatality rate around 55%, and “ready-to-use therapeutic foods, can be used in community to improve severe acute malnutrition”. Iron folate supplements is good for pregnant women and for children advised micronutrient interventions consist of supplements of vitamin A, zinc, iron supplementations and iodised salt. They applied a cohort model in children of the thirty-six countries having 90% of children with stunted linear growth to evaluate the probable outcome of these interventions. It was proved that current interventions that were planned to enhance nutrition with prevention of associated disease could decrease stunting, mortality and disability-adjusted life-years. These interventions should be enhanced to reduce stunting in the long run by amendments in the causes of undernutrition, that include affluence, poor education, disease load and lack of women's encouragement.

Lundgren and Uhrenfeldt (2014) supervised a field research to explain the health care of malnourished children by using qualitative method in Uganda. Data has been gathered by using unstructured observations, talks with parents, health care consultants by following
instructions and records utilized by the MNU, Mulago Hospital. They have utilized WHO child growth standards, “mid-upper-arm circumference tape” and clinical assessment of oedema bilaterally for determining the type and severity of malnutrition. The management comprised at the MNU were therapeutic milk and “ready to use therapeutic food (RUTF)”. Parents plays significant part for care of child in hospital as well as health experts work out preventively by increasing parent’s knowledge of malnutrition. Parents were imparted with knowledge and education focusing, nutrition, hygiene, communication of infectious diseases. They have also included preventive programme as community outreach, with purpose to enhance the health of people in rural settings. To sum up they have explored many reasons of malnutrition and found it complicated with different influencing causes. The study analysis explained the important role of parents as custodians and low economic reserves, lack of knowledge were main reasons affecting child health. They concluded that by approaching people with limited approach to health services many significant measures will accomplished in the battle of prevention of malnutrition.

Jones et al. (2015) planned a “randomized controlled trial in children having severe acute malnutrition in rural Kenya” for developing “Ready to use therapeutic food” with high short chain, n-3 polyunsaturated fatty acid. For its evaluation on outcomes, in “treatment of severe acute malnutrition” they have applied by adding and by without adding fish oil supplementation, on kid’s polyunsaturated fatty acid levels. They have enrolled sixty children from six to fifty months of age and they were randomized to take ready- to -use therapeutic food with regular composition “RUTF with high short chain n-3 PUFA or RUTF with high short chain n-3” polyunsaturated fatty acid in addition to fish oil capsules. Children’s were monitored for three months. The study primary outcome has been erythrocyte polyunsaturated fatty acid composition. They have found that “Erythrocyte
docosahexaenoic acid (DHA)” dropped in two arms who were not taking fish oil, even from the reference point. “Erythrocyte long-chain n-3 polyunsaturated fatty acid” after treatment were notably high for children’s in group taking fish oil in comparison with those in the group taking “RUTF with high short chain n-3” polyunsaturated fatty acid or regular RUTF only. To sum up, “ready to use therapeutic food with high short chain n-3 polyunsaturated fatty acid” and fish oil capsules were suitable for children’s and there were no considerable differences in safety results.

Van et al. (2016) researched in Goronyo the outcome of “supplementation with ready-to-use therapeutic food (RUTF) and a micronutrient powder (MNP)” on the prevalence of malnutrition in sick pediatric patients coming to out-patient department. A “three-armed, partially-blinded, randomized controlled trial” was piloted in pediatric patients with diagnosis of malaria, diarrhea and lower respiratory-tract infections. Children between six to fifty-nine months of age were randomized to any one arm from the three arms and received one packet per day of RUTF, two packets per day of micro-nutrients or in control arm received no supplementation around 14 days and in illness for six months. The primary outcome of the trail was the occurrence of 1st adverse nutritional outcome in six-month monitoring. Negative nutritional outcome was a study-specific measure and a sign of malnutrition and it has been classified as low “weight-for-height z-score, mid-upper arm circumference <115 mm, or edema”, whatever appear firstly. Total 2,213 children were randomized, and the incidence rate was 0.92 for “ready to use therapeutic food” vs. control, 0.87 for micronutrient powder versus control and 1.06 for “ready to use therapeutic food” vs. micronutrient powder. A subgroup evaluation revealed no relations, no confounding and not any unique efficacy for supplementing in participants in moderate malnutrition in contrast with well malnourished at admission. So it was concluded that
supplementing “RUTF or MNP” for two weeks in sick child as routine daily care had not decrease the frequency of malnutrition. The reasons may be that due to high incidence of morbidity, in Goronyo and the period for supplementing might be very short or the supplements dose might be very less to alleviate the consequences of elevated morbidity rate and already prevalent malnutrition.

Yebyo, Kendall, Nigusse and Lemma (2013) supervised a retrospective cohort study in 6–59 months old 628, SAM children in Tigray, Northern Ethiopia to evaluate “outpatient therapeutic feeding program” effects and determining factor in management of “Severe Acute Malnutrition”. This research disclosed the indictors for outcome in program along with determining feature of recovery rate. The kids were picked by applying “systematic random sampling” in different centers depending upon data of demographic features, anthropometry, “Plumpy Nut” intake, different medical problems as well as regular medications ingestions. The outcomes were anticipated by utilizing “Kaplan-Meier survival curves, log-rank test and Cox-regression, the recovery, default, mortality and weight gain rates were 61.78%, 13.85%, 3.02% and 5.23 gm/kg/day”. Regular medicines have been given partly and child who had medical problem in program were improperly handled. It was observed that if child eat 1 extra packet of “Plumpy Nut”, it will improved the recovery rate by 4% for severe acute malnutrition. The “Outpatient Therapeutic Feeding Program” was successful partly along with treatment of kids with other diseases in the program and incomplete dispensation for regular medicines were risk factors for the program efficacy.

We have discussed the literature and existing data for treating “children aged 6-59 months with severe acute malnutrition” by using CMAM model along with RUTF effect, at global level. We have tried to identify any breach in the literature for requirement of any additional research. As we came to know that the nutritional composition of RUTF and requirements
of children with SAM are largely based on expert opinion rather than trials with actual functional outcomes. World Health Organisation (WHO) has identified research priorities to identify adjunctive therapies that may improve response to RUTF so by considering this we had made an attempt to use CMAM, RUTF model in severe acute malnutrition for checking its efficacy on child growth and development along with addition of vitamin D to see its adjuvant effect.
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<th>Research Objectives</th>
<th>Study Design</th>
<th>Major Findings</th>
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<tr>
<td>Akparibo et al.</td>
<td>Upper East Region, Ghana.</td>
<td>488</td>
<td>6-59 months</td>
<td>This study investigated the performance of “community-based management of severe acute malnutrition (CMAM)” within regular healthcare services in Ghana.</td>
<td>Retrospective cohort study</td>
<td>The “average weight gain was 4.7 g−1, kg−1·day−1” which was influenced by MUAC status at baseline, presence of malaria, and length of stay. The default rate was higher and mortality rate was lower than international standards.</td>
</tr>
<tr>
<td>Defoury et al.</td>
<td>Maradi region Niger</td>
<td>Around 60,000</td>
<td>Less than 3 Years</td>
<td>To assess the efficacy of newly developed “ready-to-use food (RUF) as a dietary supplement” for SAM child.</td>
<td>Randomized trail</td>
<td>These results show the incidence of severe wasting in six to thirty six months old children is notably reduced by distribution of fortified spread.</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
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<tr>
<td>Kerac et al. (2009)</td>
<td>Malawi</td>
<td>795</td>
<td>5 to 168 months</td>
<td>To measure the “clinical and nutritional effectiveness of a probiotic and prebiotic functional food” in treating SAM.</td>
<td>Double-blind, randomised, placebo-controlled efficacy trial</td>
<td>Symbiotic did not affects “severe acute malnutrition” outcomes in HIV dominant areas.</td>
</tr>
<tr>
<td>Oakley et al. (2010)</td>
<td>Malawi</td>
<td>1874</td>
<td>Under 5 year of age</td>
<td>To compare that two locally produced RUTF having 10 % milk is less effectual than having 25% Milk for treating severe malnourished children.</td>
<td>“Randomized, double-blind, controlled, clinical, quasi-effectiveness trial”</td>
<td>Children getting 25 % milk RUTF shows more recovery and increase in their weight and height then children getting 10 % milk RUTF. It were concluded that RUTF with 25% milk is the standard home based treatment for SAM.</td>
</tr>
<tr>
<td>Irena et al. (2013)</td>
<td>Zambia</td>
<td>1927</td>
<td>Under 5 years of age</td>
<td>To assess the efficacy “of a milk-free soy-maize-sorghum-RUTF” (SMS-RUTF) to standard 25% milk RUTF (P-RUTF) in</td>
<td>“Non-blinded, parallel group, cluster randomized, controlled,”</td>
<td>This trial did not prove the hypothesis for equivalence in “SMS-RUTF and P-RUTF in SAM management”.</td>
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<tr>
<td>Country</td>
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<td>Number</td>
<td>Age Range</td>
<td>Nutritional Management</td>
<td>Equivalence Trial</td>
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<tr>
<td>Jones et al. (2015)</td>
<td>Kenya</td>
<td>60 children</td>
<td>6 months to 5 years</td>
<td>To develop a “RUTF with high short-chain n-3 PUFA” and assess its effect by giving and not giving supplements of fish oil, on kids PUFA levels to treat SAM.</td>
<td>Randomized controlled trial “RUTF with elevated short chain n-3 PUFA and fish oil capsules” were tolerate able by children’s and no noteworthy difference were observed in safety outcomes.</td>
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<tr>
<td>Van et al. (2016)</td>
<td>Goronyo, Nigeria</td>
<td>2,213</td>
<td>6 months to 5 years</td>
<td>To investigate the outcome of supplementation “RUTF and a micronutrient powder (MNP) on the incidence of malnutrition” in sick child in OTP.</td>
<td>“Three-armed, partially blinded, randomized controlled trial”. No efficacy of supplementation was observed in moderately malnourished in contrast with well malnourished children. The mean number of illness for RUTF, micro-nutrient powder and in control arms were 4.2, 3.4, and 3.6.</td>
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<td>Study</td>
<td>Location</td>
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<td>Objectives</td>
<td>Study Design</td>
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<td>Akram, Arif, Khan and Samad (2010)</td>
<td>Karachi, Pakistan</td>
<td>24</td>
<td>Under 5</td>
<td>To build up nutrition of malnourished children in the community, using home based treatment.</td>
<td>Prospective cohort study</td>
<td>11 children reached -1SD in 3 months, 10 take 4 months, 22 were at the median weight for height by the end of 5 months. Home based treatment with locally accessible foods can be used effectively to treat SAM.</td>
</tr>
<tr>
<td>Mangani et al. (2015)</td>
<td>Malawi</td>
<td>840</td>
<td>6 months old</td>
<td>To examine a “hypothesis that dietary supplementation with lipid-based nutrient supplements progresses linear growth and lowers the incidence of severe stunting in infants at risk”.</td>
<td>Randomized blinded trial</td>
<td>Finding did not depict convincing data on a causative association among the “LNS supplementation” and on the low prevalence of stunting.</td>
</tr>
<tr>
<td>Yebyo et al. (2013)</td>
<td>Tigray, Northern Ethiopia</td>
<td>628</td>
<td>6–59 months</td>
<td>To evaluate “outpatient therapeutic feeding program” effects and</td>
<td>Retrospective cohort study</td>
<td>The Outpatient therapeutic feeding program were</td>
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<td>Study</td>
<td>Location</td>
<td>Duration</td>
<td>Study Details</td>
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<tr>
<td>Bashir and Zaman (2016)</td>
<td>Lahore, Pakistan</td>
<td>60 3-120 months</td>
<td>To measure the efficacy and tolerability of RUTF between malnourished children in a tertiary care hospital.</td>
<td>Malnourished children put on weight after the short time supplementation of RUTF but had no major effect on height of the patients. Its tolerability in lieu of taste, amount consumes and demand was good. Maternal opinion was also satisfactory considering these foods.</td>
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</table>
2.4 Dietary and socio demographic predictors of malnutrition

In this section of dietary and socio demographic predictors of malnutrition we have pointed the severity and multi-dimensionality of the problems and its effects on child health and their upcoming productivity in later life.

Valente et al. (2016) piloted a “cross-sectional population-based” study in Sao Tome among 1285 children (0 to 5 years) of age with the aim to measure the nutritional parameters (acute and chronic malnutrition) of children with their predictors. Children anthropometry were done and z-score calculated for global chronic malnutrition, “height for age (HAZ), weight for height” for global acute malnutrition (WHZ) and body mass index. They have defined in their study “global acute undernutrition (weight for height < _1 Z-score and wasting < _2 Z-scores) and global chronic undernutrition as (length/height for age < _1 Z-score and stunting < _2 Z-scores”).

For collection of others related information children health records were consulted and from mothers answers were gathered on a specific questionnaire. They have revealed in their study that for global acute malnutrition % is high with 30.9% in <24 months and 21.9% in children above 24 months and global chronic undernutrition were found in 32.5% in <24 months and 41.1% in children above 24 months. They have observed that in children >12 month’s required correct birth “weight for gestational-age” is a protective factor for acute as well as chronic malnutrition along with weight improvement more than “0.67 z-score in the 1st semester of life” is also a protective factor in the same age group children. Among all socio-demographic factors in logistic regression analysis mother education were deemed to be protective factor for both acute malnutrition and chronic malnutrition in this population of 0-5 year’s old children. They have concluded that child birth weight, nutritional status with weight improvement specifically in the life first years and mother's
education were significant protecting variables against malnutrition in infancy and childhood. They have highlighted that attention should be given on these variables for preventing acute as well as chronic malnutrition in kids.

Fakir and Khan (2015) conducted a research in Bangladesh to investigate the determinants of malnutrition between children in urban slum. They have explored the effect of child and maternal along with household factors on children “weight-for-age nutritional status” by anthropometry for an ordered logistic analysis. To comprehend the effects of health knowledge in child health they have used covariates of “health-seeking practices guide, child health precautions index and medical cost information index”. To understand the gender inequalities in nutritional function difference they have also applied the gender specific regressions. They have presented in their results that child health recovers significantly by “per capita income but household assets” do not have any considerable influence on child health and indices regarding health knowledge that also appreciably enhance children health. But after adjusting “for health knowledge and health-seeking behavior”, the effect of mother’s education has no significance on children health. Moreover, in gender analysis they have notice that boys were more considerate to "child health precautions" along with "medical cost knowledge", while girls were more considerate to "health-seeking practices".

So they had made conclusion that impact of “health knowledge on child health” holds a substantial part on education effect and to enhance the nutritional parameters of girl child in comparison with boy child in study area, strategies concentrating on health-seeking practices should be promoted.
Ayana, Hailemariam and Melke (2015) piloted a case control study in public hospitals, West Ethiopia with the aim to explore the determinants of acute-malnutrition between six to fifty-nine months aged children. This “hospital based un-matched case control study” enrolled 339 children of respective age group. Data has been gathered by utilizing “a pre tested structured questionnaire and by mid upper arm circumference (MUAC)”. For statistical analysis the multivariable logistic regression to estimate “odds ratio with 95 % confidence interval” were used to recognize determining factors for acute malnutrition also known as wasting. They have found that the associating factors with acute malnutrition were having diarrheal diseases in last couple of weeks, maternal bad practices for not washing hands, no exclusive breastfeeding, having large family size, uneducated mothers, no facility of latrine and febrile because of infection in the last couple of weeks. So they wrap-up, that wasting was significantly linked with these above factors in the concerned population and they have suggested that a systematized attempt should be done on all points to enhance mothers along with children health services, for prevention of the child acute malnutrition.

Jamro, Junejo, Bouk, Lal and Jamro (2012) conducted a prospective descriptive study in Sukkur, Pakistan in six to fifty-nine months old kids to identify causes for severe acute malnutrition. Two hundred seventy children admitted in nutrition stabilization center were enrolled on WHO criteria for diagnosis of “severe acute malnutrition (weight for height measurement of < 70% of the median or > 3 SD or the presence of bilateral pitting edema of nutritional origin”). They have revealed the results that maternal illiteracy was present in 80% children, paternal illiteracy in 66.7% cases, large family size with 66.7% cases and 73.3% children parent’s income was less than 5000 monthly. Only 25.9% children were exclusively breast feed with presence of delayed weaning in 55.6% children and recurrent
diarrhea was seen in 44.4% children. So it was concluded that common risk factors linked with severe acute malnutrition were parent’s illiteracy, large family size, poverty, non-exclusive breast feeding and recurrent diarrhea. To overcome this problem of severe acute malnutrition attention should be focused on encouragement of exclusive breast feeding and parental education.

Afzal (2012) studied the determining factors for child health along with nutritional level in Punjab, Pakistan with the aim to detect the socio-economic causes that influence children health at the domestic level and suggest creating strategies forming on its results. The investigator of study has applied the instrumental variable technique for assessment purpose. The data of this study were gathered from the domestic level. Data set collected “by the Punjab Bureau of Statistics”, for multiple Indicator cluster survey in 2007 and 2008. The study’s findings suggest that among others substantial indicators mother education and health knowledge are significant determinants of child health.

Kavosi et al. (2014) planned a cross-sectional door to door survey by multistage sampling in Iran to conclude the prevalence and determinants of undernutrition in kids under six year of age. A total of 15408 children, of concerned age group were nutritionally assessed for wasting, stunting and underweight. Structured questionnaire was used for sociodemographic measures. It was determined from this survey that “prevalence of stunting, underweight and wasting were 9.53, 9.66, and 8.19%”, in that order. Stunting were more prevalent in male children with comparison to female’s children and stunting were also notably related to low income of family along with low maternal literacy. In factor analysis it was discovered that residing in urban area, coupled with poor supply of water had been significantly linked with all three types of childhood malnutrition. It was recommended that no access for getting health services had been also linked to wasting
and having big family was associated to under-weight. So conclusion was made that the population under study have low income status and focusing factors for development of the public preventive policies to constraint childhood undernutrition in the study area contains gender, residing area, big family, less family earning, mother literacy along with health services and safe source of water.

Panda, Benjamin, Singh and Zachariah (2000) piloted a descriptive study in India to evaluate health along with nutritional parameters of five to sixteen-year-old school children to discover their pattern of morbidity in a secondary school of Ludhiana city. Total of 776 students (462) boys and (314) girls were participated in study. Variables in study were “height, weight, medical history and general physical examination”. Results shows that girls of all ages except the 14 years old had lower mean weight for age in comparison to mean height, as compared to expected weight for age as per ICMR standards. The “expected height for age as per ICMR standards” were also low in both sexes in all ages, excluding only the 15 and 16 years old youngsters. The “prevalence of wasting and stunting in these children was high (52.2% wasted and 26.3% stunted”), with boys and girls suffering almost equally and 11-15 years old participants were more affected. Out of all, at examination time around 72.4% kids were having any illness as 26% had anemia, and females suffering more (30.5%) compares to males (22.9%). The analysis reveals the low nutritional profile along with health status of study school children. Isolating this class for pointed services in intention to improve their health along with nutritional status, in a highly developed and economically well off part of Punjab, the granary of India, highlighting the prerequisite for enhanced, concentrated attempts for improvement of their nutrition.

Rodríguez, Cervantes and Ortiz (2011) define the relationship among “malnutrition and immune system dysfunction” and ways by which this association effects child resistance
“to bacterial gastrointestinal and respiratory infections”. This research has been carried by México, “National Council of Science and Technology”. In this paper they examine the cyclic association among under-nutrition, immune response dysfunction, raised vulnerability towards infectious diseases, plus metabolic reactions affecting the child nutritional status. Moreover, they studied the raised mortality caused by infectious diseases in malnourished children. They have concluded that impact of malnutrition was wide and involved, low immunity against infection, delay child development, raised mortality rate and individual’s dysfunction.

Pathak and Singh (2011) inspects the associations between economic status of people and patterns of financial variations with child malnutrition in India. They have assessed figures gathered by “National Family and Health Survey (NFHS)” in the period of 1992–2006. The percentage of under-weight kids both acute as well chronic malnourished were dependent variable in this study. The wealth indicator is utilized as alternative for financial status of the people and were assessed by way of secure asset possession, approach to utilities and public services. The home characteristics of participants during survey were analyzed “by principal component” after applying different variables. They have applied “bi-variate analyses for poor-rich proportion and concentration indicators” to recognize the pattern of economic inequalities particular to child malnutrition. To analyze the changed outcome of economic status with time on child under-nutrition “pooled logistic regression models” were used. The result depicts in this regard towards children malnutrition in India in period of 1992–2006 proved slow change linked with concurrent increase in economic inequalities. The problem of malnutrition has been excessively assembled between poor class kids and mean reduction in malnutrition obscure high financial disproportion by space and time.
Mishra, Kumar, Basu, Rai and Aneja (2014) designed a “hospital-based case-control study” to reveal the potential determining factors of severe acute malnutrition in under five aged children in north India. All SAM children of concerned age group admitted in a hospital were diagnosed as per WHO criteria and were compared with age-matched controls according to WHO 2006 growth standards of weight for height < -2SD. By using “univariate and multivariate logistic regression” models data about sociodemographic factors, feeding practices and vaccination has been contrasted among the (76) cases and (115) controls. They have found that among analyzed variables maternal illiteracy, large family size, per day family income under 200 Indian rupees, lack of exclusive breast feed in first 6 months, bottle feeding, by giving pre-lacteals, depriving from colostrum and incomplete vaccination were important risk factors for SAM. Concerning complementary feeding practices, it was the consistency, rather than the age of starting complimentary feeding, frequency and variety which depict a significant effect on incidence of SAM. They have recommended that by determining these risk factors, policy makers linked with SAM children in India should give attention to these factors during health planning.

Mengistu, Alemu and Destaw (2013) directed a “community based cross-sectional study” in 820 kids aged six to fifty-nine months to measure the prevalence of malnutrition along with correlated factors at Oromia Regional State Ethiopia. Multistage sampling technique were used for selection of households and simple random sampling were utilized to select children. For collection of data structured questionnaire and anthropometric measurements (“z-scores of the indices; height-for-age, weight-for-height and weight-for-age”) were applied. For statistical analysis “bivariate and multivariate logistic regressions” have been utilized to recognize factors linked with malnutrition. In analysis they have found that the stunting was 47.6%, underweight were 30.9% and wasted were 16.7%, among
children respectively. The key variable associated with stunting have been found as child age, earning of family per month, pre-lacteal feeding along with family planning. Underweight was linked to more number of children and infants who have taken butter in per-lacteal feed. For wasting only significant variables was treatment of water. So it was concluded in this study that malnutrition still is a main problem in children aged six to fifty-nine months and special consideration is requisite for intervention of malnutrition.

The cited literature revealed the severity and multi-dimensionality of the problems and its effects on child health and their upcoming productivity in later life. This cited literature showed that this scenario is worst in Asian particularly south Asian and African countries. In developing countries this is deemed to be a serious public health issue even though these studies have just revealed the tip of the ice berg but it need serious and urgent consideration at national and global level. We had made an attempt to find predictors of malnutrition according to our sociocultural context at our local level and give clear picture to policy makers for addressing this problem for health of children in Pakistan and increasing their upcoming productivity for better future of Pakistan.
Table 7: Summary of the studies on Dietary and socio demographic factors in Malnutrition

<table>
<thead>
<tr>
<th>Authors</th>
<th>City / Country</th>
<th>Sample Size</th>
<th>Age group studies</th>
<th>Research Objectives</th>
<th>Study Design</th>
<th>Major Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valente et al. (2016)</td>
<td>Sao Tome Portugal</td>
<td>1285 children</td>
<td>0 to 5 years of age</td>
<td>To measure the nutritional status (acute and chronic malnutrition) of children and their predictors.</td>
<td>Cross-sectional population based study</td>
<td>Global acute malnutrition % is high with 30.9% in less than 24 months and 21.9% in children more than 24 months and global chronic undernutrition were found in 32.5% in &lt;24 months and 41.1% in children &gt; 24 months. Child weight at birth, nutritional status with weight gain and the maternal education were significant protecting variables.</td>
</tr>
<tr>
<td>Authors</td>
<td>Description</td>
<td>Sample Size</td>
<td>Study Group</td>
<td>Study Design</td>
<td>Findings</td>
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<tr>
<td>Fakir and Khan (2015)</td>
<td>Urban slum of Dhaka city, Bangladesh</td>
<td>174</td>
<td>Under 5 year</td>
<td>Descriptive study</td>
<td>Male child was more responsive towards &quot;child health precautions&quot; and &quot;medical cost knowledge&quot;, while female child was more responsive to &quot;health-seeking practices&quot;.</td>
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<tr>
<td>Ayana et al. (2015)</td>
<td>Oromia region, West Ethiopia</td>
<td>339 children</td>
<td>6-59 months old</td>
<td>Case control study</td>
<td>Wasting were significantly linked with diarrheal diseases in last couple weeks, mothers bad practices of not washing hands, no exclusive breastfeeding, having large family size, uneducated mothers, no access to latrine and febrile sickness in the last couple of weeks.</td>
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<tr>
<td>Mashal et al. (2008)</td>
<td>Kabul, Afghanistan</td>
<td>1400</td>
<td>Under 5 years</td>
<td>Cross-sectional survey</td>
<td>Mother’s illiteracy, child wedding, no maternal independence, absence of basic material</td>
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between the health and nutritional-status of children.”

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Sample Size</th>
<th>Age</th>
<th>Objective</th>
<th>Study Design</th>
<th>Findings/Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jamro et al. (2012)</td>
<td>Sukkur, Pakistan</td>
<td>270</td>
<td>6-59 months of age</td>
<td>To identify risk factors for severe acute malnutrition.</td>
<td>Prospective descriptive study</td>
<td>Common risk factors linked with severe acute malnutrition were parent’s illiteracy, large family size, poverty, non-exclusive breast feeding and recurrent diarrhea.</td>
</tr>
<tr>
<td>Fikree, Rabbar and Berendes (2000)</td>
<td>Karachi, Pakistan</td>
<td>78</td>
<td>Under 5 years</td>
<td>To investigate the nutritional and health status of children. To find out common diseases and sociodemographic factors responsible for them.</td>
<td>Cross-sectional health survey</td>
<td>Malnutrition have been considered as a main health problem along with parental low education, big families and less family earning were the sub-factors, causative to low nutritional profile in children.</td>
</tr>
<tr>
<td>Uzma and Muhammad (2006)</td>
<td>Karachi, Pakistan</td>
<td>In Preschool</td>
<td></td>
<td>To understand the health and nutritional-status of children in Pakistan”, and the interaction of socio-</td>
<td></td>
<td>Food accessibility, child care habits along with child health, family no, and household income were</td>
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<tr>
<td>Study</td>
<td>Country</td>
<td>Sample Size</td>
<td>Age</td>
<td>Study Design</td>
<td>Findings</td>
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<tr>
<td>Panda et al. (2000)</td>
<td>Ludhiana, India</td>
<td>776 students (462 boys and 314 girls)</td>
<td>5-16 years</td>
<td>Descriptive study</td>
<td>To assess the “health and nutritional status of school children” and to find out their morbidity pattern in a secondary school level. The prevalence of wasting and stunting in these children was high (52.2% wasted and 26.3% stunted), in the 11-15-year age group with no gender inequality. At examination 26% had anemia, with females suffering more (30.5%) than the males (22.9%).</td>
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<tr>
<td>Pathak and Singh (2011)</td>
<td>India</td>
<td>Secondary data from National Family and Health Survey</td>
<td>Under 10 children</td>
<td>Multiple indicator cluster survey</td>
<td>To investigate the child malnutrition in India affected by economic status of people and patterns of financial variations. The results depict in regard to child malnutrition in India for the period of 1992–2006 showed slow change linked with concurrent increase in economic inequalities.</td>
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<tr>
<td>Authors</td>
<td>Location</td>
<td>Sample Size</td>
<td>Age Group</td>
<td>Study Objective</td>
<td>Study Type</td>
<td>Findings</td>
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<tr>
<td>Mengistu, Alemu and Destaw</td>
<td>Oromia Regional State Ethiopia</td>
<td>820</td>
<td>Aged 6-59 months</td>
<td>To assess the prevalence and associated factors of malnutrition.</td>
<td>Community based cross-sectional study</td>
<td>Prevalence of stunting, underweight and wasting were 47.6%, 30.9% and 16.7%. Associated stunting factors were “child age, earning of family, pre-lacteal feeding and family planning”. Underweight were number of children &amp; butter as per-lacteal feed.</td>
</tr>
<tr>
<td>Afzal</td>
<td>Punjab Pakistan</td>
<td>71,507</td>
<td>Under 5 children</td>
<td>It tries to find out the socio-economic variables that alter child health at domestic level.</td>
<td>Multiple indicator cluster survey</td>
<td>The study’s finding suggest that maternal literacy along with health knowledge are main determining factors for child health.</td>
</tr>
<tr>
<td>Aslam and Kingdon</td>
<td>Pakistan</td>
<td>1000 households</td>
<td>Under 5</td>
<td>This study explores the influence of parent’s literacy on child health, nutrition and health obtaining attitude.</td>
<td>Descriptive study</td>
<td>They propose that father knowledge of health is directly linked with decisions of vaccination, but health knowledge, education and empowerment of mother in home affects child height and weight individually.</td>
</tr>
<tr>
<td>Authors</td>
<td>Location</td>
<td>Sample Size</td>
<td>Age Group</td>
<td>Study Objective</td>
<td>Study Design</td>
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<tr>
<td>Kavosi et al. (2014)</td>
<td>Fars province, Iran</td>
<td>15408</td>
<td>Under 6 years</td>
<td>To find the prevalence and determinants of undernutrition in children.</td>
<td>Cross-sectional house to house survey</td>
<td>“Prevalence of stunting, underweight and wasting were 9.53, 9.66 and 8.19%,” in that order. Gender, residing area, big family, less family earning, mother literacy, health services and secure water source were determinants of undernutrition.</td>
</tr>
<tr>
<td>Mishra et al. (2014)</td>
<td>North India</td>
<td>76 cases and 115 controls</td>
<td>Under 5 years of age</td>
<td>To reveal the&quot; potential risk factors for severe acute malnutrition in children&quot;.</td>
<td>Hospital based “case-control study”.</td>
<td>Maternal illiteracy, large family size, per day family income under 200 Indian rupees, lack of exclusive breast feed in first 6 months, bottle feeding, by giving pre-lacteals, depriving from colostrum and incomplete vaccination were important risk factors for SAM.</td>
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2.5 Discussion of Literature Review

The objective of the review in this chapter was, to search the literature and existing data for treating “children aged 6-59 months with severe acute malnutrition” by using CMAM model. RUTF effect, severe acute malnutrition impact on child development, socio-demographic predictors of malnutrition and vitamin D trials on children’s health at global level were also searched with the aim to identify any breach in the literature for requirement of any additional research. The keywords created and utilised to do the search for identification of the related papers for inclusion in the literature review were, malnutrition, severe-acute malnutrition, wasting, community-based treatment, out-patient care, ready-to-use therapeutic foods, RUTF, CMAM, development screening, development delay, DDSTII, vitamin D, randomized control trial, vitamin D trials, predictors of malnutrition, malnutrition impact on development, under 5 children.

The review of literature has recognized many research gaps and areas that need to be investigated for proper documentation and propagation in child growth and development in Pakistan and worldwide. We have designed this trial to determine if high dose vitamin D supplementation can accelerate the growth and development of malnourished children. Though there are currently no suggested guidelines for this approach. The World Health Organisation (WHO) has identified research priorities to identify adjunctive therapies that may improve response to RUTF, including administration of broad-spectrum antibiotics and high-dose vitamin A. Thus, well-designed randomized-control trials are requisite to assess vitamin D role in SAM. The results of this study should give more understanding into the possible causal relationship among vitamin D status, growth and development of severe acute malnourished children and fulfil the research gap in this area.
CHAPTER THREE

3. METHODOLOGY

This chapter defines the methodology followed in this research for assessment of developmental screening, anthropometry, identification of SAM, methods used to collect and analyse data, with recognition of risk factors for malnutrition and development delay with all complete procedure of conducting trial in the community based malnutrition treatment programme.

3.1 Research Settings

3.1.1 Study Country

Pakistan became an independent country on 14th August 1947, after the partitioning of the Indian subcontinent that were governed by the British Empire. The 4000 year’s history of area comprising Pakistan dating from brick cities such as Mohen-jo-Daro and Harappa to the Hindu civilization and the Buddhists contemporaneous to the birth of Christianity. Location of Pakistan is in the north western part of the South Asian subcontinent. The entire state area is 796,096 square Kilometers, it includes a diversified land and geography. The Indus river flows within the Pakistan approximately 2500 kilometer start off from Himalayas to the Karakoram mountain range in north and the Arabian Sea in south. The northern side of Pakistan have 5 of the world’s 14 tallest mountain peaks. Country is situated amid 24 degrees and 37-degree north latitude and amid 61 degrees and 75-degree east longitude. India is situated in Pakistan east and south east, Afghanistan lies in the north and the northwest, Iran lies to the west and Arabian Sea in the south. State has a communal frontline with China on the border of its Gilgit territory in
the north. Country is comprised of 4 provinces also have the federally administrative tribal region (FATA) and the area of Gilgit Baltistan. From population point of view Punjab is the largest province with nearly 56 percent of the country’s citizens residing there. Pakistan is an agricultural country and around 64 percent of its people’s livings in rural areas. Agriculture has key role for the country economic growth and development. As a leading sector, it denotes 21 percent of Pakistan gross domestic product. According to (PDHS, 2013), population of Pakistan is 184.5 million. The current population growth rate is 2 percent. Population density in Pakistan is 231 persons per square kilometer.

Figure 11: Map of Pakistan
3.1.2 Study Area

This study was conducted in Southern Punjab of Pakistan. Southern Punjab comprises of 4 divisions and 11 districts. The total area of South Punjab is 99,572 km² and it consist of 48.5% of the entire Punjab region. By area the South Punjab zone comprises of approximately half of Punjab Province. Population of South Punjab is 29.74 million. Most of the people are attached with farming business. There are about 15,455 primary schools and 86 health facilities are located in Southern Punjab. Southern Punjab has the highest percentage of 43% living below poverty line.

This study was conducted in District Dera Ghazi Khan of Southern Punjab, having land area around 5,306 square meter. It is a long strip of country about 198 kilometers long and it slopes steadily starting the hills, which make its western border and on the east is river Indus. This is the most backward area of southern Punjab affected by flood and hill tolerance almost every year. Out of 16, four functional outpatients therapeutic programme centre (OTPs) in DG Khan district run by the “National Program for Family Planning and Primary Health Care” at basic health Units (BHU) Samina, Jhokutra, Aaliwala and one OTP Kotchutta at rural health Centre (RHC) were included for collection of children having severe acute malnutrition. These selected areas are under developed, by substandard housing, squalor, lacking in tenure security, over-crowded have poor socio-economic conditions, illiteracy, and unhygienic living conditions.
3.2 Study Design

This study was designed in to 2 phases, 1st phase is “cross –sectional study design” and second is “Randomized controlled trial”.

Figure 12: Map of district Dera Ghazi khan
## Study Design

### Phase – I

**Objectives**
To examine the impact of malnutrition on development quotient of children
To explore the dietary and socio demographic factors responsible for severe acute malnutrition and developmental quotient of children

**Methodology**
**Study Design:** Cross sectional study design
**Place:** DG Khan
**Sample size:** 194
**Sampling techniques:** Non-probability purposive

### Phase – II

**Objectives**
To determine the effectiveness of ready to use therapeutic food (RUTF) in improving the development quotient of severe acute malnourished children under five year of age.
To investigate the outcome of vitamin D therapeutic doses intervention with RUTF rehabilitation on growth and development of malnourished children

**Methodology**
**Study Design:** Randomized controlled experimental study design
**Sampling Techniques:** purposive sampling
**Sample Size:** 194

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**Figure 13: Study Design**
3.3 Study Population

Children between “6-59 months of age” having “severe acute malnutrition” without complications living in district Dera Ghazi Khan of selected areas.

3.4 Inclusion and Exclusion Criteria

Inclusion criteria were as follows: age at enrolment between 6-59 months; severe malnutrition without complications, as defined by the World Health Organization (i.e. children with “mid-upper arm circumference (MUAC) <115 mm or weight-for-height z-score <-3” or grade 1-2 bilateral edema, who were clinically well and alert with good appetite); parental consent for child to participate. Exclusion criteria were ingestion of a dose of vitamin D >200,000 IU (5 mg) per month in the last three months or presence of complications of severe malnutrition (severe dehydration, severe anemia, severe pitting edema, anorexia, hypothermia, high pyrexia, acute lower respiratory infection or hypoglycemia).

3.5 Baseline assessment for eligibility criteria

Children aged 6-59 months whose parents gave consent for them to participate underwent the following baseline assessment. A structured socio-demographic and nutritional questionnaire was administered to capture information on participants’ demographic details, parental occupation, education and monthly income and nutritional intake. Gestational age was taken from the antenatal record where delivery occurred in hospital, and based on maternal report for home deliveries. For children aged up to 24 months of age who were born prematurely (<37 weeks’ gestation) age was corrected by subtracting the number of weeks of missed gestation from the current age. A history was
taken to assess for symptoms suggesting that the child was not clinically well (cough, shortness of breath, diarrhea, fever and anorexia); children assessed as being clinically unwell on the basis of these symptoms were excluded from the trial and referred to a stabilization center for further assessment. An appetite test was performed by offering children a small sample of ready-to-use therapeutic food to eat. Children who did not eat at least one third of a packet (3 teaspoons, 30 g) of ready-to-use therapeutic food after three feeding attempts were classified as having poor appetite, excluded from the study and referred for in-patient management. (“Child’s mother is given a sachet of RUTF and is asked to sit with the child at a calm place and give it to the child with plenty of water. The child should eat at least one third of a packet or three teaspoons from a pot of RUTF to pass the test. The health care provider observes the child eating the RUTF and decides whether the child passes or fails. This is the only reliable way to access the child’s appetite and if he/she eats half or more, it signifies a good appetite”). A physical examination was then performed. Children were assessed for the following sings of rickets: bow legs, knock knees, windswept deformity of the knees and proximal myopathy. The child’s alertness was assessed: children who were lethargic, apathetic or unconscious or who had seizures were deemed to be non-alert, and excluded from the trial. The child’s hydration status was assessed: children with a history of recent watery diarrhea associated with eyelid retraction, weak/absent radial pulse, absence of tears, cold peripheries, lethargy or absence of urinary output were deemed to have severe dehydration and excluded from the trial. Children were assessed for the presence of palmar pallor: those whose palms were very pale, or so pale that they looked white, were deemed to have severe anemia and were excluded from the trial (WHO, 2013). Children were assessed for the presence of pitting edema: thumb pressure was applied to the tops of the feet for three seconds,
and pitting edema was judged to be present where a thumb impression remained for a few seconds on both feet. Edema was graded as mild (Grade 1, affecting both feet/ankles), moderate (Grade 2, affecting both feet, plus lower legs, hands and lower limbs) or severe (Grade 3, generalized edema including both feet, plus legs, arms and face). Children with severe (Grade 3) pitting edema were excluded from the trial. Vital signs (temperature, pulse and respiratory rate) were recorded: children who were hypothermic or who had hyperpyrexia (axillary temperature <35°C or >39°C respectively) were excluded from the trial. Children with tachypnea (>50 breaths per minute for those aged <12 months, >40 breaths per minute for those aged 12-59 months), chest in-drawing, wheeze or stridor were classified as having a likely acute lower respiratory infection and excluded from the trial. A heel finger-prick was performed to check for hypoglycemia using a Dextrostix reagent strip: children with a heel-prick glucose concentration of <3 mmol/l were considered to be hypoglycemic and excluded from the trial.

3.6 Outcome measures

The primary outcome measure was the proportion of participants gaining >15% of their baseline weight at 2-month follow-up (WHO, 2013). Secondary outcomes were mean weight and mean weight-for-height/length z-score at 2 months; proportion of participants with delayed development (“global, gross motor, fine motor, language and personal/social”) at 2 months; and mean “serum levels of 25(OH)D, corrected calcium”, albumin and prealbumin at 2 months (n=90 sub-set); and proportion of participants with “serum 25(OH)D concentration ≥50 nmol/L” at 2 months (n=90 sub-set).

3.7 Biochemical Results

Vitamin D deficiency is classified as per < 50 ng/L in this randomized control trial. Other biochemical level of corrected calcium less than 2.20 -2.26 mmol/L, level of Pre albumin
less than 0.20 – 0.40 g/L and biochemical level of Albumin less than 38 – 50 g/L were considered as in normal range.

- Biochemical level of vitamin D less than <50ng/L
- Biochemical level of corrected calcium less than 2.20 -2.26 mmol/L
- Biochemical level of Pre albumin less than 0.20 – 0.40 g/L
- Biochemical level of Albumin less than 38 – 50 g/L

### 3.8 Sample size

Assuming that 76% of children in the control arm would gain >15% of baseline weight at 2 months, we calculated that a total of 158 participants (79 per arm) would need to complete follow-up in order to detect a 16% absolute increase (to 92%) in the proportion of children gaining >15% weight at 2 months in the intervention arm with “80% power at the 5% significance level”. Allowing for 25 % international acceptable standard for CMAM programme, > 75% recovery rate <15 % default rate and < 10 % death rate (Charter, 2011) this number was inflated to a total of 194 to allow for attrition due to death and loss to follow-up. No interim analyses were planned or performed.
3.9 Sampling Technique

Non-probability purposive sampling was applied for enrolment of severe malnourished children. Enrolled children were allocated randomly to one of the two dietary groups.

3.10 Procurement of Sample

Vitamin D supplementation in ampoules form (ED3) were procured from GT Pharma Lahore. Ampoule contains Cholecalciferol in 200,000 IU both for oral and intra muscular use. RUTF were procured from IRMNCH program from Dera Ghazi Khan Division. Extra virgin olive oil as placebo were taken from market.
3.11 Trial Design, Approvals, Consent Processes and Registration

We conducted a two-arm parallel randomized placebo-controlled trial with a one-to-one allocation ratio. The study was “approved by the Ethical Review and Advanced Study Research Board” of the University of Punjab Pakistan (reference 9/2352-ACAD). The “Integrated Reproductive Maternal & Newborn Child Health (IRMNCH) & Nutrition Program”, Punjab, Pakistan and the District Health Officer of the Dera Ghazi Khan District, Punjab, Pakistan grant permission to use their centers and staff for study. Parents were provided with information about the study in their native language by a doctor or a health visitor at participating outpatient therapeutic program (OTP) centers and informed consent confirmed by signature or thumb impression was taken from those who gave permission for their child to take part in the study. Sign or thumbprint consent was taken from each child’s parents at outpatient therapeutic centers after the child fulfilled the study inclusion criteria and one of the parents signed the consent form (in Urdu) or was briefed about consent in their native language (Urdu and Saraiki) to remove language barrier by the doctor or Lady Health visitor of the center (Annexure attach). “This study is registered with ClinicalTrials.gov, number NCT03170479”

3.12 Training of CMAM study staff

From May - June 2015, 4 CMAM (nutritional supervisor) already trained staff at CMAM OTP centers were briefed and given refresher training about CMAM guidelines and standard operating procedures of the trial individually at their centers by the principal investigator. Staff worked individually at center and learnt how to assess weight, height, MUAC and conducting an appetite test. Additionally, the study staff at field were given demonstration of all anthropometric measurements with assessment of clinical complications in children coming to selected CMAM, OTP centers.
3.12.1 Consent and socio-demographic form

The study staff exercised the procedure of consenting and taking written consent on center in front of principal investigator. The study staff were trained on their respective centers for the procedure of filling concerned information’s in all study questionnaires along with the consent form. Study questionnaires and consent form were briefed to participant’s parents in to their native language (saraiki) and were pretested. Throughout the practice of study staff, all staff did the interview of parents for fulfilling the socio-demographic form, and all staff interviewed parents with study forms. After every day practice, study staff discussed their results with the principal investigator and the study forms were modified if required.

3.12.2 Clinical examination of children: at CMAM centers

The research protocol along with standard operating methods and the study questionnaires were briefed to the four nutritional supervisors, study staff and local LHW of the area. Then they were guided with field workers in CMAM protocol by the help of video clips and clinical demonstration. The reliability in assessing edema, with others complications was judged by the study clinicians and principal investigator.

3.12.3 Development assessment of children at the CMAM centers

One experienced nurse and pediatricians in assessment of child development by using Denver development tool with the principal investigator were trained for applying DDST II by using Denver video demonstration with training and technical manual. The training was consisted of basic guidelines of assessing development with completion of Denver forms. After that we have pretested this DDST 11 in Mayo hospital Lahore, largest CMAM center in Punjab with more patient turn over on complicated, un complicated SAM and on children from well-baby clinic, inter-observer agreements were assessed randomly among
children. This tool was translated into local language for removing language barrier and better understanding of parents and children.

3.12.4 Collection of venous blood samples

One male nurse was instructed to obtain consent for collection of blood samples and also briefed about the method of taking care of collected blood samples before transferring for “centrifugation and freezing” to the concerned laboratory.

3.13 Screening of Children

This study was accomplished in a community setting, between 6 to 59 months old children who had “mid upper arm circumference (MUAC) ≤11.5 cm or z-score of weight for height<-3SD”. They were detected in community setting through active screening accomplished by house-to-house survey by community-based lady health workers who are an essential part of the healthcare delivery system in Pakistan. Active screening of the study areas was done after approval from district health officer in lieu to get sample for study. The lady health workers were already trained on the “Community management of acute malnutrition (CMAM) approach”, but further instructions were given by principal investigator in order to identify the malnutrition.

After the tutoring, the LHW went house-to-house to sensitized community members and detect malnourished children. LHW, s screened the children by utilizing a colour-labelle MuAC tape, along with checking for clinical signs of oedema. They send all children who fulfil the criteria for admission into the CMAM programme (<11.5 cm) of an out-patient therapeutic centre for assessment by a qualified nutritional supervisor to confirm Severe acute malnutrition (SAM). At the “basic health unit (BHU) or rural health centre (RHC)”
level, the health worker (lady health visitor or nutritional supervisor) were trained on assessment of nutritional parameters in children under the age of five years and also instructed by principal investigator to measure the weight, height and MUAC of the referred children and recognize any medical conditions, including bilateral pitting oedema and other complications of malnutrition (WHO, 2009).

An appetite assessment of all children was done by an appetite test who met the enrolment criteria before any treatment started. Children who were identified with complicated severe acute malnutrition were sent to the stabilization centre in “tertiary care hospital” for management, according to the national CMAM guidelines. As per study inclusion criteria, children who did not have a severe medical complications and who have passed an appetite test was enrolled in study and CMAM programme to get treatment (194 in study).

“Ready-to-use therapeutic food (RUTF’s)” was used with vitamin D and placebo group in the therapeutic intervention for SAM. RUTFs are now recommended by the WHO as a therapeutic diet that can be used at community level to treat children (WHO, 2009). RUTF’s have a good shelf life once opened and are also resistant to bacterial contamination (Briend et al., 1999). Trained staff at these centres supplied RUTF’s to parents weekly, the amount depending on the child's body weight. Every week, parents of the child were followed by lady health worker at home for sending child to the centre, where the nutritional supervisor assessed the child's weight, MUAC and other comorbidity.

The LHV used a CMAM OTP chart to supply RUTF to the parents according to child weight for 7 days. Parents were told to come back to the centre at 7th day for re-evaluation of the children health along with nutritional status. The mother was also counselled about
advantage of giving diet to child. Child was cured at home by their parents after taking “RUTF”, with the instruction of how to give it to their child. Identified children with non-complicated disease before enrolment have taken medication according to the given recommendations of Integrated Community Management of severe acute malnutrition. Children were followed for two months minimum or until they achieved 15% weight gain of the initial weight. Children who completed the programme cycle without meeting the recovery criteria were referred to a stabilization centre for further treatment.

3.14 Nutritional Assessment

Proforma regarding baseline data, overall health of child, history of illness, nutritional plan, intake of medications along with anthropometric assessments and other characteristics were noted before commencing the actual study. Follow up of patients for two months and for recording of their weight Community management of acute malnutrition forms was applied. Baseline data were gathered for all concerned children like demographic details, occupation, education and monthly income of parents. Clinical examination was conducted for the presence of any illness, including fever, vomiting, diarrhoea with their nutritional status assessment by anthropometric measurements and compared with Z-score chart for grading of their nutritional status. Structured socio-demographic and nutritional evaluation questionnaire were used for data collection.

The anthropometric assessments were “weight, height and mid-upper- arm circumference (MUAC)”. Anthropometric measurements were conducted by out-patient clinic staff who were specifically trained to make these measurements. Their competence in measuring weight, height and MUAC was assessed and confirmed by the principal investigator. Double measurements were taken by a staff member. If they differed from each other, additional measurements were made until an exact value was replicated. The replicated
value was then recorded. Recommended procedure and apparatus was used. Weight of the children were assessed and recorded by UNISCALE (de Onis, Onyango, Van den Broeck, Chumlea & Martorell, 2004). Children were weighed to the nearest 10g unclothed or in very light clothing with a UNISCALE, which was adjusted by a standard weight and calibrated to zero before each measurement. For infants and children who could not stand, the UNISCALE was used to measure the mother’s weight alone. The mother was then handed the undressed baby / child, while standing on the scales, and the combined weight of the mother and baby was measured. The baby / child’s weight was calculated as the difference between these two readings. Recumbent length of children ≤87 cm in height was measured to the nearest 0.1 cm using a length measuring board with an affixed headrest and a movable foot piece (SECA GmbH & Co. KG, Hamburg, Germany), placed on a flat surface. Child head was hold carefully in an “upward upright position, by stretching legs to a full extent and feet at right angles with legs”. After child proper placement foot end piece was pulled for touching the feet and the length was measured “to the nearest 0.1 cm”. Child with above 87cm height, the assessment was done with the child in standing position after removing shoes on a “horizontal flat plate” fixed to the measuring board base with both heels together. The child was carefully monitored to confirm that the “heels should be on the plate and the head in upright position” throughout the assessment. The headpiece was then carried down on the head of child and measurement recorded. MUAC of each child was “measured to the nearest 0.1 cm” with colour- labelled MUAC tape at the midpoint between the olecranon process and the acromion process. Care was taken that during measurement children arm were bent and uncovered till shoulder with the lower arm resting transversely on the stomach whereas the child should be seeing ahead straight. “The tip of the bones at the elbow and top of the shoulder had pinpointed
and the distance among the two indicated tips was calculated and divided by two to get midway point. Then the child’s arm was hang down at the sides”. The tape was positioned across the arm on the labelled mid-point and cautiously tape fitted without any discomfort round the arm that should be, not too tight or too loose. MUAC were measured for child nutritional status assessment. Children were categorized by their nutritional status. Age were asked verbally from mother because of non-availability of neonatal cards.

Standard guidelines for classification of malnutrition by WHO were applied in this randomized trial.” Wasting (acute malnutrition) is a weight for height z-score (WHZ) of < -2 as well as severe wasting is considered if WHZ was < -3 or if MUAC < 11.5 cm, Stunting (chronic malnutrition) is a height for age z-score (HAZ) of < -2 as severe stunting is considered if HAZ was < -3 and underweight (mixed acute and chronic malnutrition) is weight for age z-score (WAZ) of < -2 as severe underweight is considered if WAZ was < -3.” (Onis, 2006; WHO, 1995). Classification of nutritional status was done with WHO ANTHRO, version 3.2.2.

3.15 Development screening

Developmental screening was done by standard protocol of Denver development screening tool II. The DDST- II were created in Denver (Frankenburg, 1992) by the University of Colorado as an instrument to identify the early problems in child development. It was practiced by the health care providers, teachers and social workers (Frankenburg & Dodds, 1967). It can be conducted in home visit in community. DDST- II recognize possible developmental problems for timely intervention of child. DDST-II were classified as normal development, suspected development delay and untestable. Development profile covers, motor, personal social and language milestones of children.
(Mayson, Harris & Bachman, 2007). The purpose of tool, for use in the study was to check the outcome of energy dense food and vitamin D on child development. On random visits principal investigator also reassessed the child development by the same tool for checking the accuracy and consistency of Denver results. This tool was purchased on line for use in study from Denver website.

There is also a “testing behavior observation” section in the form end and completed by supervisor of the test. In DDST-II Child accurate age were estimated and wrote on the Denver form and for premature born infants age were adjusted by subtracting the digit of premature months from the chronological child’s age. Administrator governs the preferred task for all functional areas depending on intersection of age line. The administrator can then decide “if child’s responses fall into or outside of the normal guessed range of achievement on that task for the child’s age”. The total numbers of task on which the child attains less than the estimated age decides, that is child categorized as inside the “normal range, suspected, or delayed”. Kids with suspected scores are frequently examined and kids with delayed scores are referred for more advance evaluation.

Children who were found to be eligible to participate in the trial also underwent a baseline developmental assessment using the standard protocol of the Denver Development Screening Tool II (DDST-II), performed by a nurse with specific training in child development or a pediatrician, both of whom were blinded to participants’ allocation. This instrument assesses the ability of children aged up to 6 years to perform a range of tasks as compared with a standardized population of children of the same age. Tasks are grouped into four categories (social contact, fine motor skills, language, and gross motor skills) and include items such as ‘smiles spontaneously’ (performed by 90% of three-month-olds), ‘bangs two cubes held in hands’ (90% of 13-month-olds), ‘speaks three
words other than dada/mama’ (90% of 21-month-olds), or ‘hops on one leg’ (90% of 5-year-olds). Following a standardized algorithm, children are assessed as having ‘no delay’, ‘caution’ (an intermediate classification) or ‘delay’ in each category. These category assessments are then used to classify global developmental status as normal (no category delayed and no more than one category classified as ‘caution’), suspect (at least two cautions or at least one delay) or untestable (based on a specific pattern of refusals). Of note, some patterns of refusals may allow a category assessment but preclude a global assessment of developmental status according to DDST-II algorithms. Where children were classified as having ‘untestable’ global developmental status at screening, developmental assessments were repeated 2 days later; if global developmental status was still untestable at this point, the assessment was repeated again 2 days after that.

With regard to the timing of developmental assessments relative to commencement of ready-to-use therapeutic food and study medication: all children commenced ready-to-use therapeutic food as soon as the diagnosis of uncomplicated severe acute malnutrition was made, but no child was randomised or commenced study medication until baseline assessment of developmental status was complete.

3.15.1 How to Administer DDST - II

Tool was translated in to local language to remove language barrier before administration. “It can be done in community setting at home visit and other busy setting like day care center in the presence of teachers, caregivers or whoever will be aware of child, with or without presence of parents”. Parents were also questioned later if any other info are required for child assessment. “It can be performed through anybody familiar with the child and will pursue the guidelines of DDST-II”. However usually the para-professional and the qualified staff applied this tool (Murphy, 2001).
3.15.2 Interpretation of Denver - II

Like a growth curve the results were reported as age norms. The more task child fails to do that were passed by 90% of child age mates, showed highly significant developmental deviation that need further assessment. In each area “at least three items nearest to and left of age line should be performed by child, all tasks on intersecting the age line continue till 3 fails occur”. Three attempts were permitted to do each item and after conducting test “Test Behavior Rating” were also recorded.

3.15.3 Scoring of items

“P for PASS, the child successfully performs or the caregiver reports (as appropriate).

F for FAIL, the child does not successfully perform an item or the caregiver reports.

NO for NO OPPORTUNITY (this is used on report items only).

R for REFUSAL, the child refuses to attempt items, cannot be use on report items”.

3.15.4 Denver - II Final Interpretation

We have interpreted scores by Denver criteria as follows in

Normal

“No delay and maximum one caution

Conduct routine rescreening at next well-child visit”.

Suspect

“Two or more caution or one or more delay

Rescreening in 1-2 weeks to rule out temporary factors such as fatigue, fear, illness”.

Untestable

“Refusal scores on one or more items completely to the left of the age line or on more than one item intersected by the age line in the 75%-90% area. Rescreen in 1-2 weeks”.
Gestational age was taken from the antenatal record where delivery occurred in hospital, and based on maternal report for home deliveries. For children aged up to 24 months who were born prematurely (<37 week’s gestation) age was corrected by subtracting the number of weeks of missed gestation from the current age. Where children were classified as having ‘untestable’ global developmental status at screening, developmental assessments were repeated 2 days later; if global developmental status was still untestable at this point, the assessment was repeated again 2 days after that. In present trial in developmental screening of 185 children before intervention 69 children were have normal development, 108 have suspected development and 8 were untestable even after rescreen in to 1-2 weeks but these children were not excluded from study, nor referred to other centres, they remained in the study, followed and completed all protocol of study in the end they were screened again with other children’s and out of them only 3 remain untestable at the end in final score, these three were then referred to higher specialized centre for further assessment.

3.16 Randomization and allocation

The random allocation sequence was generated in an Excel spread sheet by a statistician who was independent of the study (Mr. Arslan Chughtai, Rashid Latif Medical Collage, and Lahore). Consecutive numbers from 001 to 200 were assigned to active vs. placebo in equal numbers. No restrictions (e.g. stratification, block size) were applied. This sequence was used by the study pharmacy to label pairs of syringes containing active and placebo medication with a study number assigned to active and placebo arms, respectively. Participants were enrolled by four health workers in “community management of acute malnutrition” (CMAM) programme. These staff assigned
consecutive ID numbers to participants according to the sequence in which they were enrolled and the hospital pharmacy then supplied syringes of placebo medication bearing this ID number. The syringes were freshly prepared and transported to the study site after every 2 weeks. The randomisation was implemented by simply assigning consecutive ID numbers to participants on their CMAM enrolment card by study staff at study site in recruitment period as they went along and then those participants received the study medication labelled with that ID number as prepared by Pharmacy. In each round every child was administered vitamin D or placebo (containing extra virgin Dalda olive oil®) by a syringe with his or her exclusive ID number by a study staff. Firstly, study staff verified the syringe label for the similar ID number recorded in the CMAM enrolment card and then once again confirmed the ID number before administrating the syringe content to the particular child.

3.17 Blinding

Parents / guardians of all study participants were blinded to allocation, as were the health workers and staff who enrolled participants and performed study assessments. Active and placebo medication were presented identically in 1 ml syringes and had identical appearance and texture.

3.18 Interventions

All participants were treated with RUTF provided by UNICEF at community centres according to WHO guidelines. (World Health Organisation, 2013 #2925). RUTF was supplied to parents according to the child’s body weight (2 sachets/day for children weighing 5-6.9 kg, 3/day for those weighing 7-9.9 kg, 4/day for those weighing ≥10 kg) on a weekly basis by suitably trained staff who provided parents with information regarding
benefits of RUTF and advice as to how it should be taken. Participants randomised to the intervention arm of the trial additionally took two oral doses of 200,000 IU (5 mg) vitamin D₃ (cholecalciferol) in 1 ml olive oil, administered via a syringe at 2 and 4 week’s post-initiation of RUTF. This solution of vitamin D was manufactured by GT Pharma (Pvt) Ltd Lahore and quality accredited by Ministry of National Health services of Pakistan. Participants randomised to the control arm of the trial received two oral doses of placebo (1 ml extra virgin Dalda olive oil®) via a syringe at 2 and 4 week’s post-initiation of RUTF. Study medication was packed and sealed in two 1 ml plastic syringes at the pharmacy in Shehroz Hospital, Dera Ghazi Khan District, by a registered pharmacist. Syringes containing active vs. placebo medication were labelled with a unique identification number according to the randomisation code, as described above and stored in a dry, cool environment for up to eight weeks as recommended by the manufacturer.

Four lady health workers of CMAM programme were trained by the principal investigator for the standard operating protocol of the study and recruited children from June 2015 to June 2016. After getting consent from caregiver first bolus dose of Vitamin D3 or placebo were given orally under direct supervision. On random interrogation of study staff and caregiver of child, no clues were detected at any stage that parents or LHW identify which child may have received placebo or vitamin D. Only principal investigator knows about allocation procedure. GT pharma and local hospital pharmacy involved for syringe preparation have no other rule in trial.

3.19 Follow-up of study participants

Study participants were given a CMAM enrolment card and family members were assisted in bringing children for visits to the study outpatient therapeutic centres. According to standard practice, children were monitored weekly throughout the whole study period at
the centres which provided their RUTF diet and assessed for any medical or nutritional complications, with recording of serious adverse events and referral to a tertiary care hospital if necessary. Parents were encouraged to come to the outpatient centres for any ailments affecting their child and treatment was free of charge for study children during the trial duration. Anthropometry and developmental assessments were performed at 2 months: all 2-month anthropometric assessments were made by CMAM health workers who were blinded to allocation. All 2-month developmental assessments were conducted by a research nurse or a paediatrician, both of whom were also blinded to allocation. A 3 ml blood sample was taken from a sub-set of 90 participants at 2-month follow-up and centrifuged after clotting; serum samples were aspirated and frozen at -20°C pending biochemical analysis. (We have requested all parents for their consent to obtain blood sample. Finally, 116 participated voluntarily. Initial 9 were examined in Pakistan, but we did not include these in our analysis because of different laboratory method. Few samples were clotted and some were unable to examine due to insufficient blood, making the final sample to 90.)

All staff members were trained in taking anthropometric measurements, identification of the malnutrition signs and symptoms. There was additional supervision by the principle investigator on random visits to the study sites to ensure that study protocol was being followed vigorously. Verbal autopsy interview with health worker of concerned child centre who have attended the child during illness and lost him during the study period were conducted after two weeks of the child's death because of absence of parents. WHO-standard verbal autopsy form was used for interrogation and questions were asked in native language.
3.20 Vitamin D or Placebo Administration

Recommended trial doses of Vitamin D3 (2 00,000 IU) and Placebo were efficaciously given to children, who were enrolled in the trial for both times of vitamin D3 administration. Only some breach in trial protocol happened and these were instantly reported and recorded by principal investigator. As we discussed earlier, the same vitamin D dose (2 00,000 IU D3) from GT pharma were filled in syringes in local hospital pharmacy by a registered pharmacist for each round of study and they filled the syringes by utilizing the same randomization list generated by an independent statistician. Random quality check of the fillings of syringes were not done because of it high cost but we have collected random blood samples from 116 children from both groups at the end of follow up to compare the serum vitamin D level in study and control group. The difference of the serum vitamin D values in both groups was utilized as an alternate to assess the quality and fillings of the syringes. The procedure of giving the syringe contents were: first removing the syringe cap, with the position of the child nearly flat in the mother's arms, put the syringe in the child's mouth and gently press the plunger so that the fluid goes in the child mouth and is swallowed by the child. Did this very slowly and carefully to avoid any child dribbling and spitting.

3.21 Vitamin D Adverse Effects

Throughout the study period of 18 months, no signs of vitamin D3 supplementation over dosage or adverse effects identified or reported. All study staff and study supervisors strictly observed the syringes content administration to identify any sign of over dosage. The contents were given to child after assessing child for any sign of vomiting. If the child has vomiting on the day of giving drug it was assessed by giving a spoon of water, if this
was vomited after ten minute’s mother was called back after a week and drug was given and completed recruitment. If child had no vomiting on the day of administration then the contents of a syringe were given to child orally for both times, the concerned study staff monitored the children for an hour for any sign and symptom of vitamin D over dosage (rash, difficulty in breathing, swelling of the body or any other unusual symptoms) and check if child vomit within half an hour of administration. Mothers or caretakers of the child were briefed to watch out for any unusual new symptoms with any delayed adverse events in the child and contact immediately the health canter staff. The finding was discussed in discussion chapter.

3.22 Collection of Blood samples for Biochemical Analysis

For biochemical outcome measures we have collected 116 venous blood samples randomly from children in each of the vitamin D and placebo groups at the end of study to measure the serum 25-hydroxyvitamin D, calcium, albumin and pre albumin concentrations, but not at baseline because of non-availability of funds for biochemical analysis. From every child approximately 3 ml blood was drawn and stored in a vacutainer tubes within the cold vaccine carrying box before transferring all samples to a local laboratory. In the laboratory samples were instantly centrifuged and the plasma was kept in a separate tube. Both the nurse and staff in the laboratory documented child's unique ID, amount of blood and plasma drawn and the date. All centrifuged samples were transferred to Microbiology laboratory of Punjab University, refrigerated and store there. A total of 116 blood samples were drawn, of which 90 were able to be tested. The remaining samples were not sufficient for processing. The samples list was provided to the lab technologist without revealing the study codes.
At the end of follow up Homerton lab participates in “Vitamin D External Quality Assessment Scheme (DEQAS)”. All Serum samples were centrifuged, before transfer to the laboratory at “Homerton University Hospital NHS Foundation Trust London UK” for measurement of 25-hydroxyvitamin D by Automated “Solid-Phase Extraction and LC/MS/MS”.

### 3.23 Laboratory Methods

Serum 25-hydroxyvitamin D concentrations were calculated by “liquid chromatography tandem mass spectrometry in the Department of Clinical Biochemistry at the Homerton University Hospital NHS Foundation Trust, London, UK, which participates in the Vitamin D External Quality Assessment Scheme (www.deqas.org/”). Serum concentrations of albumin, prealbumin and calcium were measured in the same laboratory using an Architect ci8200 analyser (Abbott Diagnostics). “Corrected calcium was calculated using the formula corrected Ca \[\text{mmol/L}\] = measured calcium \[\text{mmol/L}\] + 0.020*(40 - albumin \[\text{g/L}\])”.

#### 3.23.1 Quantitation of Vitamin D

High-pressure “liquid chromatography tandem mass spectrometry (LC-MS/MS) was applied for the testing of total serum 25[OH] D concentration”. The LC-MS/MS assays of assessment has been authenticated in contrast to other commercially available techniques and is considered as the most suitable and reliable method for analyzing the of “Vitamin D metabolites” (Snellman et al., 2010).
3.23.2 LC-MS/MS Principle

“LC-MS/MS combines the resolving capability of liquid chromatography with the mass analysis ability of mass spectrometry. Thus, the process can be normally categorized into the separation step (step 1), ionization step (step 2) and the mass analysis section (step 3). In step 1 the sample (i.e. vitamins purified from serum) is forced at high pressure by a liquid (mobile phase) into a column (solid phase) to allow separation. In step 2, the sample is charged via atmospheric pressure chemical ionization (APCI), during which a solvent reagent (to allow detection in the final step) ionizes the analyte. In step 3, the ions are separated according to their mass-to-charge ratio in an analyzer by electromagnetic fields. The ions are detected by a quantitative method and the signal is then processed into mass spectra” (Shah, James, Barker, Petroczi & Naughton, 2011).

3.23.3 Description Solid-Phase Extraction

Solid-Phase Extraction (SPE) “is a sample preparation technique that utilizes the solid particles, chromatographic packing material”, normally enclosed in a “cartridge type device, to chemically split up the different components of a sample”. At all times Samples are mostly in the liquid state (though specialty applications might be run with few samples in the gas phase).

3.24 Medications during study Period for any co-morbidity

Following medicine were allowed during the study period according to CMAM protocol (WHO, 2009) “7-day course of the antibiotics Amoxicillin (60 g·1·kg·1·day−1, three times a day) for children having a mild form of diarrhoea and other infections. A single dose of artesunate–amodiaquine combined therapy to treat children diagnosed with malaria, whereas paracetamol syrup to control fever (temp >37.5°C)”. Children who showed signs
of dehydration were given oral rehydration salt solution, made from ReSoMal (Rehydration solution for Malnourished Children).

3.25 Risk factors for Malnutrition and Development Delay

Children aged 6-59 months whose parents gave consent for them to participate underwent the baseline assessment. A structured socio-demographic and nutritional questionnaire was administered to capture information on participant’s demographic details, parental occupation, education, monthly income and nutritional intake. Data on children sociodemographic status with dietary history, medical history and anthropometry were collected once at the start of study on the child enrolment date with an interview of parents. The well-being status of the families was assessed by household total monthly income from all sources. The range from least poor to poorest was adjusted according to minimum monthly salary of a person in Pakistan, because whole study area belongs to people with low socio-economic status. Age of children (from child birth card if available, otherwise were inquired from the mothers verbally) with parents education, occupation, family structure and persons sharing the same house was assessed at enrolment as a potential risk factors. Mother’s child birth was inquired by questioning about total alive and died children and currently how many children under the age of five years she had.

Child dietary history, exclusive breastfeeding status and if not breastfeeding what type of feeding was given to the child with the frequency, quantity and dilution of milk in case of artificial feeding were asked as influencing factors for malnutrition and development delay. The time of starting complimentary feeding with the recommended quantity, variety and frequency of semisolid diet were asked. Mother knowledge were determined about “infant and young-child feeding practices (IYCF)”. Hygienic ways of the mother were assessed with the habits of hand washing of mother and child before eating food and after using
toilet. Proper utensil washing before cooking and hygienic preparation of food with food storage was also inquired. Food security of the family were assessed by the access to food for their dietary needs. Baseline nutritional parameters of the child was evaluated by measuring “weight, height and MUAC” of children at recruitment.

A detailed medical history of the child, with access to health care were also questioned in this study. Child frequent visits to hospital or any health provider for diarrhea and respiratory infections with any other illness was assessed by checking from medical records of the child. Child immunization status were also evaluated from vaccination card with physical presence of BCG scar. Child detailed history of intestinal parasites, scabies with other skin infections, measles, history of pica (ingestion of non-nutritive substances) and history of TB contact with any smear positive TB adult family member treated for tuberculosis at home were taken. Child briefed physical examination were done for any sign of malnutrition, skin and hair changes (flag sign in hairs, skin pigmentation), checking for pallor (pallor in palms of hand, conjunctiva of eyes) and presence of bilateral pitting edema. Any other child less than 5 year treated for malnutrition or have malnutrition in the family at the same time were inquired as a potential risk factor. LHW (lady health workers) visit at home after every three months, a part of primary health care in Pakistan were also questioned from families for assessment of health education of mothers from them. Families were also probed about their knowledge of vitamin D or sunlight importance with awareness of vitamin D enriched food and exposure of their houses to sunlight. Proforma attach in appendix.
3.26 Procedure for data entry and processing

On daily basis all forms used for collecting data were manually verified and mistakes were corrected by the study staff. Some forms that were incomplete or needed cross-checking were completed by consulting again with the families. Only the study staff of concerned centre who filled the form initially was permitted for correction of data in particular form. If the relevant study staff was not available, the principal investigator was allowed to rectify the data errors if applicable. All data were verified again at the time of data entry. Data were first cleaned and inserted in "excel spread sheet" and after that transferred to SPSS for further processing. All forms were entered and checked twice by the principal investigator to avoid any error in data entry. If any error were identified by the software, the investigator again rechecks the form to find the problem and correct it. To prevent any data loss, the database was backed daily. Statistical analyses were conducted by original assigned group using “Statistical Package for the Social Sciences (SPSS) version 23”. Stata/IC version 12.1 (StataCorp, Texas, USA). Z-scores for anthropometric outcomes were calculated using WHO Anthro v3.2.2. The primary outcome was analyzed by calculation of a risk ratio with 95% CI comparing the proportion of children in each arm gaining ≥15% in weight at 8-week follow-up vs. baseline. The effect of allocation on continuous outcomes that were assessed both at baseline and at the end of the study (e.g. weight and weight-for-height z-score at 8 weeks) was analyzed using linear regression, adjusting for the baseline value. The effect of allocation on categorical outcome variables that were assessed both at baseline and at the end of the study (e.g. developmental status) were analyzed with generalized linear models with a log link and binomial distribution to yield a risk ratio adjusted for the baseline value with 95% CI and P value. Mean values of continuous outcomes measured at 8 weeks but not at baseline (e.g. serum
concentrations of 25(OH) D, calcium, albumin and pre-albumin at 8 weeks) were compared between active vs. placebo groups using unpaired Student's t tests to yield a mean difference between study arms with 95% CI for that difference. Statistical significance was inferred where P <0.05. No sub-group analyses were conducted.

“Qualitative data” was represented in shape of frequency and percentage and “quantitative data” was represented in form of mean ± S.D. For analysis of predictors of development delay, univariate risk ratios for normal development were calculated, along with the chi square value and “fisher exact test”. Logistic regression were applied in multivariable analyses to calculate adjusted odd-ratios from covariates.

For analysis of predictors of malnutrition in univariate analysis for measure of malnutrition as a dependent factor with different individual sociodemographic independent factors Independent T Test / One Way ANOVA were applied. Their “numbers, mean, standard deviation mean difference with (95% CI) and p value” are presented. To analyse the effect of multiple demographic factors on malnutrition in multiple analysis linear regression were used because of continuous dependent variables. Their β with (95% CI) and p value was presented.

3.27 Study Timeline

The study started recruiting children from June 2015 to June 2016. Follow-up ended in November 2016. Data entry, cleaning, processing and analysis was completed in May 2017.
CHAPTER FOUR

4. RESULTS

In this chapter we have explained the three components of the study that were conducted, comprising the findings of these studies and the main results of these three components, such as developmental screening of severe acute malnourished children with predictors for developmental delay, results of randomized trial and results of analysis of predictors for severity of malnutrition, as well as an interpretation of these results.

4.1 Results of Developmental Screening

Table 8: Result of developmental screening by DDST II

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Development 1</td>
<td>69</td>
<td>37.3</td>
</tr>
<tr>
<td>Suspected Development 2</td>
<td>108</td>
<td>58.4</td>
</tr>
<tr>
<td>Untestable 3</td>
<td>8</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>185</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

1 “Normal: No delay and maximum one caution”
2 “Suspect: Two or more caution and/ or one or more Delays”.
3 “Untestable: Refusal scores on one or more items completely to the left of the age line or on more than one item intersected by the age line in the 75%-90% area”. 8 children were excluded from analysis because they remain untestable even after rescreen in 1-2 weeks.
Developmental screening was done by using Denver Developmental screening tool II, out of total 185 subjects, 69 (37.3%) were classified as normal development, 108 (58.4%) were classified as suspected development and 8 (4.3%) were untestable even after rescreen in 1-2 weeks. These 8 children were excluded from further analysis. So the prevalence of developmental delay in malnourished children were 58.4%. <Table 9>.

There are 4 areas of developmental milestones on the basis of which final result are concluded, in developmental subsets in personal social development only 67 (37.9%) were showed normal behaviour, 81 (45.8%) were delayed and 29 (16.4%) were in caution zone. Personal social delay percentage is higher than other three areas of fine motor, language and gross motor <Table 10>.

<table>
<thead>
<tr>
<th>Table 9: Developmental milestones subset results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Categories</td>
</tr>
<tr>
<td>Personal social</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Delay</td>
</tr>
<tr>
<td>Caution</td>
</tr>
<tr>
<td>Fine motor</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Delay</td>
</tr>
<tr>
<td>Caution</td>
</tr>
<tr>
<td>Language</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Delay</td>
</tr>
<tr>
<td>Caution</td>
</tr>
<tr>
<td>Gross Motor</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Delay</td>
</tr>
<tr>
<td>Caution</td>
</tr>
</tbody>
</table>

1: Normal: If child successfully performs the items, on the left of age line is considered normal
2: Delayed: “if child fails or refuses on item on which age line falls completely to the left of the age line, this is because child has fails an item that 90% of children in the standardization passed at an earlier”.
3: Caution: “if child fails or refuses on item on which age line falls on or between the 75th and 90th percentiles”.
4.2 Analysis of predictors of Development Delay

Data were analyzed by using the “Statistical Package for Social Sciences (SPSS version 23)”. Univariate risk ratios for normal development were calculated, along with the chi square value and “fisher exact test”. Logistic regression were applied in multivariable analyses to calculate adjusted odd-ratios from covariates.

4.2.1 Comparison of socio-demographic characteristics

In socio-demographic characteristics among normal and suspected development groups: gender, age, family income per month, mother education, mother occupation, family size, father education and family structure were inquired and analysed in univariate analysis. Among analysed variable gender with the odds-ratio (OR) and 95% confidence intervals {OR = 2.13, 95% CI 1.152 to 3.94, p=0.29}, mother occupation with {OR = 1.86, 95% CI, 0.72 to 4.85 p=0.20} family size with {OR = 2.34, 95% CI, 1.14 to 4.79 p=0.20} and family structure with {OR = 1.21, 95% CI, 0.65 to 2.32 p=0.54} did not indicate any significant correlation with developmental delay of malnourished children. All other remaining socio-demographic factors were significantly linked with development delay of malnourished children with the odd ratio and 95 % CI in age {OR = 4.10, 95% CI, 1.53 to 10.9 p= <0.00} in family income per month with the {OR =1.95, 95% CI 1.02 to 3.71, p= 0.04} in mother education with {OR =2.23, 95% CI 1.14 to 4.36, p= 0.01} and in father education were {OR =1.91, 95% CI1.02 to 3.55, p= 0.04}. All these results are presented in table (11).
Table 10: Comparison of socio-demographic characteristics in normal and suspected developmental group

<table>
<thead>
<tr>
<th>Socio-Demographic Independent Variable</th>
<th>N</th>
<th>Normal development N (%)</th>
<th>Delayed Development N (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
<td>27 (34.6)</td>
<td>51 (65.4)</td>
<td>2.13 (1.152-3.94)</td>
<td>0.29</td>
</tr>
<tr>
<td>Female</td>
<td>99</td>
<td>42 (42.4)</td>
<td>57 (57.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;12</td>
<td>107</td>
<td>36 (34.3)</td>
<td>69 (65.7)</td>
<td>4.10 (1.53-10.98)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-24</td>
<td>43</td>
<td>16 (36.4)</td>
<td>28 (63.6)</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>25-40</td>
<td>27</td>
<td>15 (68.20)</td>
<td>7 (31.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income/Month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;15000</td>
<td>121</td>
<td>41 (33.9)</td>
<td>80 (66.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15000-35000</td>
<td>56</td>
<td>28 (50.0)</td>
<td>28 (50.0)</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Mother Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>128</td>
<td>43 (33.6)</td>
<td>85 (66.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary &amp; Above</td>
<td>49</td>
<td>26 (53.1)</td>
<td>23 (46.9)</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Mother Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working lady</td>
<td>19</td>
<td>10 (52.6)</td>
<td>9 (47.4)</td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Housewife</td>
<td>158</td>
<td>59 (37.3)</td>
<td>99 (62.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 8</td>
<td>40</td>
<td>22 (55.0)</td>
<td>18 (45.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 and above</td>
<td>13</td>
<td>47 (34.3)</td>
<td>90 (65.7)</td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Father Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Education</td>
<td>109</td>
<td>36 (33.0)</td>
<td>73 (67.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary &amp; Above</td>
<td>68</td>
<td>33 (48.5)</td>
<td>35 (51.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family structure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint</td>
<td>72</td>
<td>30 (41.7)</td>
<td>42 (58.3)</td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Nuclear</td>
<td>105</td>
<td>39 (37.1)</td>
<td>66 (62.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“Chi square test and fisher exact test, OR is odd ratio with 95% confidence interval, calculated by logistic regression”.

N is number of subjects.

Income per month includes income of family in Pakistani rupees, conversion rate 10800 PKR=100$. 
4.2.2 Comparison of Medical history and Behavioral practices

In comparison of child medical history and behavioural practices between suspected and normal groups, in univariate analysis complimentary feeding practices, hygienic practices, history of TB contact, history of parasites in intestine, history of measles, history of scabies, morbidity incidence in terms of frequent hospital visits, feeding practices, immunization, history of pica and pallor were assessed and included.

Table 11: Comparison of Medical history and Behavioral practices in normal and suspected developmental group

<table>
<thead>
<tr>
<th>Variables for Medical history and Behavioural practices</th>
<th>N</th>
<th>Normal development N (%)</th>
<th>Delayed Development N (%)</th>
<th>Univariate Analysis OR (95%CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complementary feeding Practices¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>136</td>
<td>18 (43.9)</td>
<td>23 (56.1)</td>
<td>1.30 (0.64-2.65)</td>
<td>0.46</td>
</tr>
<tr>
<td>Good</td>
<td>41</td>
<td>51 (37.5)</td>
<td>85 (62.5)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Hygienic Practices²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>157</td>
<td>8 (40.0)</td>
<td>12 (60.0)</td>
<td>1.05 (0.41-2.71)</td>
<td>0.92</td>
</tr>
<tr>
<td>Good</td>
<td>20</td>
<td>61 (38.9)</td>
<td>96 (61.1)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>History of TB contact³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77</td>
<td>22 (28.6)</td>
<td>55 (71.4)</td>
<td>2.22 (1.18-4.17)</td>
<td>0.01</td>
</tr>
<tr>
<td>No</td>
<td>100</td>
<td>47 (47.0)</td>
<td>53 (53.0)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>History of Parasites in Intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>41</td>
<td>12 (29.3)</td>
<td>29 (70.7)</td>
<td>1.74 (0.82-3.71)</td>
<td>0.14</td>
</tr>
<tr>
<td>No</td>
<td>136</td>
<td>57 (41.9)</td>
<td>79 (58.1)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>History of Measles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>9 (34.6)</td>
<td>17 (65.4)</td>
<td>1.24 (0.52-2.98)</td>
<td>0.62</td>
</tr>
<tr>
<td>No</td>
<td>151</td>
<td>60 (39.7)</td>
<td>91 (60.3)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>History of scabies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>13 (50.0)</td>
<td>13(50.0)</td>
<td>Reference</td>
<td>0.22</td>
</tr>
<tr>
<td>No</td>
<td>151</td>
<td>56 (37.1)</td>
<td>95 (62.9)</td>
<td>1.70 (0.73-3.91)</td>
<td></td>
</tr>
<tr>
<td>Hospital visits⁴</td>
<td>1-7</td>
<td>95 (46.3)</td>
<td>51 (53.7)</td>
<td>Reference</td>
<td>0.03</td>
</tr>
</tbody>
</table>
### Table

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>N</th>
<th>Feeding</th>
<th>Vaccination</th>
<th>Pallor</th>
<th>History of pica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>8-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>Mixed</td>
<td>143</td>
<td>49(34.3)</td>
<td>94(65.7)</td>
<td>54(35.1)</td>
<td>22(37.9)</td>
</tr>
<tr>
<td></td>
<td>Exclusive</td>
<td>34</td>
<td>20(58.8)</td>
<td>14(41.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td>Incomplete</td>
<td>45</td>
<td>14(31.1)</td>
<td>31(68.9)</td>
<td>36(62.1)</td>
<td>58(34.3)</td>
</tr>
<tr>
<td></td>
<td>Done</td>
<td>132</td>
<td>55(41.7)</td>
<td>77(58.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pallor</td>
<td>Yes</td>
<td>76</td>
<td>31(40.8)</td>
<td>45(59.2)</td>
<td>22(37.9)</td>
<td>58(34.3)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>101</td>
<td>38(37.6)</td>
<td>63(62.4)</td>
<td>47(39.5)</td>
<td></td>
</tr>
<tr>
<td>History of pica</td>
<td>Yes</td>
<td>58</td>
<td>22(37.9)</td>
<td>36(62.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>119</td>
<td>47(39.5)</td>
<td>72(60.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1: Complementary Practices include quantity, variety, and frequency of complimentary food according to WHO recommendations in different ages.

2: Hygienic practices mean frequent hand washing after using toilet or before eating food and covering of food with utensils washing.

3: History of Tb contact means if child had contact with any smear positive or treated for tuberculosis family member.

4: Hospital visits in last 6 months because of Diarrhoea, Respiratory tract infection and other illness from their hospital records and prescriptions.

5: Exclusive breast feeding only mother milk up to the age of 6 months.

6: Vaccination status from their vaccination card, in process means child is less than 9 months only measles vaccines left according to age all other completed. Incomplete means child did not complete vaccination according to age, not done mean no vaccination at all.

7: Children were assessed for the presence of palmar pallor for looking "at the skin of the child’s palm and compare the colour of the child’s palm with your own palm and with the palms of other children”

8: History of pica is an eating disorder “defined as the persistent ingestion of non-nutritive substances for at least 1 month at an age for which this behaviour is developmentally inappropriate”.

Among the above variables significant variables with the odd ratio OR and 95% CI were history of TB contact with \{OR =2.22, 95% CI 1.18 to 4.17, p= 0.01\} frequent hospital visits with \{OR =1.96, 95% CI 1.05 to 3.65, p= 0.03\} and feeding practices with \{OR =2.74, 95%
All others variables did not show any significance on univariate analysis as weaning practices with the {OR =1.30, 95% CI 0.64 to 2.65, p= 0.46} hygienic practices {OR =1.05, 95% CI 0.41 to 2.71, p= 0.92} history of parasites in intestine {OR =1.74, 95% CI 0.82-3.71, p= 0.14} history of measles {OR =1.24, 95% CI 0.52 to 2.98 , p= 0.62} history of scabies {OR =1.70, 95% CI0.73 to3.91 , p= 0.22} vaccination of child with {OR =1.58, 95% CI 0.77 to 3.24 , p= 0.21} pallor with {OR =0.87, 95% CI 0.47 to1.61, p= 0.66} and history of pica with {OR =1.06, 95% CI 0.56 to 2.03, p= 0.84}. All these results are presented in table (12).

### 4.3 Multivariate analysis - Predictors of development delay

In order to assess the association between child delayed development, socio-demographic, behavioural and medical factors logistic regression analyses were done. In logistic regression numerous independent variables make it complex to find meaningful predictive factors, so in our study between these factors models were adjusted for any variables that were significantly associated with p<0.20 with the dependent variable in bivariate analysis (Bursac, Gauss, Williams & Hosmer, 2008). In multivariate analysis after adjusting for odd ratio following factors shows significance. Age was significant in multivariate analysis with the AOR and 95% CI {AOR 3.95, 95%CI 1.40 to 11.14. p= <0.00}.
### Table 12: Multivariate Logistic regression analysis for variables predicting development delays

<table>
<thead>
<tr>
<th>Socio demographic variable</th>
<th>Category</th>
<th>AOR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-24</td>
<td>3.95 (1.40-11.14)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>25-59</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;15000</td>
<td>1.67 (0.76-3.68)</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>15000-3500</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Education mother</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No education</td>
<td>1.76 (0.80-3.86)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Primary and above</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Occupation mother</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Working lady</td>
<td>2.06 (0.69-6.17)</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Housewife</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Family size</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Less than 8</td>
<td>2.56 (1.10-5.94)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>8 and above</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of TB contact</strong></td>
<td>Yes</td>
<td>2.25 (1.08-4.65)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of parasites</strong></td>
<td>Yes</td>
<td>0.22 (1.75-0.70)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Illness</strong></td>
<td>1-7</td>
<td>1.58 (0.77-3.25)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>8-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Feeding</strong></td>
<td>Mixed feeding</td>
<td>3.14 (1.27-7.75)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Exclusive feeding</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For calculating AOR, the age was categorised into two groups age between 6-24 (n=151) months and 25-59 (n=26) months.

Dependent variable: Denver Developmental Screening test score.

Any sociodemographic variable that were significantly associated p<0.02 with the dependent variable were entered in the model.
Family size was insignificant in univariate analysis but became significant in multivariate analysis after adjusting for others potential covariate with \( \text{AOR} 2.56, 95\% \text{CI} 1.10 \text{ to } 5.94 \text{ p= 0.02} \). History of TB contact as in univariate analysis were still significant in multivariate analysis with \( \text{AOR} 2.25, 95\% \text{CI} 1.08 \text{ to } 4.65 \text{ p= 0.02} \) and exclusive breastfeeding with \( \text{AOR} 3.14, 95\% \text{CI} 1.27 \text{ to } 7.75 \text{ p= 0.01} \). All others variables in multivariate model were insignificant. The "odds ratios (OR) and 95% confidence intervals" (C.I.) of developmental delays are presented in (Table 13).

4.4 Results of Randomized control trial

4.4.1 Trial profile

After community mobilization by CMAM staff in 4 selected study areas, total 252 children were assessed, at the CMAM centre after referral by lady health workers in pursuit of initial assessment of child at their homes, Out of 252 total, 58 were not included, either because they were ineligible (11 having severe acute malnutrition with complications, 31 moderate acute malnutrition, 3 below 6-month age and 7above 5-year age) or did not wish to participate, leaving 194 children who were randomized to either vitamin D (n=97) or placebo (n=97). Those who declined to participate (6 families), did not want to go to study hospital and some of them refused to participate without any reason. One child in the vitamin D group died (before taking the first vitamin D dose) because of diarrhoea and dehydration during the two month’s follow-up, but there were no other reported adverse events in either group that were life-threatening, that resulted in admission in hospital or prolong stay of already admitted children in hospital or that caused a persistent or major inability or considerable disorder that outcomes as incapacity to normal life function. The number of children lost to follow up and defaulters after initial two weeks of treatment was small and similar in the vitamin D (3 loss to follow-up and one death) and placebo groups.
(5 loss to follow up and no death) shown in (Figure 1). This lost to follow up occurs because of flood in the study area during study period and for that reason few families relocated and were not followed up. At the end of study time period in November 2016 in placebo group 92 children completed study per protocol and 93 children completed study per protocol in the vitamin D group. So out of the total, 185 randomized children were entered in the analysis and 9 are excluded because of non-availability of outcome data. The flow diagram for screening and recruitment shows in (Figure 15)

Figure 15: Trial Flowchart
4.4.2 Baseline characteristics of participants

4.4.2.1 Socio-demographic characteristics of study children

Total no of participants in Vitamin D group were (n=93) and in placebo group were (n=92). The mean age for study children in vitamin D group was 15.72 months (SD: 10.84) and for children in placebo group was 14.99 months (SD: 9.62) at recruitment (Table 14). In both vitamin D and placebo groups almost more than half of the children were female (54.8% and 57.6% respectively). Family monthly income below 15000 were (76.3%) in families in vitamin D and (62.0%) in placebo group families. Only (20.4%) children have exclusive breastfeeding (only mother milk up to the age of 6 month) in the vitamin D group and (18.5%) have exclusive breast feeding in placebo group. Rate of exclusive breastfeeding is pretty low in both groups. Weaning practices that include quantity, variety and frequency of complimentary food according to WHO recommendations in different ages, only (25.8%) mothers following these recommended good practices in vitamin D group and (22.8%) mothers in placebo group. Parents and care givers in both vitamin D and placebo group had no knowledge about vitamin D enriched foods and overall importance of vitamin D for health. Only (6.5%) families in vitamin D and (3.3%) in placebo group have knowledge of vitamin D. Sociodemographic characteristics shown in (Table 13).

4.4.2.2 Medical history in study children

Out of 93 children in vitamin D group (14.0%) have history of measles present and out of 92 children in placebo group (16.3%) have presence of measles history. In vitamin D and placebo group history of presence of intestinal parasites was (20.4% and 25.0% respectively). History of TB contact of child with smear positive adult TB patient was found in (35.5%) children in vitamin D group and (52.2%) in children in placebo group. History of
scabies was present in (22.6%) children in vitamin D and (7.6%) in placebo group. Vaccination status of the children were checked from their vaccination card, BCG scar was checked physically on the child arm and vaccination status was pursued according to following categories like, in process means child is less than 9 months only measles vaccine left according to age and all other completed, incomplete means child did not complete vaccination according to age, not done mean no vaccination at all. In vitamin D group vaccination done/in process was tracked in (69.9%) children and in placebo group (80.4%) children (Table 13). Hospital visits in last 6 months because of diarrhoea, respiratory tract infections and other illness from their hospital records and prescriptions were checked and asked from parents and revealed that (38.7%) children had approximately 8-15 times visits to hospital and health care providers in the vitamin D group and (53.3%) in the placebo group.

4.4.2.3 Anthropometric measurements in study children

Nutritional status was measured by baseline mid upper arm circumference (MUAC), “weight for height z-score for acute malnutrition (wasting), height for age z-score for chronic malnutrition (stunting) and weight for age z-score for underweight”. Nutritional status was analysed by their mean and SD in both groups. Mean MUAC in cm in vitamin D group was (10.47) and in the placebo group was (9.91). Mean “weight for height, z-score” in vitamin D group was (-3.76) and in the placebo group was (-4.05), mean “height for age, z-score” in vitamin D group was (-3.94) and in the placebo group was (-3.69) and mean “weight for age, z-score” in the vitamin D and placebo group was (-4.48 and -4.63 respectively) presented in (table13).
4.4.2.4 Baseline Developmental status of study children

Global development proportion delayed (%) in children from baseline assessment in vitamin D group was (57.0%) and for placebo group was (59.8%). In assessment of different areas of milestones like in fine motor milestones proportion delayed was (37.6%) in vitamin D group and (40.2%) in placebo group. In language milestones proportion delayed was assessed in (33.3%) children in vitamin D and (31.5%) in placebo group. Personal social milestones proportion delayed in vitamin D and placebo group was (63.4%) and 64.1% respectively). Gross motor milestones proportion delayed was found in (39.8%) children in vitamin D group and (34.8%) children in placebo group. 8 children were untestable even after rescreen at 1-2 visits in global development but these untestable children were also followed for whole study period for any improvement in the score. The baseline characteristics for socio-demographic status, weight, developmental milestones and medical history were similar between the two groups and presented in (Table 13).
Table 13: Baseline characteristics of children in vitamin D and Placebo group

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D₃ (n=93) N (%)</th>
<th>Placebo (n=92) N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (45.2%)</td>
<td>39 (42.4%)</td>
</tr>
<tr>
<td>Female</td>
<td>51 (54.8%)</td>
<td>53 (57.6%)</td>
</tr>
<tr>
<td><strong>Income/month</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15,000 PKR</td>
<td>71 (76.3%)</td>
<td>57 (62.0%)</td>
</tr>
<tr>
<td>≥15,000 PKR</td>
<td>22 (23.7%)</td>
<td>35 (38.0%)</td>
</tr>
<tr>
<td><strong>Mean Age in months (S.D)</strong></td>
<td>15.72 (10.84)</td>
<td>14.99 (9.62)</td>
</tr>
<tr>
<td><strong>Mean MUAC in cm (S.D)</strong></td>
<td>10.47 (0.83)</td>
<td>9.91 (0.99)</td>
</tr>
<tr>
<td><strong>Mean weight for height, Z-Score (S.D)</strong></td>
<td>-3.76 (1.41)</td>
<td>-4.05 (1.28)</td>
</tr>
<tr>
<td><strong>Mean height for age, Z-Score (S.D)</strong></td>
<td>-3.94 (1.73)</td>
<td>-3.69 (1.39)</td>
</tr>
<tr>
<td><strong>Mean weight for age, Z-SCORE (S.D)</strong></td>
<td>-4.48 (1.11)</td>
<td>-4.63 (1.05)</td>
</tr>
<tr>
<td><strong>Global Development Proportion delayed (%)</strong></td>
<td>53/93 (57.0%)</td>
<td>55/92 (59.8%)</td>
</tr>
<tr>
<td><strong>Fine motor milestones Proportion delayed (%)</strong></td>
<td>35/93 (37.6%)</td>
<td>37/92 (40.2%)</td>
</tr>
<tr>
<td><strong>Language milestones Proportion delayed (%)</strong></td>
<td>31/93 (33.3%)</td>
<td>29/92 (31.5%)</td>
</tr>
<tr>
<td><strong>Personal Social Milestones Proportion delayed (%)</strong></td>
<td>59/93 (63.4%)</td>
<td>59/92 (64.1%)</td>
</tr>
<tr>
<td><strong>Gross Motor Milestones Proportion delayed (%)</strong></td>
<td>37/93 (39.8%)</td>
<td>32/92 (34.8%)</td>
</tr>
<tr>
<td><strong>History of measles</strong></td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13/93 (14.0%)</td>
<td>15/92 (16.3%)</td>
</tr>
<tr>
<td>Category</td>
<td>Response</td>
<td>Count 1</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>History of Parasites</td>
<td>Yes</td>
<td>19/93 (20.4%)</td>
</tr>
<tr>
<td>History of TB Contact</td>
<td>Yes</td>
<td>33/93 (35.5%)</td>
</tr>
<tr>
<td>History of Scabies</td>
<td>Yes</td>
<td>21/93 (22.6%)</td>
</tr>
<tr>
<td>History of Weight loss</td>
<td>Yes</td>
<td>64/93 (68.8%)</td>
</tr>
<tr>
<td>Vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Done/In process</td>
<td></td>
<td>65 (69.9%)</td>
</tr>
<tr>
<td>Incomplete/Not done</td>
<td></td>
<td>28 (30.1%)</td>
</tr>
<tr>
<td>Hospital visits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7</td>
<td></td>
<td>57 (61.3%)</td>
</tr>
<tr>
<td>8-15</td>
<td></td>
<td>36 (38.7%)</td>
</tr>
<tr>
<td>Exclusive breastfeeding</td>
<td>Yes</td>
<td>19/93 (20.4%)</td>
</tr>
<tr>
<td>Weaning Practices</td>
<td>Good</td>
<td>24/93 (25.8%)</td>
</tr>
<tr>
<td>Knowledge of vitamin D</td>
<td>Yes</td>
<td>6/93 (6.5%)</td>
</tr>
</tbody>
</table>

1 Income per month includes income of family in Pakistani rupees, conversion rate 10800 PKR=100$.
2 S.D, Standard deviation, n means total no and % percentages.
3 MUAC (Mid upper arm circumference).
4 Vaccination status from their vaccination card, in process means child is less than 9 months only measles vaccines left according to age all other completed. Incomplete means child did not complete vaccination according to age, not done mean no vaccination at all.
5 Hospital visits in last 6 months because of diarrhoea, respiratory tract infections and other illness from their hospital records and prescriptions.
6 Exclusive breastfeeding only mother milk up to the age of 6 months.
7 Weaning practices include quantity, variety and frequency of complimentary food according to WHO recommendations in different ages.
8 Knowledge of vitamin D include knowledge of vitamin D enriched foods and overall importance of Vitamin D.
9 Untestable means “refusal scores on one or more items completely to the left of the age line or on more than one item intersected by the age line in the 75%-90% area by the child”. 8 children were untestable even after rescreen at 1-2 visits in global development but these untestable children were also followed for whole study period for any improvement in the score.
4.5 Statistical analysis for primary and secondary outcome

“Statistical analyses were conducted using STATA/IC version 12.1 (StataCorp, Texas, USA)”. “Z-scores for anthropometric outcomes were calculated using Who Anthro v3.2.2”. The primary outcome was analysed by calculation of a risk ratio with 95% CI comparing the proportion of children in each arm gaining ≥15% in weight at 2-month follow-up vs. baseline. The effect of allocation on continuous outcomes that were assessed both at baseline and at the end of the study (e.g. weight and weight-for-height z-score at 2 months) was assessed using linear regression, adjusting for the baseline value. The effect of allocation on categorical outcome variables that were assessed both at baseline and at the end of the study (e.g. developmental status) were analysed with generalised linear regression with a “log link and binomial distribution” to yield a risk ratio adjusted for the baseline value with 95% CI and P value.

Table 14: Outcome anthropometric measurements in the vitamin D and placebo groups

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Vitamin D (n=93)</th>
<th>Placebo (n=92)</th>
<th>Risk Ratio (95% CI)</th>
<th>Adjusted mean difference (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion with weight gain &gt; 15% at 2 months</td>
<td>84/93 (90.3%)</td>
<td>80/92 (87.0%)</td>
<td>1.04 (0.94 to 1.15)</td>
<td>-</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean weight at 2 months in kg (S.D)</td>
<td>7.50 (1.95)</td>
<td>6.49 (1.58)</td>
<td>-</td>
<td>0.26 (0.11 to 0.41)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean Weight for height/length Z-Score at 2 months (S.D)</td>
<td>0.15 (2.83)</td>
<td>-1.22 (2.00)</td>
<td>-</td>
<td>1.07 (0.49 to 1.65)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Proportion of children > 15 percent weight gain were measured by following protocol of “Community management of acute malnutrition (CMAM) by WHO. Weight for length/height Z-SCORE were calculated by using WHO ANTHRO software”. 
Mean values of continuous outcomes measured at 2 months but not at baseline (e.g. “serum concentrations of 25[OH] D”, calcium, albumin and prealbumin at 2 months) were compared between active vs. placebo groups using unpaired Student’s t test to yield a mean difference with 95% CI for that difference. “Statistical significance was inferred where P <0.05”. No sub-group analyses were conducted. The proportion of participants with weight gain > 15 % of baseline at 2 months was not significantly different between vitamin D3 and placebo group (84/93 (90.3%) versus 80/92 (87.0%) respectively {RR = 1.04, 95% CI 0.94 to 1.15, p=0.47}. However, mean weight at 2 months was higher in the vitamin D group compared to placebo, with a mean difference of 0.26 kg {95% CI (0.11 to 0.41) p=0.001}, after adjusting for baseline weight. Mean Weight for height/length z-Score at 2 months was 1.07{95% CI (0.49 to 1.65) p=<0.001. (Table 14).

Table 15: Outcome measures: risk ratios (RR)

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D (n=93)</th>
<th>Placebo (n=92)</th>
<th>Adjusted Risk Ratio (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global Development</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion delayed¹ at 2 month (%)</td>
<td>19/91 (20.9%)</td>
<td>36/91 (39.6%)</td>
<td>0.49 (0.31 to 0.77)</td>
<td>0.002</td>
</tr>
<tr>
<td>Personal Social milestones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion delayed at 2 months (%)</td>
<td>32/93 (34.4%)</td>
<td>41/92 (44.6%)</td>
<td>0.78 (0.58 to 1.04)</td>
<td>0.093</td>
</tr>
<tr>
<td>Fine motor milestones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion delayed at 2 month (%)</td>
<td>15/93 (16.1%)</td>
<td>28/92 (30.4%)</td>
<td>0.59 (0.38 to 0.91)</td>
<td>0.018</td>
</tr>
<tr>
<td>Language milestones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion delayed at 2 month (%)</td>
<td>12/93 (12.9%)</td>
<td>19/92 (20.7%)</td>
<td>0.57 (0.34 to 0.96)</td>
<td>0.036</td>
</tr>
<tr>
<td>Gross Motor milestones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion delayed at 2 month (%)</td>
<td>6/93 (6.5%)</td>
<td>18/92 (19.6%)</td>
<td>0.29 (0.13 to 0.64)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

¹: Delayed: “if child fails or refuses on item on which age line falls completely to the left of the age line, this is because child has fails an item that 90% of children in the standardization passed at an earlier”.  
2: 3 children were excluded from analysis two from vitamin D group and one from Placebo group in the global development delay because they remain untestable even after rescreen in 1-2 weeks.
Secondary outcome analysis showed that the proportion of children with delay in global development at 2 months was significantly lower in vitamin D3 group compared to placebo: 20.9% vs 39.6%, \( p = 0.002 \) \( \{ \text{RR 0.49; 95\%CI 0.31 to 0.77} \} \) adjusted for the baseline value. Analysis of individual milestones of DDTS II adjusted for baseline showed that children taking vitamin D3 had a lower prevalence of delayed development in fine motor, \( p=0.018 \) \( \{ \text{RR 0.59; 95\%CI 0.38 to 0.91} \} \) language, \( p=0.036 \) \( \{ \text{RR 0.57; 95\%CI 0.34 to 0.96} \} \) and gross motor milestones at 2 months compared to placebo \( p= \text{value 0.002 \{RR 0.29; 95\%CI 0.13 to 0.64\}} \) in (Table 15).

**Table 16: Biochemical outcomes of participants at 2 months**

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D3 (n=45)</th>
<th>Placebo (n= 45)</th>
<th>Mean difference (95% CI)</th>
<th>Risk Ratio (95%CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean serum 25(OH)D concentration, nmol/L(SD)</td>
<td>99.4 (39.7)</td>
<td>46.6 (14.1)</td>
<td>52.7 (40.3 to 65.2)</td>
<td>_</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proportion with serum 25(OH) D ≥ 50nmol/L (%)</td>
<td>45/45 (100.0%)</td>
<td>19/45 (42.2%)</td>
<td>_</td>
<td>2.37 (1.68 to 3.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean serum corrected calcium concentration, mmol/L(SD)</td>
<td>2.30 (0.19)</td>
<td>2.28 (0.26)</td>
<td>0.02 (-0.08 to 0.12)</td>
<td>_</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean serum albumin concentration (g/L; SD)</td>
<td>38.0 (6.38)</td>
<td>38.4 (5.71)</td>
<td>0.40 (-2.27 to 3.06)</td>
<td>_</td>
<td>0.77</td>
</tr>
<tr>
<td>Mean serum prealbumin concentration, g/L (SD)</td>
<td>0.17 (0.04)</td>
<td>0.15 (0.05)</td>
<td>-0.02 (-0.04 to 0.00)</td>
<td>_</td>
<td>0.11</td>
</tr>
</tbody>
</table>

When biochemical variables were compared in the subgroup who provided blood samples at the end of two months follow up, mean 25-hydroxyvitamin D3 levels were significantly higher \( \{ 52.7 \text{ nmol/L 95\%CI 40.3 to 65.2 } p=\text{<0.001} \} \) in the vitamin D group compared to placebo (Figure 2), showing that an adequate dose of vitamin D supplementation was
given, while mean levels of calcium, albumin and prealbumin were similar in both groups (Table 16).

![Serum 25(OH)D concentrations in Vitamin D3 vs. Placebo groups](image)

**Figure 16: Serum 25(OH) D concentrations in intervention vs placebo group at last follow-up**

The scatter plot shows that “Serum 25(OH) D level in nmol/L” was higher in Vitamin D3 group as compared to placebo group with the (p- value < 0.001) at the time of last follow-up. P value derived from unpaired t test.
4.6 Analysis of Risk factors for severity of malnutrition

**CONCEPTUAL FRAME WORK FOR ACUTE&CHRONIC MALNUTRITION**

**Demographic**
- GENDER
  - Male  (81)
  - Female  (104)
- AGE
  - <12  (112)
  - 13-24  (45)
  - >25  (28)

**Medical history**
- Ho of parasite
  - Yes (42)  No (143)
- Ho of TB contact
  - Yes (81)  No (104)
- Ho of measles
  - Yes (28)  No (157)

**Behavioural Practices**
- Mother knowledge of weaning
  - Good (88)  Poor (97)
- Feeding Practices
  - Exclusive (36)  Not exclusive (149)
- Hygienic practices
  - Good (20)  Poor (165)
- Weaning practices
  - Good (45)  Poor (140)

**Socioeconomic**
- Family monthly income
  - <15000  (128)
  - 15000-35000  (57)
- Mother Education
  - No education  (133)
  - Primary & above (52)
- Father Education
  - No education  (112)
  - Primary & above (73)
- Under 5 siblings
  - 2 and low  (144)
  - 3 and high  (41)
- House hold food security
  - Yes (162)  No (23)

**Access to health care**
- Hospital Visits
  - 1-7 (100)  8-15 (85)
- Immunization
  - Done (62)  incomplete (34)
  - In progress (77)  Not done (12)

Figure 17: Factor Analysis Flowchart
4.6.1 Statistical analysis for predictors for severity of malnutrition

The nutritional status was evaluated by the “weight for age, length/height for age and weight for length/height indices, expressed by mean z scores, according to the WHO reference standard”. Length were measured for children less than two years and height for children more than two years. The classification of nutritional status was performed with WHO ANTHRO, version 3.2.2. For univariate analysis for measure of malnutrition as a dependent factor with different individual sociodemographic independent factors Independent T Test / One Way ANOVA were applied. Their “numbers, mean, standard deviation mean difference with (95% CI) and p value” are presented. To analyse the effect of multiple demographic factors on malnutrition in multiple analysis linear regression were used because of continuous dependent variables. Their β with (95% CI) and p value was presented. The result also revealed that “weight-for-age z-score mean -4.55 length for age” mean were -3.82 and weight for height mean were -3.90 respectively.

Figure 18: Scatter plot for age in months
Figure 18 illustrates scatter plot for age in months. In this study 112 (60.5%) age was below 12 months, 45 (24.3%) age was below 13-24 years and 28 (15.1%) were above 25 months and above.

Figure 19 illustrate the gender of child. Among the subjects 81 (43.8%) were males and 104 (56.2%) were females. Males mean age was 15.40 ± 10.74 months and among female mean age was 15.33 ± 9.87 months.

Figure 19: Pie chart for child gender

4.6.2 Analysis of risk factors for weight for length/height Z-score

“Z-score for weight for length/ height” were calculated according to WHO criteria as a measure of severe acute malnutrition. “Weight for length/height” was used as a dependent variable with sociodemographic independent variables in univariate analysis. In univariate analysis family monthly income was have significant association with severe acute
malnutrition with the mean difference of 0.43 \{95\% CI (0.01 to 0.85) p=0.04\}. Weaning practices that include quantity, variety and frequency of complimentary feeding according to WHO recommendation was also show statistical significance with measure of acute malnutrition with the mean difference of 0.51 \{95\% CI (0.05 to 0.96) p=0.03\}. None of others variable shows any statistical significance with “weight for length /height z-score” on univariate analysis. By applying linear regression for multivariate analysis both family monthly income and weaning practices still shows significance but no other factor show any significance. In multivariate analysis family income have $\beta$ - 0.16 with \{95\% CI (-0.89 to -0.04) p=0.03\} p value is slightly increase in multivariate analysis as compared to univariate. Weaning practices on multivariate analysis have $\beta$ -0.21 \{95\% CI (-1.14 to 0.19) p=0.01\} p value also increases in weaning practices in multivariate analysis. Others non-significant factors in both univariate and multivariate analysis were gender, age, history of parasites in Intestine, TB contact history, measles history, immunization status and hospital visits in last 6 months. Their mean difference with (95\% CI) and p value in univariate and $\beta$ with (95\% CI) and p value in multivariate analysis were presented in (Table 17).
Table 17: Determinants of Weight for length/height Z-score

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>N</th>
<th>Mean (S.D)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight for Length/Height z-score</td>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>81</td>
<td>-3.89 (1.41)</td>
<td>0.03 (-0.37 to 0.43)</td>
<td>-0.05 (-0.53 to 0.27)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>104</td>
<td>-3.92 (1.32)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>(Acute malnutrition)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 12</td>
<td>112</td>
<td>-3.85 (1.43)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13-24</td>
<td>45</td>
<td>-4.17 (1.07)</td>
<td>-0.32 (-0.89 to 0.25)</td>
<td>0.29 (-0.845 to 0.138)</td>
</tr>
<tr>
<td></td>
<td>&gt;25</td>
<td>28</td>
<td>-3.71 (1.46)</td>
<td>0.14 (-0.54 to 0.81)</td>
<td>0.01 (-0.54 to 0.64)</td>
</tr>
<tr>
<td></td>
<td>Family monthly income</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>&lt;15000</td>
<td>128</td>
<td>-4.04 (1.36)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>15000-35000</td>
<td>57</td>
<td>-3.61 (1.32)</td>
<td>0.43 (0.01 to 0.85)</td>
<td>-0.16 (-0.89 to 0.04)</td>
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<tr>
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<td>History of Parasites in Intestine</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>42</td>
<td>-3.88 (1.47)</td>
<td>0.03 (-0.44 to 0.50)</td>
<td>-0.07 (-0.70 to 0.26)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>143</td>
<td>-3.91 (1.33)</td>
<td>Reference</td>
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<td>TB contact history</td>
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</tr>
<tr>
<td></td>
<td>Yes</td>
<td>81</td>
<td>-4.09 (1.34)</td>
<td>-0.31 (-0.71 to 0.08)</td>
<td>0.11 (-0.12 to 0.69)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>104</td>
<td>-3.77 (1.36)</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td></td>
<td></td>
<td>28</td>
<td>-4.07 (1.12)</td>
<td>-0.19 (-0.74 to 0.36)</td>
<td>0.04 (-0.43 to 0.69)</td>
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Measles History

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<td></td>
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<td>0.64</td>
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</tr>
<tr>
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<td>No</td>
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</tr>
<tr>
<td></td>
<td>157</td>
<td>-3.88 (1.40)</td>
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Immunization status

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<td></td>
<td>0.23</td>
<td>0.29</td>
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<tr>
<td></td>
<td>Incomplete</td>
<td>-4.12 (1.49)</td>
<td>-0.28(-0.74 to0.18)</td>
<td>0.08 (-0.21 to 0.72)</td>
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</table>

Hospital visits last 6 months

<table>
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<tr>
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</thead>
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<td></td>
<td></td>
<td>0.58</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 – 15</td>
<td>-3.97 (1.46)</td>
<td>-0.12(-0.52 to0.27)</td>
<td>0.08 (-0.16 to 0.62)</td>
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</table>

Weaning practices

<table>
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<tr>
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<th>Poor</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.03</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>-4.03 (1.34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.51 (0.05 to 0.96)</td>
<td>-0.21(-1.14 to 0.19)</td>
<td></td>
</tr>
</tbody>
</table>

UNIVARIATE ANALYSIS METHOD INDEPENDENT T TEST/ ONE WAY ANOVA, MULTIVARIATE BY LINEAR REGRESSION

Income per month includes income of family in Pakistani rupees, conversion rate 10800 PKR=100$

Immunization status from their vaccination card, in process means child is less than 9 months only measles vaccines left according to age all other completed. Incomplete means child did not complete vaccination according to age, not done mean no vaccination at all.

Hospital visits in last 6 months because of diarrhoea, RTI and other illness from their hospital records and prescriptions.

Weaning Practices include quantity, variety and frequency of complimentary food according to WHO recommendations in different ages.

Length were measured for children less than two year of age or less then 85cm and height for children above two years of age or more than 85 cm. All anthropometric measurements were calculated by WHO standard protocol.
Figure 20 illustrate scatter plot for weight for length/height z-score with the mean of -3.91, standard deviation 1.358 and total no of children 185.

4.6.3 Analysis of risk factors - Weight for Age Z-Score

“Z-score for weight for age” were calculated according to WHO criteria as a measure of acute and chronic malnutrition. Weight for age was used as a dependent variable with sociodemographic independent variables in univariate and multivariate analysis. Variables as an independent were gender, age of child, food Security at home, children less than 5 years age in family, history of parasites in Intestine, hygienic practices, exclusive breast feeding, weaning practices and hospital visits in last 6 months. Out of these variables no one has statistical significance on univariate and multivariate analysis with weight for age z-core. Only food security at home shows borderline significance with the mean difference of -0.42 {95%CI (-0.89 to 0.05) p=0.08} in univariate analysis. Weaning practices shows border line significance in both univariate and multivariate analysis by the mean difference
of 0.31\(\{95\% \text{CI} (-0.05 \text{ to} 0.68)\ p=0.09\} \) in multivariate \(\beta -0.14 \{95\% \text{CI} (-0.72 \text{ to} 0.03)\ p=0.07\}. \) “Determinants of Weight for Age Z- Score” (Measure for chronic and acute malnutrition) were presented in (Table 18).

**Table 18: Determinants of Weight for Age Z-Score**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>N</th>
<th>Mean (S.D)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean difference(95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Weight for Age z-score</td>
<td>Male</td>
<td>81</td>
<td>-4.47 (1.18)</td>
<td>0.15(-0.17 to.046)</td>
<td>0.36</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>81</td>
<td>-4.47 (1.18)</td>
<td>0.15(-0.17 to.046)</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>104</td>
<td>-4.62 (0.99)</td>
<td>Reference</td>
<td>0.36</td>
</tr>
<tr>
<td>Age</td>
<td>&lt; 12</td>
<td>112</td>
<td>-4.58 (1.12)</td>
<td>Reference</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>13-24</td>
<td>45</td>
<td>-4.64 (0.98)</td>
<td>Reference</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>&gt;25</td>
<td>28</td>
<td>-4.31 (1.05)</td>
<td>Reference</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>162</td>
<td>-4.50 (1.08)</td>
<td>Reference</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>23</td>
<td></td>
<td>-0.42(-0.89 to 0.05)</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Children less than 5 Years age in family

<table>
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<tr>
<th>Group</th>
<th>n</th>
<th>Value</th>
<th>95% CI</th>
<th>Reference Value</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 and Lower</td>
<td>144</td>
<td>-4.60</td>
<td>(-0.55 to 0.59)</td>
<td>0.26</td>
<td>0.24</td>
</tr>
<tr>
<td>3 and Higher</td>
<td>41</td>
<td>-4.38</td>
<td>(-0.55 to 0.16)</td>
<td>0.17</td>
<td>-0.09 (-0.63 to 0.16)</td>
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</table>

History of parasites in Intestine

<table>
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<tr>
<th>Group</th>
<th>n</th>
<th>Value</th>
<th>95% CI</th>
<th>Reference Value</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>42</td>
<td>-4.69</td>
<td>(-0.55 to 0.19)</td>
<td>0.064 (-0.22 to 0.55)</td>
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</tr>
<tr>
<td>No</td>
<td>143</td>
<td>-4.52</td>
<td>(-0.71 to 0.31)</td>
<td>0.36</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Hygienic practices

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Value</th>
<th>95% CI</th>
<th>Reference Value</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>20</td>
<td>-4.73</td>
<td>(-0.32 to 0.72)</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>165</td>
<td>-4.53</td>
<td></td>
<td>0.44</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Yes | 36 | -4.40 | 0.19 (-0.21 to 0.59) | -0.06 (-0.59 to 0.24) |
<table>
<thead>
<tr>
<th>Exclusive breast feeding</th>
<th>0.35</th>
<th>0.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0 149 -4.59 Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Poor 140 -4.63 Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Good 45 -4.32 Reference</td>
<td>0.09 0.07</td>
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</tr>
<tr>
<td>1-7 100 -4.49 Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>8 – 15 85 -4.63 Reference</td>
<td>0.11 (-0.09 to 0.56)</td>
<td></td>
</tr>
</tbody>
</table>

Univariate Analysis Method Independent t Test/ One Way ANOVA, Multivariate by Linear Regression

Hygienic practices of the mother was assessed by the habits of hand washing of mother and child before eating food and after using toilet. Proper utensil washing before cooking and hygienic preparation of food with food storage.

Exclusive breast feeding only mother milk up to the age of 6 months.

Food security of the family were assessed by the access to food for their dietary needs.
Figure 21: Scatter plot for weight for Age z-score

(Mean = -4.56  Std.Dev = 1.082  N =185)

Figure 21 illustrate scatter plot for weight for age z-score with the mean of -4.56, standard deviation 1.082 and total no 185.

4.6.4 Analysis of risk factors - length/height for age Z- Score

Z-score for length/height for age were calculated according to WHO criteria as a measure of chronic malnutrition (stunting). Length/height for age was used as a dependent variable with sociodemographic independent variables in univariate and multivariate analysis. Independent sociodemographic variables were gender, age, monthly income of family, father education, mother knowledge of complimentary diet, house hold food security, TB contact history, history of parasites in Intestine, feeding practice and immunization status. In univariate analysis mother knowledge of complimentary diet was significant variable
with the mean difference of -0.50 \{95\% CI \(-0.95\) to \(0.05\)\} \(p=0.03\), household food security also show statistical significance in univariate analysis with the mean difference of 0.79 \{95\% CI \(-1.48\) to \(-0.11\)\} \(p=0.02\) and exclusive breast feeding practices was significant with the mean difference of 0.75 \{95\% CI \(0.18\) to \(1.31\)\} \(p=0.01\).

History of TB contact in univariate analysis showed borderline significance with the mean difference of 0.41 \{95\% CI \(-0.53\) to \(0.86\)\} \(p=0.08\). In multivariate analysis by using linear regression family monthly income become significant with the \(\beta\) -0.16 \{95\% CI \(0.26\) to \(1.08\)\} \(p=0.04\) mother knowledge of complimentary diet with the \(\beta\) 0.15 \{95\% CI \(0.25\) to \(0.96\)\} \(p=0.03\) household food security with the \(\beta\) 0.16 \{95\% CI \(0.11\) to \(1.48\)\} \(p=0.02\) and exclusive breast feeding practices with the \(\beta\) -0.22 \{95\% CI \(-1.47\) to \(-0.30\)\} \(p=0.00\) also show statistical significance in multivariate analysis. Father education show borderline significance in multivariate analysis with the \(\beta\) -0.15 \{95\% CI \(-0.99\) to \(0.01\)\} \(p=0.05\). All other independent variables did not show significant association with length/height for age z-score for measure of chronic malnutrition. Analysis of length/height for age z-score with socio-demographic variables was presented in (Table 19).
<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>N</th>
<th>Mean (S.D)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
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<tbody>
<tr>
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<td>Mean difference (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Length/Height for age z-score (Stunting)</td>
<td>Gender</td>
<td>Male</td>
<td>81</td>
<td>-3.93 (1.66)</td>
<td>-0.20 (-0.66 to 0.27)</td>
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<td>Female</td>
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<td>-3.73 (1.50)</td>
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<td>Age</td>
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<td>112</td>
<td>-3.86 (1.55)</td>
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<td>13-24</td>
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<td>-3.64 (1.63)</td>
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<td>Monthly income of family</td>
<td>&lt;15000</td>
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<td>15000-35000</td>
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<td>-3.92 (1.61)</td>
<td>-0.15 (-0.65 to 0.34)</td>
</tr>
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<td>Father Education</td>
<td>No education</td>
<td>112</td>
<td>-3.96 (1.54)</td>
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<td>Primary &amp; Above</td>
<td>73</td>
<td>-3.59 (1.60)</td>
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<td>Mother knowledge of complementary diet</td>
<td>Good</td>
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<td>-4.08 (1.53)</td>
<td>-0.50 (-0.95 to 0.05)</td>
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<td>Poor</td>
<td>97</td>
<td>-3.58 (1.58)</td>
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<td>Reference</td>
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<td>TB contact history</td>
<td>81</td>
<td>-3.59 (1.50)</td>
<td>0.41 (-0.53 to 0.86)</td>
<td>104</td>
<td>-3.99 (1.61)</td>
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<td>History of parasite in intestine</td>
<td>42</td>
<td>-3.93 (1.66)</td>
<td>-0.14 (-0.69 to 0.40)</td>
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<td>Exclusive breast feeding</td>
<td>36</td>
<td>-3.22 (1.69)</td>
<td>0.75 (0.18 to 1.31)</td>
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<td>Immunization status</td>
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<td>Incomplete</td>
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<td>-3.77 (1.74)</td>
<td>0.05 (-.47 to 0.58)</td>
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UNIVARIATE ANALYSIS INDEPENDENT T TEST/ ONE WAY ANOVA, MULTIVARIATE BY LINEARREGRESSION

Length were measured for children less than two year of age or less then 85cm and height for children more than two years of age or more than 85 cm. All anthropometric measurements were calculated by WHO ANTHRO software.
Figure 22: Histogram for Length/height for Age z-score

Figure 22 illustrate histogram for length/height for age z-score with the mean of -3.82, standard deviation of 1.576 and total number of children 185.
CHAPTER FIVE

5. DISCUSSION

In this chapter we have discussed study findings in to three parts, developmental screening of severe acute malnourished children with predictors for developmental delay, discussion on randomized trial, discussion on analysis of predictors for severity of malnutrition in integration with previous literature cited and summarizes the main findings of the different study parts, as well as an explanation of how this study results were incorporated to stipulate an explanation of what worked, how and why intervention should be done in the community-based programmes. Study limitations and strengths are also discussed in this section.

5.1 Developmental screening of severe acute malnourished children

Different brain functions in early childhood are affected by the deficiency of various nutrients, like protein-energy malnutrition is a reason for global deficits (Chattopadhyay & Saumitra, 2016). Protein calorie malnutrition shrinks brain size, causes dendritic arborization and cell maturation (Chertoff, 2015). Iron deficiency in the neonatal period causes myelination, monoamine neurotransmitter synthesis and hippocampal energy metabolism have an effect on motor development, cognition and memory. Vitamin D deficiency is the reason of delayed motor milestones because of it neuromuscular effect. Zinc insufficiency modifies autonomic nervous system control and hippocampal with cerebellar development. For synaptogenesis, membrane function and potentially for myelination essential role is played by long-chain polyunsaturated fatty acids (Chattopadhyay & Saumitra, 2016). The time period of start of diet constraint is significant to understand the damaging effects of low protein calorie diet on brain function (Chertoff,
2015). Thus, early childhood development is affected by deficiency of various nutrients, overall nutritional status, child attributes with family socio demographic characteristics, and environment factors. There are insufficient national indicators on the developmental outcome of severe acute malnourished children in Pakistan. We thus, explored the early childhood wasting in families having low socioeconomic status in rural setting to assess the impact of malnutrition and sociodemographic risk factors on developmental potential of children. This study aimed to stipulate intuition into the early detection and timely intervention for child development by concentrating and analysing risk factors to which children are exposed under 5 years of age and that affect large numbers of young children in developing countries.

5.1.1 Developmental Screening

The one objective of the current study was to examine the impact of malnutrition on developmental milestones of children with their sociodemographic predictors. Thus in our study we found that out of total 185 subjects, 69 (37.3%) classified as normal development, 108 (58.4%) classified as having suspected developmental delays and 8 (4.3%) were untestable even after rescreen in 1-2 weeks. So the prevalence of developmental delay in malnourished children was 58.4%. In present study we have revealed that severe uncomplicated malnutrition was significantly linked with increased risk for developmental problems. As previously, (Grantham, 1999) concluded in his study that severe malnourished children showed significant behavioural abnormalities in the acute stage. They are more apathetic, less active and least interested in the surrounding environment, both in quantity and complexity, in comparison with other children. They show less agony on hospital admission and more petulant when disturbed in contrast with children with other diseases. Walker et al. (2007) too stated that in young children,
underweight and stunting are also linked with apathy, low positive affect, reduce levels of play and increase insecure attachment.

In studies from Jamaica and Bangladesh also revealed that under nutrition also influences the temperament and behaviour of children. Children having less height for age z-score (stunted) were found to be less happy and passionate, more apathetic, less sociable than well-nourished children (Liu & Raine, 2006). Malnutrition effects brain development and specifically, protein calorie malnutrition is the main cause of abnormal development with behavioral effects. More over as an outcome of failure in normal development, social and behavioral debilities occur and it’s also effects the adulthood period (Chertoff, 2015). Sitaresmi, Ismail and Wahab (2016) observed that children having malnutrition in infant age have increased risk for developmental delay. The have also stated that malnourished child has poor attention and poor social relations at school age with their normal fellows. Our study results correlate with these studies results because in milestones assessment, 45.8 % children showed delayed response in personal social behavioural development and 16.4% were in caution zone in personal-social development.

As well as, low weight-for-age (Underweight) and height-for-age (Stunting) were also reported to be associated with developmental delay from studies piloted in India, Ethiopia and Bangladesh assessing that malnutrition is associated with poor development, in both cognitive and motor milestones (Vazir, Naidu & Vidyasagar, 1998; Hamadani et al., 2001; Hamadani, Fuchs, Osendarp, Huda & Grantham, 2002). Furthermore (Hill, 2001) proved in his study that lower z-scores for height, weight and head circumference were linked with higher incidence of delayed motor and language skills. Cheung, Yip and Karlberg (2001) in his longitudinal study conducted in Pakistan concluded that in larger perspective both fetal and early postnatal growth can affect motor development of infants. They have
evaluated age at start of independent walking and age at onset of building a 3-cube tower individually as a markers of gross and fine motor development. They revealed that shortness and thinness at birth with postnatal wasting were inversely linked to the age at onset of development of gross and fine motor milestones. Other longitudinal studies piloted in, Guatemala, Peru and Jamaica also indicated effect of malnutrition in relation with age of walking (gross motor milestones), cognition, intelligence quotient (IQ), school enrolment and failure to school performance (Kuklina, Ramakrishnan, Stein, Barnhart & Martorell, 2004; Berkman, Lescano, Gilman, Lopez & Black, 2002; Martorell, 1992). Stunting in postnatal period was also inversely associated to the age at initiation of a gross motor milestone. Our study results also correlate with this previous study in Pakistan and other studies because in our study, 36.2% children in fine motor milestones and 35% children in gross motor milestones were in delayed/caution zone because of severe wasting and moderate to severe stunting.

International adopted children (IAC) do research on a large extend in association between malnutrition and poor development and found significant association between growth retardation, global development delay with particularly delay in motor and cognitive development, such as attention deficit disorder, reduced school performance, low scores in intelligent quotient (IQ), impaired language development, social skills, reduced memory with learning disabilities and problem solving skills, which probably is a consequence of the malnutrition because of hidden infections and psychosocial deprivation (Johnson et al., 1992; Albers, Johnson, Hostetter, Iverson, & Miller 1997). In our study with others delays, 23 (13.0%) shows delayed language milestones and 29 (16.4%) were in caution zone for language milestones so our results concedes with these findings as malnutrition impact the language development of children.
In one research, malnourished children were examined on Gesell’s development schedule between four to fifty-two weeks of age and determined that children having grades 2 and 3 malnutrition had poor development in all areas including behavior, gross motor, fine motor-adaptive, language and personal social skills (Upadhyay, Agarwal & Agarwal, 1989). Researches on the developmental outcome in children with malnutrition were done in many countries and severe malnutrition were identified as a primary risk factor for developmental delays (Grantham, 1995). Our findings are consistent with other studies because 58.4% children with severe acute malnutrition, shows suspected global developmental delays with individual delays in motor, personal-social skills and language milestones. Malnutrition and developmental challenges are main health problems of childhood, specifically affecting the developing world (Chattopadhyay & Saumitra, 2016).

Developmental delays hamper child’s educational attainment and reproductive health, therefore deteriorating future productivity and more over worsen the situation in already resource scarce countries. Despite the fact that malnutrition is a serious problem in Pakistan, there are insufficient national indictors for the development of severe malnourished children. Early detection of developmental disabilities is essential for the welfare of children and their families in country like Pakistan so, developmental screening for early detection and regular examination are prerequisite for high risk groups such as malnourished children and should be the part of primary and secondary health care.

5.1.2 Predictors of development Delays

Consequences of severe malnutrition are intricately mingled by the drifts of socio-cultural disadvantage in the deprived families. The effect of different nutrients on permanent cognitive damage is also induced by several other factors for example level of deficiency, timing of deficiency, environmental stimulation, wealth less, inadequate health care and
maternal education (Chattopadhyay & Saumitra, 2016). So, compromised development in the early five years of life are commonly linked to various social factors in relation with nutritional status like, exclusive breast feeding, socioeconomic status, large family size and history of morbidity incidence (Bradley & Corwyn, 2002). Various environmental factors supposed to cause delay in child development are linked with the socioeconomic status of families because child from low income families are assumed to be more susceptible to malnutrition and its associated complications. This risk may increase because low socioeconomic status mothers mostly do not have satisfactory adequate prenatal care and are at risk of giving birth to preterm or low birth weight children (Moore, Bocchini & Raphael, 2016).

Child from low income families are more subject to have less access to medical care, malnourished and have lack of immunizations coverage (Herbst & Baird, 1983). Our study results correlate with these findings because our study children belongs to low income families with illiterate mothers or having very low educational status of mothers. In present study age of child, family size and parental education, low family income, hospital visits, exclusive breast feeding and history of TB contact were significantly associated with developmental delay in univariate analysis and these results also supports other studies like in one study in Korea, mother education and presence of mother illness were linked among normal and suspected delay developmental groups (Bang, 2008). Sonnander and Claesson (1999) conducted, development screening on children at 18 months and monitored school performance later and determined that prenatal and postnatal condition and mother education comprised the best analysts of school learning problems. Education of mother is directly related with good ante-natal care, small family size, optimal nutrition and healthcare of the child. Offspring of educated mothers have high levels of cognitive
development (Barros, Matijasevich, Santos & Halpern, 2009). Even high-risk children exhibit better developmental outcome when born to educated mothers (Wang & Huang, 2008). In our study almost all mothers were illiterate or have just a primary education with dependence on a husband as housewife and family size, age of child, exclusive breastfeeding and history of TB contact were significantly associated with developmental delay in multivariate analysis after adjusting for other factors.

In many studies correlation between breastfeeding and development of child has been confirmed because of the nutritive value of mother milk and enhanced emotional bonding between mother and child. Around the globe researches have explained strong association between duration of exclusive breast feeding and progressed developmental outcome in all age groups and at all geographical settings. Duration of exclusive breastfeeding also has a remarkable impact on cognitive development (Rao, Hediger, Levine, Naficy & Vik, 2002). In Republic of Belarus “a cluster-randomized trial of a breastfeeding promotion intervention” also generated significant finding yielding on the long-term health and neuro-developmental outcome (Kramer et al., 2008). In this study on multivariate logistic regression model breast feeding shows significant effect on development so this study results co-relate with other studies.

In present study, history of tuberculosis contact is significant in both univariate and multivariate model not proved by other studies, so further research in this context is needed. International adopted children revealed characteristics of growth retardation and developmental delays as a result of the psychosocial deprivation, under nutrition and illnesses, which probably is a consequence of the malnutrition with infections (Johnson et al., 1992; Albers et al., 1997). As discussed above, that more researches should be done to understand this as TB is infectious diseases and probability is that these children may
be suffering from latent tuberculosis because of low immune status, malnutrition and presence of smear positive contact, so detailed physical examination for tuberculosis with laboratory investigation should be required to diagnose these children further but this is beyond the scope of our study, so studies should be conducted to understand this phenomenon completely.

In multivariate result of our study, it is proven that young age children have more risk of development delay. Rydz et al. (2006) stated that delayed developmental in younger children is an indicator of serious physical or psycho social problems. As development throughout infancy and in toddler period is rapid and cumulative and easily effected by social-demographic factors. Delayed development sometimes also known as “failure to thrive” because of multiple physical and psycho social problems may also signal the presence of serious neglect or maltreatment in this age group (Wet herby et al., 2004). So this age group is most vulnerable for early screening and detection of problem for timely intervention. (Bradley & Corwyn, 2002) describe the relationship between socio-economic status and family size with child development delay as low family economic status and large family size is directly related in term of child malnutrition and development delay as in our study large family size are associated with delay development of children consistent with other studies.

In findings of our study, it is proven that malnutrition with sociodemographic risk factors such as age, large family size, exclusive breast feeding and history of TB contact are associated with child developmental delay. Though children already have nutritional risk factors, it can be intruded by other environmental factors. It is recommended that developmental screening should be used in a primary health care setting for children in
Pakistan, specifically for the susceptible group of children under five year of age to identify developmental problems timelier and provide proper interventions.

The findings from this study reveal that severe malnutrition, age, family size, with exclusive breast feeding and history of TB contact were the most meaningful prognostic factors for child development. These results propose that malnutrition had adverse effect on early child development and developmental screening is crucial for all children, specifically for malnourished children. Replication study with large sample size and prospective studies are required to confirm our findings and to advancement of further interventions.

5.2 Randomized Trial

We have designed this trial to determine if high dose vitamin D supplementation can accelerate the growth and development of malnourished children. Though there are currently no suggested guidelines for this approach. The World Health Organisation (WHO) has identified research priorities to identify adjunctive therapies that may improve response to RUTF, including administration of broad-spectrum antibiotics and high-dose vitamin A. The results of this study give more understanding into the possible causal correlation among between vitamin D levels and growth and development of severe acute malnourished children.

5.2.1 Primary and secondary outcomes

To our knowledge, this is the first randomised controlled trial to investigate the effects of high-dose vitamin D supplementation in children with severe acute malnutrition. Among children with uncomplicated SAM in Pakistan, we have found that administrating two oral doses of 200,000 IU (5 mg) vitamin D₃ in addition to RUTF resulted in clinically significant improvements in mean weight and mean weight-for-height z-score at 2-month follow-up. High-dose vitamin D supplementation also resulted in substantial reductions in the
proportion of children with delayed global developmental status, delayed gross and fine motor development and delayed language development at 2 months.

Vitamin D supplementation has previously been reported to improve weight gain and growth in children: in a randomised controlled trial conducted in 2,079 low birthweight term infants in New Delhi, India, Kumar and colleagues reported that a weekly oral dose of 1,400 IU vitamin D$_3$ improved z-scores for weight, length and arm circumference by 0.11-0.12 points at 6 months (Kumar et al., 2011). Our study extends this finding that, in a clinically distinct population of children with uncomplicated SAM, administration of a much higher dose of vitamin D was well-tolerated and resulted in substantial and clinically meaningful increases in weight (0.26 kg absolute increase in weight, 1.07-point increase in weight-for-height/length z-score). In other clinical contexts, vitamin D has been shown to protect against acute infections and accelerate resolution of inflammation: both infections and increased systemic inflammation associate with adverse outcome in SAM (Attia et al., 2016) and it may be because of these immunomodulatory actions of vitamin D that cause improvement in weight gain that we observed.

In keeping in view, the reports of other trials in children being treated for SAM (Grandham, Schofield & Harris, 1983; Nahar et al., 2009) we have observed longitudinal improvements in developmental status in both arms of the study. However, the improvements that we observed among participants randomised to high-dose vitamin D$_3$ were significantly greater than those seen in the control arm. Improvements in gross motor development that we observed in intervention vs. control arms are likely – at least in part - to reflect recognised benefits of vitamin D supplementation for skeletal muscle function (Hazell et al., 2012). However, our finding of a favourable effect of vitamin D supplementation on language development provides novel and unequivocal evidence of neurodevelopmental
benefits of vitamin D supplementation in children. This finding complements results of studies demonstrating the importance of vitamin D for brain development in rats (Eyles et al., 2009) and lends weight to the emerging paradigm that vitamin D has significant effects on development and functioning of the central nervous system in humans (Wrzosek et al., 2013). Taken together, our positive findings suggest that current vitamin D content of RUTF is not optimal, at least in the population that we studied.

In a follow-up study of DIVIDS 2 (Kumar et al., 2015) found that vitamin D supplementation in infancy reduced body mass index (BMI) at three to six years of age, although no long-term effects from early vitamin D supplementation were seen on body composition. In addition, a few long-term differences in motor development were observed later in follow-up but these were small. In contrast, our study found significant effects of vitamin D supplementation on motor developmental milestones at 2 months, but long term follow up, such as by Kumar et al, is beyond the scope of our present study.

Our study results are also strengthened by the results of (Dhesi et al., 2004) who showed in a double-blind RCT that vitamin D administration enhances neuro-muscular or neuro-protective function in older patients and protect them from falls. We found significant differences in fine and gross motor milestones between vitamin D and placebo group, showing a neuromuscular effect from vitamin D supplementation in SAM children.

A related outcome relevant to our findings is the decreased risk of pneumonia relapse in Afghan children aged 1-36 months residing in areas of high vitamin D deficiency after supplementation with vitamin D$_3$ (Manaseki et al., 2010) showing anti-inflammatory and immune modulatory effect of vitamin D. Vitamin D has vital function in calcium metabolism and bone health (White, 2008). A randomized control trial, in India reported that
supplementation with vitamin D in low birthweight infants improved growth, probably because of vitamin D function in bone health, but did not reduce mortality and severe morbidity (Kumar et al., 2011). Additionally, vitamin D performs an active part in the immune system (Van Etten, Stoffels, Gysemans, Mathieu & Overbergh, 2008) and vitamin D deficiency is linked with enhanced risk of infectious disease (Roth, Shah, Black & Baqui, 2010).

Our findings are potentially important because vitamin D is crucial for calcium and phosphorous homeostasis and has an important role in skeletal mineralization. Inadequacy of vitamin D is a reason for rickets in children and osteomalacia in adults (White, 2008). Previous research has shown that 1, 25-dihydroxyvitamin D3, the active metabolite of vitamin D, is essential for raising and controlling immune responses (Rockett et al., 1998; Cantorna, 2000; Pichler, 2002). Subclinical vitamin D insufficiency has been linked with an increase odds of tuberculosis in adults by alteration of polymorphisms in the vitamin D receptor (Wilkinson et al., 2000). For children, serum levels of vitamin D less than 20 ng/ml are a known index of suboptimal vitamin D level and levels less 12 ng/ml are associated with rickets (Misra et al., 2008).

Low vitamin D levels has also linked with malnutrition. A case-control research conducted in India has previously reported that malnourished children, both with and without rickets, are at increased risk of severe vitamin D deficiency compared to normal children (Raghuramulu & Reddy, 1980). Walli et al. (2017) piloted a cross-sectional research in Tanzania reported that 30.6% of malnourished children aged <5 years had co-existing severe vitamin D deficiency (<50 nmol/l). In Pakistan study of micronutrient deficiency in malnourished children, 36% of malnourished children were found to be suffering from nutritional rickets (Ejaz & Latif, 2010). Increase frequency of rickets (61%) was also
reported in study of malnourished children in western Kenya (Kwena et al., 2003). In contrast, a cross-sectional study of hospitalized children in Uganda did not observe any significant difference in vitamin D levels between well-nourished and malnourished children (Nabeta, Kasolo, Kiggundu, Kiragga & Kiguli, 2015).

A study conducted in Ayub medical college Abbottabad, Pakistan found that rickets is common in Hazara division children, affecting them to different diseases and skeletal deformities, they are lacking in vitamin D even in the presence of abundant sun shine. Lack of awareness to sun, malnutrition and antenatal factors may be the important reasons for the progression of nutritional rickets.

Another study conducted in department of pediatrics, services institute of medical sciences Lahore, Pakistan in June 2008 for analysis of predictors of vitamin D deficiency rickets in children below two years of age concluded that main risk factors for vitamin D deficiency rickets in children are aged six to eleven months, male gender, prematurity, not timely complementary feeding, drinking unfortified animal milk, lack of sunlight exposure and absence of dietary supplementation of vitamin D.

Department of pediatrics post graduate medical institute Lady reading hospital, Peshawar Pakistan, conducted study in March 2004 on vitamin D deficiency to find out of rickets in admitted patients and found that most cases of rickets are nutritional and receptive to treatment of vitamin D supplementation.

The Kharadar general hospital arranged a seminar “on the prevalence of vitamin D deficiency in Pakistan” with collaboration of Aga Khan University Hospital Karachi discussed that” In Pakistan 90% population is suffering from vitamin D deficiency and this deficiency does not only affect the growing bone of children but also contributes to develop
diabetes, hypertension, pneumonia, depression and cancer”. All these studies strengthen our study finding, our positive findings suggest that due to high prevalence of vitamin D deficiency in Pakistani children and among them in most vulnerable SAM children current vitamin D content of RUTF is not optimal, for treatment and additional doses needed to enhance the growth and developmental status of children at least in the population that we studied.

5.2.2 Serum Biochemical levels

A 3 ml blood sample was taken at 2-month follow-up from a sub-set of 116 participants whose parents gave additional consent for blood sampling and out of them sufficient serum for biochemical analyses was available for 90/116 sampled participants. In the sub-set of 90 participants for whom biochemical analyses were performed, mean 2-month serum 25(OH) D levels were significantly high in participants randomized to intervention verses control group. High-dose vitamin D3 was effective in eliminating vitamin D deficiency among those whose vitamin D status was tested: 45/45 (100%) of sampled participants randomized to the intervention arm had serum 25(OH)D concentrations >50 nmol/L, as compared with 19/45 (42%) sampled participants randomized to placebo. Children in intervention group had a high concentration of serum vitamin D3 after supplementation of vitamin D3 contrasted to that in placebo group. This advocates that there was no protocol breach of allocation to particular study groups and also the vitamin D administrations was effectual to raise the serum vitamin D status.

Furthermore, a high serum vitamin D3 status in intervention group recommend the reliability of syringe contents. Despite the relatively high dose of vitamin D administered, no suspected or actual adverse reactions were reported and no hypercalcaemia was observed among a sub-set of 90 participants who gave safety blood samples at 2 months.
But, if there were any chronic toxic outcome of vitamin D3 after the study, we were not able to trace them. In respect to previous researches reveling that 100,000 IU vitamin D could give a good protection against vitamin D3 deficiency without any risk of adverse effects in infants (Zeghoud et al., 1994). Our trial also noticed a required level of vitamin D between intervention groups with no adverse effect. Our positive findings suggest that current vitamin D content of RUTF is not optimal, at least in the population that we studied and additional therapeutic doses needed in this SAM group for sufficient level of vitamin D. This proposes that such dosages might enhance vitamin D status to a sufficient level in SAM children with certain socio-demographic and health background and this should be deemed in future studies.

Moreover, no statistically significant difference in mean serum concentrations of corrected calcium, albumin or pre-albumin were seen for participants randomized to intervention vs. control arms of the trial at 2-month follow-up.

5.2.3 **Vitamin D adverse effects**

Only one serious adverse event occurred during this study: one participant died, because of severe dehydration secondary to acute gastroenteritis. This event occurred after randomisation but before any dose of study medication was administered. No actual or suspected adverse reactions arose during the trial. Even though vitamin D excess is theoretically possible, it can be minimized by utilizing standard guidelines of supplementation for vitamin D (Zeghoud et al., 1994). Previous studies have not reported adverse outcomes from the high dose of vitamin D supplementation similar to that used in our study. A study from low socioeconomic areas in sunny Istanbul found that one single
intramuscular injection of (300,000 IU) was safe and effective for treating nutritional rickets in children aged six to thirty months (Kutluk et al., 2002).

Another study in France found that giving daily supplementation of vitamin D in a recommended dose (500–1000 IU/day), in addition to 120 000 IU for 3 months through milk fortified with vitamin D, did not cause toxicity in infants with baseline 25-hydroxyvitamin D in the normal range (Vervel et al., 1997) even after continuous maternal antenatal vitamin D supplementation during summer (Zeghoud et al., 1997). In other study one high dose for 3 months in a controlled environment, were more effective for compliance than a daily dose and was successful for maintaining the serum vitamin D level in normal range for two to three months in French high-risk infants (Manaseki et al., 2010). Hence, these findings from other studies indicate that the dosing regimen used in our study is likely to be safe in SAM children. Many developing countries like Pakistan do not have the opportunity to test people for vitamin D levels before giving supplementation because of the high cost of the test. In this scenario and because of insufficient published data for safety of higher doses in children, more trials are needed before justifying giving high doses of vitamin D to young SAM children.

5.2.4 Experiences of health care providers

During the course of study, we did discussions with nutrition experts and pediatricians from large tertiary care hospitals treating malnourished children and others nutritional disorders to sightsee their mindsets about adverse outcome of vitamin D after supplementation of the dose we have administer in our study children. They have shared their views from their clinical experience, that they have never faced a case of vitamin D over dosage even after 3 consecutive doses of 600,000 IU vitamin D in their patients especially in treatments of rickets. In discussion with CMAM stabilization center staff in
tertiary care hospital it was found that they give vitamin D mega dose on admission to all patients with complicated SAM with therapeutic milk based on their clinical experience and due to the fact that there is severe deficiency of vitamin D in common peoples in developing countries as well as in Pakistan according to different local studies and national nutritional survey 2011.

Furthermore, according to their experiences most children with SAM have malabsorption due to a variety of reasons (zinc deficiency, recurrent diarrheas, TB etc.) and immune system is compromised because of reductive adaptation. So RDA is not sufficient to meet the demands and they required therapeutic doses. Due to poor economic status and lack of facilities, they are not in position to document vitamin D levels of each child before and after treatment. So there is no documented evidence in support of this practice but clinical improvement is highly favorable. They advocated that they have never confronted any vitamin D adverse effect after giving therapeutic dose of vitamin D with therapeutic milk which contain 3 times RDA dose of vitamin D on daily basis as RUTF. They have proposed that some children may develop in long term some chronic conditions, such as kidney diseases, which might require added investigation after the completion of study. They believed that, it would be a good notion to compare between the study and control group, level of vitamin D in serum tests incorporated in this study at the end of follow up to find out if there might be any correlation of over dosage with high therapeutic dose.

5.2.5 Strength and limitation of trial

Our study has several strengths. Developmental testing was conducted by well-trained clinical staff using established protocols (Frankenburg et al., 1992) in order to minimise missing data, they repeated developmental assessments up to two times in children whose status was initially assessed as being ‘untestable’. Administration of study
medication was directly observed, allowing for 100% adherence, and the intervention regimen was effective in elevating 25(OH) D concentrations >50 nmol/L in all participants assigned to take it. Rate of loss to follow-up were low and serum 25(OH) D concentrations were measured using gold standard methodology in a laboratory participating in a reputable external quality assessment scheme. Our study also has some limitations. The proportion of participants gaining >15% of their baseline weight at 2 months was higher than anticipated, making it difficult to demonstrate an additional benefit of the intervention on the primary outcome.

The study duration was relatively short and this precluded an assessment of whether the striking early benefits on anthropometric and developmental outcomes that we demonstrated could be sustained and translated into long-term benefits on growth and neuropsychiatric function. It would have been interesting to conduct exploratory analyses to determine whether effects of vitamin D supplementation varied according to baseline serum 25(OH) D concentrations or developmental status. However, vitamin D status was not measured at baseline and the study was powered to allow for detection of biologically plausible interaction effects in sub-group analyses. Recognition of these limitations suggests potential directions for future research: larger trials with longer follow-up are needed, both in this population (to explore long-term outcomes and potential sub-group effects) and in others (to determine whether favourable effects of high-dose vitamin D are demonstrable in populations where vitamin D deficiency is less prevalent).

5.2.6 Generalizability of the findings

Recognition of these limitations suggests potential directions for future research: larger trials with longer follow-up are needed, both in this population (to explore long-term outcomes and potential sub-group effects) and in others (to determine whether favourable
effects of high-dose vitamin D are demonstrable in populations where vitamin D deficiency is less prevalent). Results from this study could be inferred on people with same background features residing in similar settings, but due to possibility of genetic variations, these results might not be generalizable to children in different settings, specifically to settings where the incidence of malnutrition and risk of vitamin D deficiency is low. Due to deficit research in Pakistan, it is tough to recognize likenesses and differences of socio-economic status between different parts within the country. In conclusion, it is misappropriate to generalize results of this study done in few low socio-economic rural areas of southern Punjab to the whole population in country.

Pakistan is a poor country with lack of quality food, access to health care, illiteracy and proper living conditions, but results from this study showed that population in our study areas were extremely poor and a large proportion of study children were severely wasted with high rate of severe stunting, revealed they are also suffering from acute on chronic malnutrition. For that reason, they possibly will not be a good representative of children of same age group residing in other areas of the country, where socio-economic conditions with access to health facilities are much better like in urban areas of country. We observed that vitamin D supplementation with RUTF enhanced serum vitamin D in intervention group in comparison with control arm (i.e. after taking up to 2,400 IU vitamin D per day via RUTF) indicates that baseline vitamin D status is likely to have been very low in this cohort. Thus biologically, it may give same findings if we conduct such trials in other areas of the country in severely malnourished children with the hypothesis that Pakistani malnourished children are sharing same genetic features, however it may not be appropriate for children in neighboring countries where their immune system may respond differently in reaction to vitamin D administration.
In spite of all these realities vitamin D enhance growth and developmental status in malnourished child; vitamin D supplementation could be a low-cost and effective intervention for improvement of vitamin D status in poor settings like Pakistan, predominantly due to the fact that such countries have poor access to quality food, such as fortified milk and calcium enriched food sources and normal level of serum vitamin D is a crucial for proper intestinal absorption of calcium. Children in poor countries could benefit if they are provided with oral vitamin D supplementation because we have reveled in our study that administration of high-dose vitamin D in addition to RUTF, safely and significantly enhanced weight gain and developmental status of children with uncomplicated SAM living in Pakistan. Larger trials with longer follow-up in different settings are needed to explore these promising findings.

This trial showed that 200,000 IU supplementation of vitamin D3, given in bolus doses, improved weight gains and developmental status at 2 months among children with SAM in Pakistan. These results advocate that vitamin D administration can improve rehabilitation from malnutrition when targeted at children with high risk of vitamin D deficiency. However, more studies needed to determine whether effects can be replicated. The outcome of vitamin D supplementation for children in other diseases should be researched to evaluate the full potential of this intervention to enhance children health at a global level.

5.3 Analysis of predictors for severity of malnutrition

Malnutrition is anticipated to cause around more than one third of all child deaths globally (Black et al., 2013) and among them acute child malnutrition in children under the age of five years is the major public-health concern in the developing territories (Black et al., 2008). Because of their inherent frailty, susceptibility and dependency, the nutritional
status of children specifically under the age of 5 years, is a complex indicator of country health condition (WHO, 2011). Thus, growth assessment is commonly utilized not only for the evaluation of the personage nutritional-status, health and development, on the other hand also for the assessment of nutritional-status and health of inhabitants (Srivastava, Mahmoud, Srivastava, Shrotriya & Kumar, 2012).

In underdevelop countries, malnutrition in children aged under 5 years be contingent on the interaction of multiple factors like poverty with ill health and decreased energy and protein consumption (Munthali, Jacobs, Sitali, Dambe & Michelo, 2015; Egata, Berhane & Worku, 2014). The severity and degree of malnutrition is directly linked with increase mortality rate in these countries (Mohamed, 2015). Literature from different studies has already recognized that numerous other factors were closely linked with child malnutrition, such as poverty and family income (Van de, Hosseinpoor, Speybroeck, Van Ourti, & Vega, 2008; Vitolo, Gama, Bortolini, Campagnolo & Drachler, 2008; Fotso & Defo, 2005) number of siblings (Black et al., 200; Vitolo et al., 2008) and poor infant and child feeding practices (Jones et al., 2014).

Dereje (2014) stated that severe acute malnutrition (SAM) is very lethal condition in children. It kills children by increasing the case mortality of common childhood infections and consequently it is directly related to an immediate reason of child death. Malnourished children, who are suffering from illness die just because of their malnutrition. Mortality rates in children with severe acute malnutrition are 9 times high in comparison with well-nourished children. To prevent this, it is very important to find its determinants for policy makers to implement prevention strategies. So we make an attempt in our study to find predictors responsible for this problem in study area.
5.3.1 Determinants of Weight for height z–score

In our finding of risk factor for severe acute malnutrition (wasting), family monthly income have significant association with severe acute malnutrition with the mean difference of 0.43 \(95\% \text{ CI (0.01 to 0.85)}\ p=0.04\). So our finding is consistent with others studies from different regions of the world that poverty and family income is directly related with the severity and degree of child malnutrition. All our children’s in study have severe acute malnutrition (wasting) weight for age z-score less than -3 SD belongs to low income families. Our result is strengthening by the literature stated on low income from others regions of world on degree of malnutrition.

Other significant factor for wasting in our study on multivariate analysis were weaning practices have \(\beta-0.21\ \{95\% \text{ CI (-1.14 to 0.19)}\ p=0.01\}\) that include quantity, variety and frequency of feeding by WHO recommended guidelines on infant and young child feeding practices (WHO, 2014). Dereje (2014) discovered that severe acute malnutrition is linked with sub optimal frequency of complementary feeding such as less than or equal to two times per day were on three times increase risk of severe wasting than children who took complimentary feeding 3-5 times in one day. This complimentary feeding practices was also related with lower child weight for height z-scores in Zambia and Zimbabwe (Jones et al., 2014). So our findings are also consistent with the finding of others studies that complimentary feeding practices are associated with severe acute malnutrition (wasting).

(Dereje, 2014; Vitolo et al., 2008; Ayana et al, 2015) reveled in their studies about the association of maternal illiteracy and maternal autonomy in decision making with wasting. Well in our study almost all mothers are illiterate or have very basic education of primary with mostly house wife and head of the family is male partner with autonomy of decision making so these two factors are already present in our study population. Jamro et al.
(2012) in Pakistan also relate mother illiteracy and poverty with severe acute malnutrition in children (Ayana et al, 2015) in their study also find that frequent diarrhea and febrile illness association with wasting, but our study did not find it significant, it may be because of different geographical and genetic reason of study population. Smith, Ruel & Ndiaye (2005) state that many others factors should be investigated for child malnutrition in rural area as compared to urban areas like family health seeking behavior. Our study population belongs to rural areas but in severe acute malnutrition, we did not find any other significant factor in relation to wasting like health seeking behavior and gender in respect of more gender discrimination in low income rural, uneducated family in Pakistan and other under developed countries (Shaikh & Hatcher, 2004; Momsen, 2004).

Hazarika (2000), stated in gender discrimination in children's nutrition and access to health care in Pakistan in respect to revealing parents drives in favor of sons in South Asia that between 0 to 5-year-old children, boys are more valued in the provision of health care though, girls seems to be as nourished as or well-nourished than boys. This finding seems to be consistent with ours, though the number of boys in our study were 81 and no of girls were 104 but we did not find any significant difference between their nutritional profiles. To sum up this study has recognized low family income, sub optimal practices of complementary feeding with others risk factors as significant determinants of severe acute malnutrition in children under the age of 5 years.

5.3.2 Determinants of height for age z – score

Child malnutrition is universally acknowledged important public health concern and its consequences are justifiably recognized in regard to human functioning, health and existence (Grantham et al., 2007; Sudfeld et al., 2015; Adair et al., 2013). In spite of the economic development in recent days in emerging countries, a high frequency of
malnutrition is noticed, specifically stunting, chronic malnutrition (Said, Micklesfield, Pettifor & Norris, 2015). Even in Pakistan this figure of stunting increases from previous years in 2001 from 41.5 % to 44.8% in 2011 (NNI, 2011). The age range of 0 to 5 years recognized as the time of high susceptibility for growth and development in children. Chronic malnutrition (stunting) in this duration, particularly in the 1st two years of life, can cause irreparable damage with significant after-effects for the potential health of the people in upcoming period of life (Onis & Branca, 2016; Wamani, Åström, Peterson, Tumwine & Tylleskär, 2007; Uauy, Kain & Corvalan, 2011). Furthermore, stunting too rises the odds of obesity and non-communicable diseases in future life (Keino, Plasqui, Ettyang & van den Borne, 2014). So it turn out to be indispensable to describe the nutritional-status in this particular age period and detect potential elements happening in this critical period of life, thus in this study we have tried to explain the possible determinants of chronic malnutrition in this geographical area.

Kavosi et al. (2014) reveled in their study in multivariate model in Iran that stunting is associated with family income, child gender, type of settlement, low maternal education. Well these finding are consistent with our study regarding to family income, low maternal education and type of settlement because in our study mothers belongs to rural areas having no education or very basic primary level of education with low income in family. In our multivariate analysis by using linear regression family monthly income become significant with the β -0.16 (95%CI (0.26 to 1.08) p=0.04) mother knowledge of complimentary diet with the β 0.15 (95%CI (0.25 to 0.96) p=0.03) which was directly linked with mother education as educated mothers have adequate knowledge about their children’s health and nutrition.
Another study in a rural community of Osun state, Nigeria, investigated the effect of socio-economic factors on nutritional status of children and found that children of mothers who were not educated more than secondary school level had one and a half to two times the prevalence rate of stunting in contrast with well-educated mothers. House hold food security with the $\beta$ 0.16 {95%CI (0.11 to 1.48) $p=0.02$} was significant linked also with low family income. But gender difference is not significant in our study in comparison with (kavosi et al., 2014) study. Mengistu et al. (2013) in their study indicated that child age, family monthly income was significantly associated with stunting, but in our study we did not find any significant association of age with stunting, this may be because of different area or genetic reason of study population children (Asres & Eidelman, 2011).

Jesmin, Yamamoto, Malik and Haque (2011); Souza, Benício, Castro, Muniz and Cardoso (2012) in their studies present significant association of father education with chronic malnutrition consistent with our study as father’s education can affect the child’s health and nutritional status, for its role in family income and his decision in purchasing family food. Father education show significance in multivariate analysis with the $\beta$ -0.15 {95%CI (-0.99 to 0.01) $p=0.05$}. In our study exclusive breast feeding practices with the $\beta$ -0.22 {95%CI (-1.47 to -0.30) $p=0.00$} show statistical significance in multivariate analysis contrary to finding of these studies by (Asres & Eidelman, 2011) that no association were found by pattern or duration of breastfeeding and with stunting in infants above 6 months of age. Valente et al. (2016) findings are also not consistent with our study, they found exclusive breast feeding association with acute malnutrition not with stunting. History of TB contact in univariate analysis showed borderline significance with the mean difference of 0.41 {95%CI (-0.53 to 0.86) $p= 0.08$}. This finding was not described in others studies according to our knowledge but this may be linked with the presence of infectious
diseases, as these children are at increased risk of active tuberculosis or latent tuberculosis because of low immune status and history of smear positive TB person contact with child malnutrition. But we did not conduct blood and other examination for diagnosis of TB in these children because this is beyond the scope of our present study. Moreover, research in this context is needed to clarify this finding in our study.

Black et al. (2013) revealed that maternal undernutrition during pregnancy is a contributing factor in fatal growth restriction, which enhances the risk of neonatal deaths and, for those who survive the risk of stunting increases up to 2 years and in childhood. But, it was not possible in our study to retract the nutritional status in pregnancy and because of lack of most mother’s antenatal records as this is least developed area of country. Therefore most deliveries occur at home so no hospital data were available for antenatal record and we did not collect this information of mother’s undernutrition in relation to child stunting. In short we have found socioeconomic status, mother’s knowledge about complimentary feeding practices, food security, father education as a significant contributing factor with chronic malnutrition and these findings are consistent with others studies. Few of our finding like TB contact history and exclusive breastfeeding practices are not consistent with other studies, hence further explanation needed in this context to find out the geographical difference. Because of our study limitations, we missed the significant finding of association of mother undernutrition with child malnutrition (stunting) as presented in other studies.

5.3.3 Determinants of Weight for Age Z-Score

The economic growth in South Asian region in the past years have not been sufficiently manifested in improving the figures in child nutrition (Lau et al., 2007). Undernutrition not only constitutes the child more susceptible to morbidity and mortality (Fenske, Burns,
Hothorn & Rehfuess, 2013) but also has been associated to poor educational achievement (Islam, Angeles, Mahbub, Lance & Nazem, 2006) delayed mental development (Moestue & Huttly, 2008) low intellectual and physical capacities in adult hood (Siddiqi, Haque & Goni, 2011). Height for- age and weight-for-age thus assess child growth in relation to suggesting chronic and acute nutritional deficiency.

Frost, Forste and Haas (2005) and Hall et al. (2001) both investigated broadly to recognize the reasons of malnutrition in children, in combination with the UNICEF malnutrition model, isolating them as immediate (at individual level), intermediary (individual and domestic level) and basic (maternal, domestic and local) factors. Nutrient insufficiencies in utero (Fenske et al., 2013) insufficient nutrition after breastfeeding and early life infections were considered as immediate reasons. After these immediate factors were the intermediary and basic factors which contain, but are not constrained to child care practices, food security, family income, maternal literacy, health services and hygienic practices, all of these are surrounded with the larger socio-economic, environment and political sphere (Goudet et al., 2015). In review with these finding in our study, we were not successful to prove these factors as a significant relation with weight for age z-score but only household food security with the mean difference of -0.42 {95%CI (-0.89 to 0.05) p=0.08} in univariate analysis and weaning practices shows borderline significance in both univariate and multivariate analysis by the mean difference of 0.31{95%CI (-0.05 to 0.68) p=0.09} in multivariate β -0.14 {95%CI (-0.72 to 0.03) p=0.07}. All others factors are not proven in our study.

Siddiqi, Haque, and Goni (2011) disclosed that poor fetal growth or malnutrition in the early child years causes irreparable damage triggering shorter adult height as well as
lower weight but as we discussed above this is our limitation of study to retract the nutritional status in pregnancy and because of lack of most mother's antenatal records. Fakir and Khan (2015) presented in their study for analysis of weight for age z-scores the impact of maternal education on child nutritional status is gender specific and children with older siblings have better nutritional status and this is suggestive for both girls and boys. These results are also not consistent with our results. To sum up in weight for age z-score our study did not prove any significant association with factors which are proven in others studies, this may be because of different region or sociodemographic back ground of our study population from others studies population.

In summary, family monthly income, food security, paternal knowledge, mother knowledge and practices about infant and young child feeding like appropriate practices for complimentary feeding and exclusive breast feeding, were important variables for both acute (wasting) chronic malnutrition (stunting) and undernutrition in infancy and childhood period. So policy makers looking for improvement in the nutritional status of children should promote health-seeking practices and knowledge of families in this regard.
CHAPTER SIX

6. SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

6.1.1 Developmental Screening and its Predictors

Malnutrition in the first few years of life can have long-term deleterious effects that may prevent behavioural, motor, cognitive development, educational achievement and reproductive health. There are insufficient national statistics on the developmental outcome of severe acute malnutrition (SAM) among children in Pakistan. We, therefore, explored the effect of severe malnutrition along with socio-demographic risk factors on developmental potential of children.

We screened 194 uncomplicated SAM children having weight for height < -3 standard deviation in rural areas of southern Punjab. The children were screened by cross sectional study using Denver developmental screening tool 2 (DDST2) covering all four major domain of development: personal social, gross motor, fine motor and language. We interpret their overall development by using these scores according to DDST2 protocol and analyse socio-demographic risk factors by using a pretested structured questionnaire.

Out of 185 children, 69 (37.3%) have normal developmental, 108 (58.4%) had suspected delayed development and 8 (4.3%) had untestable profile in overall developmental score. In multivariate logistic regression model, significant variable was age with \{AOR 3.95, 95%CI 1.40 to 11.14. p= <0.00\}, family size \{AOR 2.56, 95%CI 1.10 to 5.94 p= 0.02\} history of TB contact \{AOR 2.25, 95%CI1.08 to 4.65 p= 0.02\} and exclusive breastfeeding \{AOR 3.14, 95%CI 1.27 to 7.75 p= 0.01\} were significant predictors of suspected delay.
The findings from this study reveal that age, exclusive breast-feeding, family size, and history of tuberculosis contact were significant predictors of suspected delay in severe malnourished child development. Furthermore, developmental screening should be vital part of primary health care system, specifically for high risk malnourished children.

6.1.2 Randomized Clinical Trial

Children with severe acute malnutrition (SAM), which is associated with delayed growth and development, often have multiple micronutrient deficiencies, including vitamin D deficiency. Randomized control trials of vitamin D supplementation for growth and development in SAM children are lacking. We therefore investigated whether vitamin D₃ (cholecalciferol) supplementation, in combination with ready-to-use therapeutic food (RUTF), would increase child growth and developmental status during the rehabilitation phase of SAM (SAM). Clinical trials of vitamin D supplementation have not been conducted in this population.

We carried out a randomised, placebo-controlled, trial of vitamin D₃ supplementation in 185 children aged 6-59 months with uncomplicated SAM, in southern Punjab, Pakistan. Children were randomly allocated to receive either two oral doses of 200,000 IU vitamin D₃, or placebo, along with RUTF, at 2 and 4 weeks. Participants and study staff were unaware of treatment assignment. The primary outcome was the proportion with weight gain >15% of baseline and the secondary outcome were mean weight-for-height/length z-score and global developmental status. (Assessed with the Denver Development Screening Tool II) at 2 months, adjusted for baseline. This study is registered with ClinicalTrials.gov, number NCT03170479.
185/194 randomized children completed follow-up and were included in the analysis. Patients were randomly allocated to vitamin D$_3$ (n=93) or placebo (n=92). Vitamin D$_3$ did not influence the proportion of children gaining >15% of baseline weight (relative risk [RR] 1.04, 95% CI 0.94-1.15, p=0.47) but it did increase weight-for-height/length z-score (adjusted mean difference 1.07, 95% CI 0.49-1.65, p<0.001) and reduce the proportion of participants with delayed global development (adjusted RR [aRR] 0.49, 95% CI 0.31-0.77, p=0.002), delayed gross motor development (aRR 0.29, 95% CI 0.13-0.64, p=0.002), delayed fine motor development (aRR 0.59, 95% CI 0.38-0.91, p=0.018) and delayed language development (aRR 0.57, 95% CI 0.34-0.96, p=0.036).

There was no significant difference between the two groups in the primary outcome, however high-dose vitamin D$_3$ supplementation increased mean weight gain and the developmental status of children receiving standard therapy for uncomplicated SAM in Pakistan. Further studies are needed to determine whether positive findings can be replicated' in other settings.

6.1.3 Predictors of severity of malnutrition

Malnutrition is a prime public-health issue all over the developing world. Current global appraisal suggests that more than 150 million children, under the age of 5 years are malnourished, most of these children live in South Asia and Sub-Saharan Africa. According to UNICEF and WHO joint malnutrition estimates for 2016 in Pakistan, 10.5% of children are wasted, 45% are stunted and 31.6% are underweight. If untreated, severe under-nutrition can progress to irreversible effects, with delay in development thereby declining upcoming productivity of these children and worsen the economic burden of country. So it important to find predictors for malnutrition to properly address this problem.
We conducted a cross sectional study in 4 tehsils of southern Punjab and identified 194 acute malnourished children to find the predictors for severity of malnutrition. Structured sociodemographic and nutritional questionnaire were used to collect information. Before entering in study anthropometry were done and nutritional status was evaluated by the weight for age, length/height for age, and weight for length/height indices, expressed by mean z- scores according to the WHO standard reference. Nine, children were excluded from analysis because of incomplete and missing data.

For analysis Independent t Test / One Way ANOVA and linear regression were used. In findings mean, weight-for-age z-score were -4.55, length for age mean were -3.82, and weight for height mean were -3.90 respectively. Results indicate that weight for height is strongly associated with the family income $\beta$ - 0.16 with $95\%$ CI (-0.89 to -0.04) $p=0.03$ and weaning practices $\beta$ -0.21 $95\%$ CI (-1.14 to 0.19) $p=0.01$. In length/height for age z-score the significant factors are family monthly income $\beta$ -0.16 $95\%$CI (0.26 to 1.08)$p=0.04$ mother knowledge of complimentary diet $\beta$ 0.15 $95\%$CI (0.25 to 0.96) $p=0.03$ house hold food security $\beta$ 0.16 $95\%$CI (0.11 to 1.48) $p=0.02$ and exclusive breast feeding practices, $\beta$ -0.22 $95\%$CI (-1.47 to -0.30) $p=0.00$. In weight for age z-score no variable show any significant association with the independent variables.

These results emphasize the importance of women's appropriate knowledge and practices on infant and young child feeding with improved economic status of family in order to prevent global acute and chronic malnutrition in infancy and childhood. Emphasis should be given at national level for creating families awareness in this context.
6.2 Conclusion

We concluded that malnutrition has high impact in causing delayed development in malnourished children and developmental screening with other strategies is crucial for all children, specifically for malnourished children. Therefore nutritional and environmental stimulation interventions need to be created and utilized for facilitating the child nurturing environment. The problem of poor child development will remain exist until extensive effort is made to affix proper integrated strategies. This is evident that timely interventions can help to avert the loss of potential in concerned children and recoveries can happen speedily. In regard of the high cost of delayed child development, economically in terms of justice, individual well-being and the accessibility of effectual interventions, we can no longer defend inactivity in country like Pakistan.

In this regard high dose therapeutic supplementation 200.000 IU vitamin D with RUTF at 2 and 4 weeks after initiation of RUTF treatment shown to be a safe intervention for maintaining a preferred level of serum vitamin D for protection against vitamin D deficiency and in growth and development of SAM children. Additional investigations are desirable in order to establish a better dosage and dosage frequency to produce a desirable vitamin D functions and addition of vitamin D therapeutic doses like vitamin A in CMAM guidelines. In appropriate complimentary feeding practices with lack of exclusive breastfeeding, maternal knowledge and low family income were significant contributing factors in causation of severity of malnutrition.

These findings highlight the significance of women's appropriate knowledge and practices on infant and young child feeding with improved economic status of family for child growth during the initial vulnerable years of life and for prevention of global acute and chronic malnutrition during infancy and childhood period. In this respect policy makers working in
Pakistan on improvement of nutritional outcomes of children along with development should promote health-seeking practices and knowledge of families.

Results from this study could be generalized on a same setting and on a same populace and may not be generalized to settings with different socio-demographic and biological features because of differences in genetic traits among peoples of different countries, it might be worth replicating such a study in other parts of the world to improve child health at global level.

6.3 Recommendations

This present study is consequential because it studies multifaceted nutritional and environmental variables all together, like screening of children for delayed development and explores risk factors for malnutrition along with developmental delays. Also did interventions for child growth combined with development and because this kind of study is still very limited in Pakistan.

6.3.1 Development Screening:

Developmental screening is unique approach in the prevention and improvement of developmental delays. In this context, to detect developmental disorders and to avoid complications, the estimates of risk factors are also crucial. Multifaceted associations among biological and environmental factors are identified effecting the developmental sequences and outcomes. Early detection of developmental disabilities is essential for the welfare of children and their families. It is a vital function of the primary care medical home and an apt liability of all paediatric health care experts to identify this delays as early as possible.
The result of this study propose that malnutrition had adverse effect on early child development and developmental screening is crucial for all children, specifically for malnourished children. So proper strategies should be made with full support of government for early screening. For this all private and government organizations should work in collaboration to combat this problem. Government can integrate screening as a regular part of primary and secondary health care by training doctors, social worker, childcare workers, teachers and paramedics.

By advancing school health care with proper training of teachers for initial development assessment will be very helpful and economical for Pakistan. Different workshops and training sessions with parent and teacher’s interaction should be conducted at regular interval. For high risk children this should be done in hospital as a routine care and even government can incorporate this in to existing health care CMAM services as with growth, development should be the part of their follow up and treatment. Print and electronic media can also be used for awareness of families and society at broad level. In regard of the high cost of delayed child development, both economically and in terms of justice and individual well-being and the accessibility of effectual interventions, we can no longer defend inactivity in countries like Pakistan.

6.3.2 Randomized Trial
In our study, CMAM guidelines were used for the treatment of malnutrition and development outcome. We observed a successful treatment of malnutrition in the OTP component in both intervention and controls groups likened to the previous appraisals. The marked improvement in the nutritional status of children is might be because of quality of services we delivered, such as community mobilization, accurate anthropometry, proper referral to stabilization center uninterrupted supply of ready to use therapeutic food, proper
follow up of children at their homes to reduce default rate, providing treatment for associated illness like diarrhea, using a standard protocol for diagnosis and treatment of the cases and conclusively accessibility to health care in need. The last point is more important that, I have observed while working in these remote area of southern Punjab is interrupted supply of RUTF, shortage of recommended medicine, not proper diagnosis and referral for illness because health staff could not be accessed always except the official time they work in a basic health unit.

So we strongly recommend based on our knowledge we have gained in this study that government of Pakistan should give appropriate attention to above mentioned problems at OTP’s and CMAM centers to increase the ratio of children with successful treatment of malnutrition at national level. Furthermore long term follow up at home after completion of treatment should be the part of CMAM project to prevent and decrease the proportion of children with relapse.

As we find that vitamin D administration was successful for improving growth along with development, so after conducting more trials at government level, vitamin D should be included in the routine CMAM guidelines recommendations as iron, vitamin A and zinc. This should be counted as an essential micronutrient for SAM children after considering the NNS 2011 report of prevalence of vitamin D deficiency in Pakistani population as this is already overlooked micronutrient in Pakistan.

This story should not be ended here we should try more trials with combination of multiple micro and macronutrients along with environmental stimulation for improving the growth parameters and development status of this high risk SAM population.

According to UNICEF Pakistan has the third highest proportion of stunted children globally (9.6 million), with considerable disparity among urban and rural areas (37% vs 46%,
respectively). There is an utmost requirement to search for new approaches to prevent stunting and treat wasting, but we deficit in required knowledge of the utmost tractable pathways for intervention. Our aim should be the better understanding of the pathogenesis of stunting and wasting and identify mechanisms to target them. So with SAM attention should be given to combat the problems of chronic malnutrition known as (stunting) in Pakistan. Vitamin D role with other interventional strategies should be designed for this huge problem to enhance child survival, health and potential by finding new interdisciplinary tactics for stunting and wasting, to effect the sustainable development goals. We need new approaches to management of complicated SAM to reduce mortality and improve long-term outcomes.

6.3.3 Predictors of malnutrition

Caregiver attitudes, behaviors and capabilities during recovery from SAM: The psychosocial environment gravely affects retrieval from SAM. Child stimulation through caregivers in hospital and in rehabilitation phase is recommended by WHO to fasten recovery; but, its adoption is probably reliant on conducive maternal attitudes, behaviors and capabilities.

We should therefore give valuable attention to impart this knowledge among mothers of children recovering from SAM to characterize maternal attitudes, behaviors and capabilities. Maternal capabilities mostly signify the skills and attributes that conclude a mother’s ability to care for a young child, comprising her “mental health; social support; autonomy; workload and time stress; gender norms attitudes; and mothering self-efficacy”. Caregiving habits and attitudes are decisively shaped by caregiver capabilities. Maternal attitudes, behaviors and capabilities that hamper best childcare may need particular interventions to modulate them before an infant stimulation intervention can be effective.
Thus by reinforcing caregiver capabilities and accomplishing by behavior change to provide a package of care for the child could improve long-term outcomes during SAM.

To combat malnutrition in Pakistan an effectual culturally applicable behavioral change communication approach must be executed and maintained. For this to avoid extra financial burden on country we can utilize already existing programmes in Pakistan like, the “National Program for Family Planning and Primary Health Care” working in Pakistan. With the objective to access reproductive health and nutrition services, improving maternal, new-born and child health, providing family planning services (family planning reduces the number of unplanned pregnancies, abortions among women, and allows women the opportunity to choose when the time is right to have a child) for improving healthy pregnancy and healthy fetal outcome.

The other programme are “National Maternal and Newborn Child Health (MNCH) Program and Leady health worker program” (each LHW conducted one health session with married women of reproductive age on daily basis for promotion of family planning and exclusive breast feeding up to six months). “Poverty alleviation programs” also working in country like social safety nets like (Baitul Maal, Zakat programs or the Benazir Income Support programs) have massive potential for reaching those trapped in the spiral of food poverty.

“Integrated management of childhood illness (IMCI)” is also working in country was first introduced in mid 1990s by WHO and UNICEF. Pakistan adapted it in 1998-2000 to contribute to healthy growth and development of children and in order to decrease mortality due to pneumonia, diarrhea, measles, malaria and malnutrition through
immunization, counseling and case management components. In immunization WHO recommends polio, tuberculosis, measles, pertussis, diphtheria, tetanus, vaccines for prevention of childhood mortality and morbidity. Hib and pneumococcal conjugate vaccines are newly introduced in Pakistan for prevention of pneumonia.

Nutrition Program by giving preventive services are being delivered in 36 districts of Punjab through lady health workers (LHWs) which include screening of under 5 children and pregnant and lactating mothers (PLWs), infant and young feeding (IYCF) counselling, provision of iron, vitamin-A and MMS to mother and child. Improvement of child nutritional status by implementing CMAM program in all districts of Pakistan including stabilization center, OTP, supplementary feeding program with community mobilization. By using this already existed programme in Pakistan, we should therefore give valuable attention to impart this knowledge among mothers of children recovering from SAM to characterize maternal attitudes, behaviors and capabilities for prevention of malnutrition. Healthcare providers must be proficient in strategies that support nutrition sufficiency for mothers and children, comprising those that promote healthy lifestyles, nutrition and physical activity.

In country like Pakistan religious leaders, school teachers and social mobilisers can also show an influential role in endorsing exclusive breastfeeding and recommended complementary feeding. Consistent monitoring and liability is important if Pakistan have to break the deadlock for focusing on malnutrition issue. There is a requisite to confirm regular data on nutrition indicators with more distinct regional or district level info. This scenario is apt for change with more importance on nutrition and invention of several
national and provincial nutrition focused policies. Moreover, there is demand for incorporating many different segments and plans to attain the preferred outcomes effectively and efficiently as various determining and influencing factors are outside the health sector. It is very important that policymakers recognize the significance of improvement in child health and nutritional status for national growth and as a vital contributor to accomplishing Pakistan’s sustainable development goals.
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Pichler, J., Gerstmayr, M., Szépfalusi, Z., Urbanek, R., Peterlik, M., & Willheim, M. (2002). 1α, 25 (OH) 2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. Pediatric research, 52(1), 12-18.


# ANNEXURE 1 - ENROLLMENT PROFORMA

## A. Basic Demography

<table>
<thead>
<tr>
<th>Name</th>
<th>Reg. No</th>
<th>Date of Admission</th>
<th>Mother Alive</th>
<th>Sex*</th>
<th>M / F</th>
<th>Mother Alive</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother/Caregiver Name</td>
<td>Mother Alive</td>
<td>Date of Admission</td>
<td>Sex*</td>
<td>M / F</td>
<td>Mother Alive</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Address (Village)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission*</td>
<td>Direct from Community</td>
<td>From SFP</td>
<td>From SC</td>
<td>Readmission (Relapse)</td>
<td>SC Refusal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additional Information:</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Father’s Occupation
- Monthly Income ________________
- No. of Siblings ________________
- Mother’s Education: Nil □ <= 5 □ 5-10 □ Higher □
- Father’s Education: Nil □ <= 5 □ 5-10 □ Higher □

## B. Admission Anthropometry

<table>
<thead>
<tr>
<th>Bilateral Pitting Oedema</th>
<th>++</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUAC (cm)</td>
<td>Weight (kg)</td>
<td>Height/Length (cm)</td>
<td>Weight for Height (WFH)</td>
</tr>
<tr>
<td>Admission Criteria</td>
<td>Oedema</td>
<td>MUAC</td>
<td>WFH</td>
</tr>
</tbody>
</table>
### C. History

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
<th>Stools / Day</th>
<th>1-3</th>
<th>.4-5</th>
<th>&gt;5</th>
<th>Passing Urine</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appetite</td>
<td>Good</td>
<td>Poor</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reported Problems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### D. Physical Examination

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
<th>Chest In drawing</th>
<th>Yes</th>
<th>No</th>
<th>Severe</th>
<th>Palmer Pallor</th>
<th>Severe</th>
<th>Palmer Pallor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration Rate (# min)</td>
<td>&lt;30</td>
<td>30 – 39</td>
<td>40 – 49</td>
<td>50+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
<td>Anaemia</td>
<td></td>
<td>No</td>
<td></td>
<td>Palmer Pallor</td>
<td></td>
<td>Severe Palmer Pallor</td>
</tr>
<tr>
<td>Eyes</td>
<td>Normal</td>
<td>Sunken</td>
<td>Discharge</td>
<td>Dehydration</td>
<td>None</td>
<td>Some</td>
<td>Severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ears</td>
<td>Normal</td>
<td>Discharge</td>
<td>Mouth</td>
<td>Disability</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph Nodes</td>
<td>None</td>
<td>Neck</td>
<td>Maxilla</td>
<td>Groin</td>
<td></td>
<td></td>
<td></td>
<td>Extremities</td>
<td>Normal</td>
</tr>
<tr>
<td>Skin Changes</td>
<td>None</td>
<td>Scabies</td>
<td>Peeling</td>
<td>Ulcers / Abscesses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**ANNEXURE 2 - QUESTIONNAIRE**

**SOCIO-DEMOGRAPHIC AND NUTRITIONAL EVALUATION OF CHILD**

<table>
<thead>
<tr>
<th>Serial #</th>
<th>Name of Child:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Child Age:</th>
<th>Sex:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Address:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Father’s Name:</th>
<th>Father Occupation:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Father Education:**
- NO Education
- Primary
- Middle
- Secondary
- Higher

<table>
<thead>
<tr>
<th>Mother Name:</th>
<th>Mother occupation:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mother Education:**
- NO Education
- Primary
- Middle
- Secondary
- Higher

<table>
<thead>
<tr>
<th>Socio-Economic Status:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest</td>
</tr>
<tr>
<td>Second</td>
</tr>
<tr>
<td>Middle</td>
</tr>
<tr>
<td>Fourth</td>
</tr>
<tr>
<td>Highest</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Living Children:</th>
<th>Child Number in Siblings:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Children less than 5 years of age:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family Size:</th>
<th>Family:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Joint</td>
</tr>
<tr>
<td></td>
<td>Nuclear</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Child Weight (kg):</th>
<th>Length/Height (cm):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Z Sore/WH%:</th>
<th>MUAC:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Initial Assessment of Child**

**History**

<table>
<thead>
<tr>
<th>Intestinal parasites:</th>
<th>Yes No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oedema:</th>
<th>If yes for how long:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes/ No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Skin changes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
</tr>
<tr>
<td>Scabies</td>
</tr>
<tr>
<td>Peeling</td>
</tr>
<tr>
<td>Ulcer</td>
</tr>
<tr>
<td>Abscess</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hair changes:</td>
</tr>
<tr>
<td>Weight loss:</td>
</tr>
<tr>
<td>Clubbing:</td>
</tr>
<tr>
<td>Pallor:</td>
</tr>
<tr>
<td>History of contact:</td>
</tr>
<tr>
<td>TB:</td>
</tr>
<tr>
<td>Personal hygiene:</td>
</tr>
<tr>
<td>Number of repeated illness in 6 months:</td>
</tr>
<tr>
<td>Do Mothers have knowledge of all these dangerous sings:</td>
</tr>
</tbody>
</table>

**Child Feeding Practices & Dietary History**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Do the LHWs visit home regularly:</td>
<td>Yes/No</td>
<td></td>
</tr>
<tr>
<td>If yes how often:</td>
<td>1-3-month 4-6months 9-12months</td>
<td></td>
</tr>
<tr>
<td>Do Mother have knowledge of Exclusive Breast Feeding:</td>
<td>Yes/No</td>
<td></td>
</tr>
<tr>
<td>DO mother know Age appropriate of breast feeding:</td>
<td>Yes/No</td>
<td></td>
</tr>
<tr>
<td>Mother perception about importance of breast feeding:</td>
<td>Good Average Poor</td>
<td></td>
</tr>
<tr>
<td>Mother source of knowledge for IYCF:</td>
<td>Family Friend Media</td>
<td></td>
</tr>
<tr>
<td>Exclusive Breast feeding:</td>
<td>Yes/No</td>
<td></td>
</tr>
<tr>
<td>If No:</td>
<td>Predominated feeding Mixed/Partial feeding artificial feeding</td>
<td></td>
</tr>
<tr>
<td>Type of food with Breast feeding:</td>
<td>Water Gripe water Arq Honey other</td>
<td></td>
</tr>
<tr>
<td>Total duration of breast feeding:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artificial feeding - Fresh Milk Type:</td>
<td>Cow Buffalo Goat</td>
<td></td>
</tr>
<tr>
<td>Formula / Commercial Milk Type:</td>
<td>Tetra pack Powder Milk</td>
<td></td>
</tr>
<tr>
<td>Amount per day:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilution:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Complementary Feeding

Do Mother have knowledge of Timely Complementary Feeding: Yes/No

Does Mother have knowledge of variety and frequency of Complementary Feeding: Good/Average/Poor?

Age of starting of semi solid diet: ______________________________

Type of diet: Home made ______________________________

Commercial ______________________________

Amount and diversity of Diet: ______________________________

Would you describe his appetite: Good Fair Poor?

Immunization: Done Incomplete in progress not done

B.C.G Scar: Present Absent

Social and Cultural aspects of Family

Any special food preferences in family: ______________________________

Food security: Yes No

Food storage system: Yes No

House exposure to sun light Yes No

Knowledge of sun light importance Yes No

Knowledge of vitamin D enriched food Yes No

Knowledge of Vitamin D importance Yes No
## ANNEXURE 3 - TIMETABLE AND OUTCOME MEASURES

<table>
<thead>
<tr>
<th>Measures</th>
<th>Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>✓</td>
</tr>
<tr>
<td>Height</td>
<td>✓</td>
</tr>
<tr>
<td>MUAC</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Development Assessment</strong></td>
<td></td>
</tr>
<tr>
<td>Global development</td>
<td>✓</td>
</tr>
<tr>
<td>Fine motor milestone</td>
<td>✓</td>
</tr>
<tr>
<td>Personal social milestone</td>
<td>✓</td>
</tr>
<tr>
<td>Language milestone</td>
<td>✓</td>
</tr>
<tr>
<td>Gross motor milestone</td>
<td>✓</td>
</tr>
</tbody>
</table>
ANNEXURE 4 - CONSENT FORM
### ANNEXURE 5 - RUTF RATION FOR OTP

<table>
<thead>
<tr>
<th>Weight of child (kg)</th>
<th>Packets per week</th>
<th>Packets per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 - 3.9</td>
<td>11</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>5.5 - 6.9</td>
<td>18</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>8.5 - 9.4</td>
<td>25</td>
<td>3.5</td>
</tr>
<tr>
<td>9.5 - 10.4</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>10.5 - 11.9</td>
<td>32</td>
<td>4.5</td>
</tr>
<tr>
<td>≥ 12</td>
<td>35</td>
<td>5</td>
</tr>
</tbody>
</table>

**RUTF**: 92g packets containing 500 kcal

**Source**: Pakistan National Guidelines for the Community Based Management of Acute Malnutrition 2014
# ANNEXURE 6 - NUTRITIONAL COMPOSITION OF RUTF

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>For 100g</th>
<th>Per Sachet of 92g</th>
<th>Nutrients</th>
<th>For 100g</th>
<th>Per Sachets of 92g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>545keal</td>
<td>500keal</td>
<td>Vitamin A</td>
<td>910ug</td>
<td>840ug</td>
</tr>
<tr>
<td>Protein</td>
<td>13.6g</td>
<td>12.5g</td>
<td>Vitamin D</td>
<td>16ug</td>
<td>15ug</td>
</tr>
<tr>
<td>Lipids</td>
<td>35.7g</td>
<td>32.86g</td>
<td>Vitamin E</td>
<td>20mg</td>
<td>18.4mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>300mg</td>
<td>276mg</td>
<td>Vitamin B1</td>
<td>53mg</td>
<td>49mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>300mg</td>
<td>276mg</td>
<td>Vitamin B2</td>
<td>0.6mg</td>
<td>0.55mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,111mg</td>
<td>1,022mg</td>
<td>Vitamin B6</td>
<td>1.8mg</td>
<td>1.66mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>92mg</td>
<td>84.6mg</td>
<td>Vitamin B12</td>
<td>0.6mg</td>
<td>0.55mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>14mg</td>
<td>12.9mg</td>
<td>Vitamin K</td>
<td>1.8ug</td>
<td>1.7ug</td>
</tr>
<tr>
<td>Copper</td>
<td>1.8mg</td>
<td>1.6mg</td>
<td>Vitamin</td>
<td>21ug</td>
<td>19.3ug</td>
</tr>
<tr>
<td>Iron</td>
<td>11.5mg</td>
<td>10.6mg</td>
<td>Biotin</td>
<td>65ug</td>
<td>60ug</td>
</tr>
<tr>
<td>Iodine</td>
<td>100ug</td>
<td>92ug</td>
<td>Folic acid</td>
<td>210ug</td>
<td>193ug</td>
</tr>
<tr>
<td>Selenium</td>
<td>30ug</td>
<td>27.6ug</td>
<td>Pantothenic acid</td>
<td>3.1mg</td>
<td>2.85mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>&lt;290mg</td>
<td>267mg</td>
<td>Niacin</td>
<td>5.3mg</td>
<td>4.88mg</td>
</tr>
</tbody>
</table>

Source: Brined et al, 1999
## ANNEXURE 7 - ROUTINE MEDICAL PROTOCOL FOR OTP

<table>
<thead>
<tr>
<th>Drug</th>
<th>When</th>
<th>Age / weight</th>
<th>Prescription</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>On admission</td>
<td>2-12 months (4-10kg)</td>
<td>Syrup 125 mg 5ml</td>
<td>3 times/day for 5 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 months-5 years (10-19kg)</td>
<td>Syrup 125 mg 10ml</td>
<td></td>
</tr>
<tr>
<td>Anti-Malarial</td>
<td>On admission (as required)</td>
<td>&gt; 2 months old</td>
<td>See malaria protocol</td>
<td>See malaria protocol</td>
</tr>
<tr>
<td>Mebendazole</td>
<td>Second visit</td>
<td>&lt; 1 year</td>
<td>DO NOT GIVE</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-23 months</td>
<td>250mg</td>
<td>Single dose on second visit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500mg</td>
<td></td>
</tr>
<tr>
<td>Measles Vaccination</td>
<td>On week 4</td>
<td>From 6 months</td>
<td>Standard</td>
<td>Once on week 4</td>
</tr>
<tr>
<td>Iron/Folic Acid</td>
<td>On day 14 for mild/moderate anemia</td>
<td>&gt; 2 months old</td>
<td>See iron/folic acid protocol</td>
<td>Give one dose daily for 14 days</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Only give if signs of vitamin A deficiency or history of measles (do NOT give if edema)</td>
<td>6 months to 1 year</td>
<td>100 000 IU</td>
<td>Single dose on admission (for children with edema - single dose on discharge)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 year</td>
<td>200 000 IU</td>
</tr>
</tbody>
</table>

**Source:** Pakistan National Guidelines for the Community Based Management of Acute Malnutrition 2014
DIRECTIONS FOR ADMINISTRATION

1. Try to get child to smile by smiling, talking or waving. Do not touch him/her.
2. Child must stare at hand several seconds.
3. Parent may help guide toothbrush and put toothpaste on brush.
4. Child does not have to be able to tie shoes or button/pin in the back.
5. Move yarn slowly in an arc from one side to the other, about 8" above child's face.
6. Pass if child grasps rattle when it is touched to the backs or tips of fingers.
7. Pass if child tries to see where yarn went. Yarn should be dropped quickly from sight from tester's hand without arm movement.
8. Child must transfer cube from hand to hand without help of body, mouth, or table.
9. Pass if child picks up raisin with any part of thumb and finger.
10. Line can vary only 30 degrees or less from tester's line.
11. Make a fist with thumb pointing upward and wiggle only the thumb. Pass if child imitates and does not move any fingers other than the thumb.

12. Pass any enclosed form. Fall continuous round motions.
13. Which line is longer? (Not bigger.) Turn paper upside down and repeat. (pass 3 or 3 of 5 or 6)
15. Have child copy first. If failed, demonstrate.

When giving items 12, 14, and 15, do not name the forms. Do not demonstrate 12 and 14.

16. When scoring, each pair (2 arms, 2 legs, etc.) counts as one part.
17. Place one cube in cup and shake gently near child's ear, but out of sight. Repeat for other ear.
18. Point to picture and have child name it. (No credit is given for sounds only.)
   If less than 4 pictures are named correctly, have child point to picture as each is named by tester.

19. Using doll, tell child: Show me the nose, eyes, ears, mouth, hands, feet, tummy, hair. Pass 6 of 8.
22. Ask child: What do you do with a cup? What is a chair used for? What is a pencil used for?
   Action words must be included in answers.
23. Pass if child correctly places and says how many blocks are on paper. (1, 5).
   (Do not help child by pointing, moving head or eyes.)
25. Ask child: What is a ball?...lake?...desk?...house?...banana?...curtain?...fence?...ceiling? Pass if defined in terms
   of use, shape, what it is made of, or general category (such as banana is fruit, not just yellow). Pass 5 of 8, 7 of 8.
26. Ask child: If a horse is big, a mouse is ___. If fire is hot, ice is ___. If the sun shines during the day, the moon shines
   during the ___. Pass 2 of 3.
27. Child may use wall or rail only, not person. May not crawl.
28. Child must throw ball overhand 3 feet to within arm's reach of tester.
29. Child must perform standing broad jump over width of test sheet (8 1/2 inches).
30. Tell child to walk forward_→ heel within 1 inch of toe. Tester may demonstrate.
   Child must walk 4 consecutive steps.
31. In the second year, half of normal children are non-compliant.

OBSERVATIONS:

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Denver, Colorado 80237-5075
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(800) 419-4729

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ANNEXURE 9 - DPCC LETTER

UNIVERSITY OF THE PUNJAB
Quaid-i-Azam Campus, Lahore – Pakistan

Deputy Registrar (Academic)

Dr. Javeria Saleem,
Ph.D. scholar,
Institute of Social & Cultural Studies,
University of the Punjab,
Lahore.

It is a pleasure to inform you that the Advanced Studies & Research Board at its meeting held on 07-05-2015 approved the synopsis of your thesis titled “Developmental Screening and Nutritional Intervention of Severe Acute Malnourished Children in Southern Punjab, pakistan” for Ph.D. research in the subject of Public Health. Dr. Rubeena Zakar, Associate Professor (Responsible for overall correspondence) and Prof. Dr. Muhammad Zakria Zakar, Director, Institute of Social & Cultural Studies, University of the Punjab, Lahore have been appointed as your Research Supervisors.

During the course of your studies, you will be desired to comply with the rules of Doctoral Programme, which are available from the office of the undersigned/DPCC.

Deputy Registrar (Academic) for Registrar
Subject: - Review of the Research Proposal Submitted by Dr. Javeria Saleem

The Institutional Review Board (IRB) has reviewed the research proposal titled “Developmental Screening and Nutritional Intervention of Severe Acute Malnourished Children in Southern Punjab, Pakistan” submitted by Dr. Javeria Saleem, for his Ph.D (Public Health) dissertation.

The Board thoroughly reviewed the research objectives and methodology of the proposal. The Board is satisfied with the ethical dimensions of the proposed research and approves the proposal accordingly.

Chairman
Institutional Review Board (IRB)
OFFICE OF THE
District Coordinator
IRMNCH and Nutrition Program,
O/O District Health Development Center,
District, Dera Ghazi Khan.

No. 232 /IRMNCH, Dated D.G. Khan the 06/04/2015.

To,
Dr. Javeria Saleem,
Consultant Pediatrician and PhD Scholar.

Subject: Request for Technical Support of Nutrition Intervention Research

Reference to the Letter No. 838/ADGHS/IRMNCH Punjab, Lahore dated 03-04-15, it is stated that you are
consultant pediatrician at the children Hospital Lahore. Currently you are a PhD Scholar at the
Department of Public Health (University of Punjab). The research you are conducting for your PhD
involves, "Developmental Screening and Nutritional Intervention of Severe Acute Malnourished Children
in Southern Punjab".

I am hereby giving my consent to approach the centers (RHC Kot Chutta, BHU Samina, BHU Aali Wala,
BHU Jhoke Utra) in my District to provide participants for this research.

You have provided me with a copy of your research proposal and a copy of the approval letter which you
received from the main office of IRMNCH Punjab, Lahore.

If you require any further information, please do not hesitate to contact AOCO (Shazia Mughal) on
(+923336371733). Thank you for your time and consideration in this matter.

Good Luck with your Research.

Yours sincerely,

[Signature]
District Coordinator, IRMNCH
Dera Ghazi Khan.
ANNEXURE 12 - HIGHER EDUCATION COMMISSION LETTER

HIGHER EDUCATION COMMISSION

H-9, Islamabad (Pakistan) Phone: 0092-51-90400000, Fax: 0092-51-90400000, E-mail: jehan@hec.gov.pk

Project Director

No: 1-8/HEC/HRD/2016/6029
PIN: ISSIP 32 3.5c 02
Dated: August 22nd, 2016

Dr. Javeria Saleem Malik
66 K-3, WAPDA Town, Lahore

Subject: Award of scholarship under “International Research Support Initiative Program”

Dear Dr. Malik,

I am delighted to inform you upon your selection as a recipient of a grant under the International Research Support Initiative Program (IRSSIP) for Queen Mary University of London, UK. Details of the award, along with terms and conditions are as follows:

a. Return air travel (Economy Class) at actual and up to Rs. 150,000/- (One Hundred Fifty Thousand) whichever is less.

b. Stipend/living allowance UK£ 750/- per month for a maximum period of six months.

c. UK£ 1750/- as bench fee to be paid to the foreign university. (Payment is subject to the provision of the fee invoice from foreign university)

2. All other expenses are to be covered by you.

3. You will be required to submit sureties, bonds, undertaking and guarantee to HEC to return and complete your PhD studies at your parent Universities/Institutions.

4. You are required to apply for visa before October 15th, 2016. The offer will stand withdrawn without any further notice in case not availed till December 31st, 2016.

5. Before return to Pakistan, you will be required to submit us a progress report on your visit on prescribed Performa duly endorsed by your foreign supervisor.

6. Any other terms/conditions and TORs of the program issued by HEC from time to time will be binding for all purposes.

Yours Sincerely,

Jehanzeb Khan
Project Director

Cc:
1. Prof. Dr. Adrian Martinou, School of Medicine and Dentistry, Queen Mary University of London, UK.
2. Dr. Muhammad Zakir Zakar Dr. Rubaera Zakar, Public Health, University of the Punjab, Lahore.
3. Head of Department, Public Health, University of the Punjab, Lahore.
4. The Honorable Ambassador, Consulate General of Pakistan, 34 – 36 Lowndes Square, London, SW1X 9JN.
5. Personal file.

7th April 2017

Professor Jahanzib Khan
Project Director
Higher Education Commission,
Islamabad, Pakistan.

Dear Professor Khan

Re: Dr. Javera Malik, IRSP scholarship recipient

I am writing to confirm that Dr. Malik spent a productive six months here at Queen Mary University of London under my supervision.

She has worked tirelessly and to the highest standards, analysing and writing up data from her clinical studies into the effects of vitamin D in malnourished children. She was also involved in biochemical analysis of serum samples from study participants.

I have no doubt that she has made the most of the opportunity of visiting the UK to study, and we will all miss her dynamism and commitment when she returns to Pakistan. I am hopeful that her visit will be the start of future collaborative projects between the University of Punjab and the University of London.

Yours sincerely,

Adrian Martineau

Adrian Martineau B Med Sci MB BS DTM&H MRCPI PhD FRSB
Professor of Respiratory Infection and Immunity
## ANNEXURE 14 - CLINICAL TRIAL REGISTRATION

### ClinicalTrials.gov PRS

Protocol Registration and Results System

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**ClinicalTrials.gov Protocol Registration and Results System (PRS) Receipt**

**Release Date:** May 25, 2017

**ClinicalTrials.gov ID:** NCT03170479

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### Study Identification

- **Unique Protocol ID:** UPunjab
- **Brief Title:** Developmental Screening and Nutritional Intervention of Severe Acute Malnourished Children in Southern Punjab, Pakistan
- **Official Title:** Developmental Screening and Nutritional Intervention of Severe Acute Malnourished Children in Southern Punjab, Pakistan
- **Secondary IDs:**

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### Study Status

- **Record Verification:** May 2017
- **Overall Status:** Completed
- **Study Start:** June 2015 [Actual]
- **Primary Completion:** November 2016 [Actual]
- **Study Completion:** May 2017 [Actual]

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### Sponsor/Collaborators

- **Sponsor:** University of the Punjab
- **Responsible Party:** Principal Investigator
  - Investigator: Dr. Javeria Saleem [jsaleem]
  - **Official Title:** Principal Investigator
  - **Affiliation:** University of the Punjab

- **Collaborators:**

---

### Oversight

- **U.S. FDA-regulated Drug:**
- **U.S. FDA-regulated Device:**
- **U.S. FDA IND/IDE:** No
- **Human Subjects Review:**
  - **Board Status:** Approved
  - **Approval Number:** 07/05/2015
  - **Board Name:** Advanced Studies and Research Board
  - **Board Affiliation:** University of the Punjab
  - **Phone:** +92 423 9231106
  - **Email:** dracademic@pu.edu.pk
  - **Address:**
Quaid-i-Azam Campus  
Lahore  
Pakistan

Data Monitoring: Yes  
Plan to Share IPD: Yes  
FDA Regulated Intervention: No

Study Description

Brief Summary:  
i. To examine the impact of malnutrition on development quotient of children  
ii. To determine the effectiveness of Ready to Use Therapeutic Food (RUTF) in improving the development quotient of severe acute malnourished children under five year of age.  
iii. To investigate the outcome of Vitamin D therapeutic doses intervention with RUTF rehabilitation on growth and development of malnourished children.

Detailed Description:  
1. Research Instrument: For Development quotient Denver 2 screening form and tools will be used. Follow up of patients for three months and for recording of their weight Community management of acute malnutrition forms will be used. Denver screening tool will be used for child development quotient covering all areas of development, fine motor, gross motor, language and personal social contact.  
2. Research Settings: The study will be conducted in Dera Ghazi Khan division at, Basic Health Units (BHU).  
3. Study design: Randomized Controlled Trial.  
4. Data Collection: Lady Health Visitors (LHWs) will screen the children aged 6 months to 59 months in their community and will refer the severely malnourished children to Outpatient Therapeutic Programs (OTPs). Two groups of malnourished children will be made one study and one control group; one group will be treated with RUTF and extra virgin olive oil as placebo. Other will with RUTF and two mega doses of Vitamin D randomly, first after 15 days of enrollment and second after 15 days of first dose.

Conditions

Conditions: Malnutrition in Children

Keywords:

Study Design

Study Type: Interventional  
Primary Purpose: Supportive Care  
Study Phase: Phase 2  
Interventional Study Model: Parallel Assignment  
Number of Arms: 2  
Masking: Participant, Care Provider, Investigator  
Allocation: Randomized  
Enrollment: 185 [Actual]
Arms and Interventions

<table>
<thead>
<tr>
<th>Arms</th>
<th>Assigned Interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental: RUTF with Vitamin D</strong>&lt;br&gt;Two groups (arms) of malnourished children will be made, one study and one control group; Experimental arm will use RUTF and two mega doses of 200,000 IU vitamin D randomly first after 15 days of enrollment and second after 15 days of first dose.</td>
<td><strong>Dietary Supplement: Vitamin D</strong>&lt;br&gt;Two doses of Vitamin D supplementation in ampoules form (ED3) were procured. Ampoule contains Cholecalciferol in 200,000 IU both for oral and intramuscular use. <strong>Dietary Supplement: Ready to Use Therapeutic Food (RUTF)</strong>&lt;br&gt;RUTF is recommended by WHO for severe malnourished children in community settings as a therapeutic diet. The quantity depends upon the child body weight.</td>
</tr>
<tr>
<td><strong>Placebo Comparator: RUTF with Placebo</strong>&lt;br&gt;Placebo arm will receive Ready to Use Therapeutic Food (RUTF) and extra virgin olive oil as Placebo.</td>
<td><strong>Dietary Supplement: Ready to Use Therapeutic Food (RUTF)</strong>&lt;br&gt;RUTF is recommended by WHO for severe malnourished children in community settings as a therapeutic diet. The quantity depends upon the child body weight.</td>
</tr>
</tbody>
</table>

Outcome Measures

Primary Outcome Measure:

1. Weight gain in children
   More than 15% weight gain from enrollment date
   [Time Frame: 2 Months]

Secondary Outcome Measure:

2. Developmental Milestones of children
   Normal or delayed development using Denver Developmental Screening Tool 2 (DDST2)
   [Time Frame: 2 Months]

Eligibility

Minimum Age: 6 Months
Maximum Age: 59 Months
Sex: All
Gender Based: No
Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria

- All the selected children of Severe uncomplicated acute malnutrition of concerned age group whose parents or guardians have given written consent for the study
- Mid Upper Arm Circumference (MUAC) less than 11.5 cm or Weight For Height (WFH) less than minus 3 standard deviation.

Exclusion Criteria

- Children above the concerned age group
- Refusal of parents for taking part in the study.
• Child having Severe Acute Malnutrition (SAM) with complications have loss of appetite, lower respiratory tract infection indicated by chest in drawing, severe vomiting.
• Temperature greater then 39°C or hypothermia less then 35°C, very pale, oedema, unconsciousness.

Contacts/Locations

Central Contact Person: Javeria S Malik, PhD Scholar
    Telephone: +92 300 4366011
    Email: javeria.hasan@hotmail.com

Central Contact Backup: Rubeena Zakar, PhD
    Telephone: +92 332 4337299
    Email: rubeena499@hotmail.com

Study Officials: Muhammad Z Zakar, PhD
    Study Director
    University of the Punjab

Locations:

References

Citations:

Links:

Study Data/Documents: