NEURODEVELOPMENT OF LOW BIRTH WEIGHT BORN INFANTS IN RELATION TO THEIR IRON STATUS IN DAR ES SALAAM, TANZANIA.

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A Dissertation Submitted in Partial fulfillment of the Requirements for the Degree of Master of Medicine (Pediatrics and Child Health) of the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, a dissertation entitled: Neurodevelopment of low birth weight born infants in relation to their iron status in Dar es salaam, Tanzania, in partial fulfillment of the requirements for the degree of Master of Medicine (Pediatrics and Child Health) of the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam.

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Date.....

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(Supervisor 2

DECLARATION AND COPYRIGHT

I, Albion Kasasa, declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature.....

ACKNOWLEDGEMENTS

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Glory, praise and honors are to GOD for taking me through this work and my studies.

AMEN.

DEDICATION

To My Mother and father.

My brothers and sisters.

ABSTRACT

Background: Iron plays an essential role in many important biochemical processes. As with all nutrients, the requirement for iron is greater during periods of rapid growth and differentiation such as in the late fetal and neonatal period. Poor iron homeostasis during this period can result in disordered development. Inadequate tissue iron levels can lead to reduced erythropoiesis and poor oxygen carrying capacity. The nervous system, which develops rapidly during the late fetal and early neonatal period, seems to be particularly susceptible to iron deficiency.

Objective: To assess neurodevelopment of low birth weight infants in relation to their iron status.

Methodology

Hospital based descriptive cross-sectional study of 270 infants who were born premature/low birth weight aged less than 12 months, attending the high-risk children clinic, whose mothers consented. Infants were recruited on Mondays and Fridays during their clinic visit using convenient sampling technique. Infants with known congenital anomalies, born with birth asphyxia, meningitis and jaundice and those who were acutely ill during the clinic visit were excluded from the study. Bayley Mental Developmental Scoring was done on all infants recruited in the study, along with a thorough history, physical examination and laboratory tests including serum ferritin and complete blood count. Cognitive, motor and language development scores were considered normal with score of ≥85 and poor with score of <85. Iron deficiency were considered if serum ferritin was <12µ/dl.

Results

Of the 270 infants, 124 (45.9%) were male and 146 (54.1%) were female providing a male to female ratio of 0.8:1.

The prevalence of poor cognitive, motor and language development were 90%, 88.2% and 59.2% respectively. Poor cognitive, language and motor development were noted with

increasing age with p=0.0001 in all categories. Language and motor development were more poorly developed in females than males with p=0.05 and p= 0.006 respectively.

Infants with iron deficiency and poor cognitive percentile score were 37% compared with 18% of iron deficient infants with normal cognitive percentile scores (p=0.007). More infants with poor language percentile scores (35%) were observed in iron deficient infants than iron sufficient infants (p=0.04). Motor development percentile score was not significantly affected among the iron deficient infants

Infants with a history of not receiving iron supplementation had poor cognitive, language and motor development with p=0.006, 0.001, and 0.0001 respectively.

The prevalence of iron deficiency was 34.1%. The iron status of the baby was significantly associated with the gestational age (p=0.0004), the birth weight (p=0.0013) and the nutrition status of the infant (p=0.013).

Whereas maternal age, gestation age and maternal education were not significantly associated with poor neurodevelopment outcomes, birth weight, nutritional status and iron supplementation status were significantly associated with neurodevelopment. Infants born with very low birth weight of 0.8-1.49kg were significantly associated with poor cognitive development. However, wasting was a positive predictor of poor motor development and not predictive of the status of cognitive and language development.

Conclusion and Recommendations

Neurodevelopment was poor among infants born with low birth weight and was more pronounced among iron deficient infants. Neurodevelopment was significantly associated with birth weight, iron deficiency and wasting. Therefore the study recommends early neurodevelopment assessment done on all preterm/low birth weight infants to identify neurologically challenged infants and to direct them for further stimulation and supplementation, in order to improve neurodevelopment.

The study also recommends an improvement in iron supplementation program through universal iron supplementation in the right doses and appropriate duration to all low birth weight infants as well as exploring for other causes of poor neurodevelopment and intervening as needed.

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ABBREVIATIONS

BSID – Bayley Scale of infant Development

CDC – Centre for Diseases Control

CPL – Central Pathology Laboratory

EDTA – Ethylene Diamine Tetra Acetic Acid

EPI – Expanded Programme of Immunization

FBP - Full Blood Picture

Hb – Hemoglobin

Hct – Haematocrit

MCH – Mean Corpuscular Hemoglobin

MCHC – Mean Corpuscular Hemoglobin Concentration

MCV – Mean Cell Volume

LBW – Low Birth Weight

SGA – Small for Gestation Age

SDA – Seventh Day Adventist

IUGR- Intrauterine Growth Retardation

WHO – World Health Organization

MNH – Muhimbili National Hospital

RCH - Reproductive and Child Health

RDW – Red Blood Cell Distribution Width

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background and definition of low birth weight infant and prematurity.

Low birth weight (LBW; birth weight of <2500g) is due to prematurity, and or intrauterine growth retardation (IUGR, also referred to as SGA). Preterm birth is birth before 37 weeks of gestation[1] and a "premature" infant is one that has not yet reached the level of fetal development that generally allows life outside the womb. In the normal human fetus, several organ systems mature between 34 and 37 weeks, and the fetus reaches adequate maturity by the end of this period. Therefore, a significant overlap exists between preterm birth and prematurity: generally, preterm babies are premature and term babies are mature. Prematurity and IUGR are associated with increased neonatal morbidity and mortality [2]

1.2 Epidemiology of preterm delivery and low birth weight infant.

Many infants in developing countries are not weighed at birth. However, available data from 100 developing countries, reports a low birth weight rate below 10 per cent.[3]

An estimated 18 million babies worldwide are born each year with low birth weight 9.3 million in South Asia and 3.1 million in sub-Saharan Africa. Low-birth weight babies face a greatly increased risk of dying during their early months of life. Those who survive have impaired immune function and face increased risk of disease, including that of diabetes and heart disease later in life. They are also likely to remain malnourished, have lower intelligence Quotient (IQ) and cognitive disabilities leading to school failure and learning difficulties.[3]

The incidence of low birth weight (LBW) in Dar es salaam is 16%[4] It is higher among female infants and infants born to parents of a low socioeconomic status [5]. Similarly, based on the demographic health survey of Tanzania done in 2004-2005, the incidence of the low birth weight infants countrywide was found to be 10 per 100 live births.[6] Factors associated with low birth weight in Dar es Salaam are first pregnancy to mother, short stature of the mother, multiple pregnancy, and "toxemia" of pregnancy. These factors influence the growth velocity of the fetus leading to an increased incidence of small-for-dates infants.[7] Low maternal age and antepartum hemorrhage mainly affect the duration of gestation and lead to a preponderance of preterm appropriate-for-gestational-age.[5, 7, 8]

In developing countries, approximately 70% of LBW infants have IUGR. Infants with IUGR have greater morbidity and mortality than do appropriately grown, gestational age –matched infants[9]

1.3 Neurodevelopment in the low birth weight and preterm infants

The developing brain is one of the organs in the human body most sensitive to damage. Functional manifestations ranging from frank mental retardation to milder learning disabilities are the most common class of birth defects. The maturation of the central nervous system requires a more complex sequence of processes than any other structure, making this organ uniquely vulnerable to environmental influences[2]. The low birth weight infants usually have mild to severe neurodevelopmental abnormalities in motor, cognitive, behavioral and language skills[10]. Premature birth in itself may prejudice later development. The greater the immaturity and the lower the birth weight, the greater the likelihood of intellectual and neurologic deficit; as many as 50% of 500–750 g infants have a significant neurodevelopmental impairment (blindness, deafness, mental retardation, cerebral palsy)[11]. Many surviving LBW infants have hypotonia before 8 months corrected age, which improves by the time they are 8 months–1 yr old. This transient hypotonia is not a poor prognostic sign[12]. Thirty to fifty percent of VLBW children have poor school performance at 7 yr of age (repeat grades, special classes, learning disorders, poor speech and language), despite a normal IQ[13-15]. Iron deficiency is common in low birth weight infants and it has been suggested to have role on poor neurodevelopment performance. The other factors posing a risk for poor academic performance include birth weight below 750 g, severe intraventricular hemorrhage, periventricular leukomalacia, bronchopulmonary dysplasia, cerebral atrophy, posthemorrhagic hydrocephalus, IUGR, low socioeconomic status, and, possibly, low thyroxine levels[2].

1.4. Role of iron in neurodevelopment

The regulation of the availability of micronutrients is particularly critical during periods of rapid growth and differentiation such as the fetal and neonatal stages. Iron deficiency during the early weeks of life can have severe effects on neurodevelopment that may persist into adulthood and may not be corrected by restoration of normal iron levels.

Iron deficiency anemia has been conclusively seen to delay psychomotor development and impair cognitive performance of infants in Costa Rica[16, 17], and the USA[18]

Iron plays an essential role in many important biochemical processes.[19] As with all nutrients, the requirement for iron is greater during periods of rapid growth and differentiation such as late fetal and neonatal period. Consequently, poor iron homeostasis during this period can result in disordered development. Inadequate tissue iron levels can lead to reduced erythropoiesis and poor oxygen-carrying capacity.[19] The nervous system, which develops rapidly during the late fetal and early neonatal period, seems to be particularly susceptible to iron deficiency. Consequently, iron deficiency, anemia, or iron excess can have severe effects on neurodevelopment. The mechanism by which iron deficiency affects brain development is incompletely understood, but it may involve general metabolic deficiencies, disordered myelination, disordered synaptogenesis, changes in specific monoamine neurotransmitter synthesis, neuronal and glial cell energy metabolism in hippocampus of the neonate[20]. Neural assessment of effects of iron deficiency include tests for speed of processing (myelination), changes in motor and affect (monoamines), and recognition memory[21]. Since the myelination, synapse formation, neuronal and glial energy metabolism at hippocampus begins before term/ birth; the premature infants with iron deficiency in uteral will be having impaired neurodevelopment. In the other study looking for the effects of micronutrient effects on neurodevelopment noted that the iron deficiency is associated with delay in cognitive development[22] .

Iron status can be considered as a continuum from normal iron status with varying amount of iron, then iron deficiency with no anemia and iron deficiency with anemia. Iron deficiency is the result of long-term negative iron balance. Iron stores in the form of haemosiderin and ferritin are progressively diminished and no longer meet the needs of normal iron turnover.[23]

Iron deficiency is the most common single-nutrient deficiency disease in the world: While iron deficiency was once presumed to exert most of its deleterious effects only if anemia was present[24], it is now clear that many organs show morphological, physiological, and biochemical changes before there is any drop in hemoglobin concentration[25]

In the brain the iron is mostly found in the nucleus accumbens, substantial nigra, deep cerebellar nuclei, the red nucleus and portions of the Hippocampus and can approach the concentration of iron seen in the liver.[26] The functions of iron in the brain include neurodevelopment, metabolic activity and neurodegenerative pathologies associated with aging[27]. Oligodendrocytes are cell predominantly containing iron which is required for myelination of the spinal cord and white matter of the cerebellar folds and is a cofactor for enzymes involved in neurotransmitter synthesis, including tryptophan hydroxylase and tyrosine hydroxylase. Iron is also responsible for the catabolism of neurotransmitters and works as cofactor for ribonucleotide reductase, the rate-limiting step in DNA synthesis. Iron deficiency in infancy is associated with alteration in affect and activity, as a result infant has poor neurodevelopment[16]

1.5 Iron deficiency

In study done in Dar es salaam-Tanzania 1994, iron deficiency was the most common cause of severe anemia in the first one year of life. Iron deficiency was prevalent in both severe anemic group (89.3%) and in a group who didn't have severe anemia (78.6%) at the end of their first year of life[28]. The estimated prevalence of anemia in children younger than 4 years in developing countries is 46-66%, and iron deficiency anemia accounted for half of these children[29]

Dietary iron deficiency results in biochemical changes in the blood and reduced concentrations of iron in tissues. Iron deficiency without anemia is generally considered to correspond to a degree of dietary iron deficiency sufficient to deplete ferritin stores and to decrease iron concentrations in some tissues, but not sufficient to reduce hemoglobin to the point of developing anemia. Individuals with depleted iron stores and hemoglobin concentrations below the 10th percentile of a normally distributed population are generally considered to have iron deficiency anemia[30].

Iron deficiency is public health important nearly in every country in the world. Programmes for the prevention of iron deficiency, particularly iron supplementation for pregnant women, were being implemented in 90 of 112 countries that reported to WHO in 1992[31]. A study in Niger

randomized second trimester mothers to receive either 100mg elemental iron throughout the remainder of their pregnancies or a placebo. Iron treatment reduced iron deficiency, serum ferritin concentrations were higher in infants of women in the iron supplemented group and their neurodevelopment was significantly better than the infants of placebo treated mothers.[32]

Cochrane review of routine iron supplementation in pregnancy did not draw conclusions about either beneficial or harmful effects to mother or infant[33] .Iron deficiency is highly prevalent in many malaria-endemic regions of Africa, and it is clear that many infants, particularly those born prematurely or with low birth weight, have low total body iron, and is at particular risk of iron deficiency during the first six months of life. A short period of iron supplementation in the first few months of life might replenish iron stores at a time when there is less pressure from infectious diseases such as malaria.[34]

Iron supplementation has been seen to have its role in the process of motor development. The supplementation of iron has beneficial effects on infant motor development and cognitive development.[35]

Since the premature infants are prone to get the iron deficiency by the mechanism of high utilization of iron in the fast growing and regaining body weight, then they are likely to have poor neurodevelopment score. The evidence of causal relationship of the poor neurodevelopment due to iron deficiency is mostly worked well in the animal models than in human [36].

1.6 Neurodevelopment assessment of infants [37]

The neurodevelopment assessment is done using the Bayley scale of infant and toddler development version III. .

The Bayley Scales of Infant and Toddler Development–Third Edition (Bayley-III)

The Bayley Scales of Infant and Toddler Development–Third Edition (Bayley-III) is a revision of the frequently used and well-known Bayley Scales of Infant Development–Second Edition (BSID-II; Bayley, 1993). Like its prior editions, the Bayley-III is an individually administered

instrument designed to measure the developmental functioning of infants and toddlers. Other specific purposes of the Bayley-III are to identify possible developmental delay, inform professionals about specific areas of strength or weakness when planning a comprehensive intervention, and provide a method of monitoring a child's developmental progress. The Bayley-III is appropriate for administration to children between the ages of 1 month and 42 months (although norms extend downward to age 16 days).

Description of the Bayley-III Scales

The most significant revision to the Bayley-III is the development of five distinct scales (as compared to three scales in the BSID-II) to be consistent with areas of appropriate developmental assessment for children from birth to age 3. Whereas the BSID-II provided Mental, Motor, and Behavior scales, the Bayley-III revision includes Cognitive, Language, Motor, Social-Emotional, and Adaptive Behavior scales.

Cognitive

The Cognitive scale of the Bayley-III contains 72 out of the 178 items that were previously included in the mental scale of the BSID-II. Additionally, 19 new items were added to the Cognitive scale, resulting in a total of 91 items. Forty-five of the items from the Mental scale were completely removed from the Bayley-III, whereas the remaining items either remained the same or were slightly modified and moved to a different scale (i.e., 27 items were moved to the Expressive Communication subtest of the Language scale, 23 items were moved to the Fine Motor subtest of the Motor scale, and 11 items were moved to the Receptive Communication subtest of the Language scale).

Language

Recognizing the significance of assessing a child's language development, the Bayley-III added a Language scale consisting of Receptive and Expressive Communication subtests. Items in the Receptive Communication subtest are designed to provide information regarding the child's auditory acuity and ability to understand and respond to verbal stimuli. This subtest

includes 11 items (some slightly modified) from the BSID-II Mental scale and an additional 38 new items. The Expressive Communication subtest assesses the individual's ability to vocalize, name pictures and objects, and communicate with others. This subtest contains 27 items from the BSID-II (some slightly modified) and 21 new items.

Motor

The Bayley-III Motor scale, consisting of Fine Motor and Gross Motor subtests, is similar to the Motor scale of the BSID-II. The Fine Motor subtest contains 66 items (18 items are new) and is supposed to measure skills associated with eye movements, perceptual- motor integration, motor planning, and motor speed. The Gross Motor subtest contains 72 items (4 items are new) and is designed to measure movements of the limbs and torso.

Social-Emotional

The Behavior Rating scale in the BSID-II was replaced by the Greenspan Social-Emotional Growth Chart: A Screening Questionnaire for Infants and Young Children (Greenspan, 2004) and is intended to be completed by the child's primary caregiver. For each of the 35 items, which measure emotional development and related behaviors, the respondent selects one of six ratings: 0 (can't tell), 1 (none of the time), 2 (some of the time), 3 (half of the time), 4 (most of the time), or 5 (all of the time).

Adaptive Behavior

A significant addition to the Bayley-III is the inclusion of the Adaptive Behavior Assessment System–Second Edition as a measure of adaptive skills. By having the child's primary caregiver complete the ABAS-II, estimates of the child's functioning in the areas of Communication, Community Use, Health and Safety, Leisure, Self-Care, Self-Direction, Functional Pre-Academics, Home Living, Social, and Motor can be obtained. (Children younger than 1 year do not receive scores in the areas of Community Use, Functional Pre-Academics, or Home Living.) Within the ABAS-II, caregivers indicate the extent to which the child performs the adaptive skills when needed. Response options include 0 (is not able), 1

(never when needed), 2 (sometimes when needed), or 3 (always when needed). The inclusion of the ABAS-II facilitates a more comprehensive assessment as caregivers are more involved in completing the ABAS-II than they would be in completing the BSID-II.

Materials

Whereas many of the Bayley-III stimulus materials will look familiar to a user of the BSID-II, the current edition contains additional items such as a bank, a bear, a bracelet, a connecting block set, a lacing card, memory cards, a set of seven ducks, and a wider stepping path. Some notable items not contained in this edition include the map, sugar pellets, jumping rope, pull toy, and separate visual stimulus cards. Examiners must provide more materials than were required for a BSID-II administration, including facial tissue, five small coins, food pellets, several blank 3 × 5 in. index cards, safety scissors, and blank unlined white paper. The stimulus book of this edition is more user-friendly in that it contains a built-in easel that folds low to the table, which aids in assessing an examinee's responses and allows for ease in switching between tasks. A wider stepping path is included and is considered an improvement over the previous edition, providing a more developmentally appropriate guide for assessing gross motor skills.

Administration and Scoring Procedures

Examiners who administer the Bayley-III should be familiar with and have training in developmental assessment and interpretation. The age range for which the measure is designed requires that the examiner have the ability to establish and maintain rapport with infants, toddlers, and caregivers. Because of these factors, examiners should have completed relevant graduate training or professional experiences that include formal individual assessment preparation and supervision so that the measures can be administered consistent with the Standards for Educational and Psychological Testing. To gain an accurate impression of an infant or toddler's optimal performance and to avoid negative behavioral reactions to separation, a caregiver (generally a parent) is encouraged to remain in the testing room for the duration of the Bayley-III administration. However, caregivers should not encourage,

influence, or interfere with item administration to the point that standardization procedures are violated.

Administration times range from approximately 50 min for children aged 12 months and younger to 90 min for children aged 13 months and older. Consistent with the BSID-II, the examinee's chronological age (adjusted for prematurity if necessary) corresponds to a starting point, designated by a letter A through Q. This letter should be used to determine the starting item for the Cognitive, Language, and Motor scales. Each scale has an identical requirement for establishing basal and ceiling levels: The first three items administered must be correct (examinees receive credit for un administered items below the basal), and scoring of the scale should discontinue when the examinee receives no credit on five consecutive items. In the event that a basal is not established with the first three items administered, the examiner must reverse to the previous starting point and continue administration until the ceiling criterion is met. Although it may be necessary to reverse to an earlier starting point on one scale, the examiner should use the original age-determined starting letter to determine the starting point for subsequent scales. No items should be re-administered during the course of a testing session; however, if a correct response was not initially elicited but is observed later in a session (e.g., during the Gross Motor subtest or some items on the Expressive Communication subtest), some items may then be scored as correct. Scoring for every item is either 1 (credit) or 0 (no credit). The item scoring in this edition is more straightforward and manageable than in the previous edition, and it allows for a more efficient method of calculating a total raw score. Additionally, examiners should be aware of specific behaviors that are indicative of delayed or atypical development (referred to as developmental risk indicators) within the areas of social behavior, attention, motor and movement, hearing, and vision.

The examiner's record form contains items for the Cognitive, Language, and Motor scales, with a separate questionnaire that contains items for both the Social-Emotional and Adaptive Behavior scales. This questionnaire is to be completed by the primary caregiver. The examiner's record form provides item titles, materials needed for each item, scoring criteria, and space for noting additional comments about an examinee's responses. Similar to the BSID-

II, some items are part of a series that use the same materials, and the examinee can demonstrate varying levels of proficiency. For example, for the pegboard series, Item 47 requires the child to place at least one peg two or more times in the same or different hole or holes. The pegboard should also then be used to administer Item 55, in which the child receives credit for placing all six pegs in the pegboard within 70 seconds. Examiners should score such series items concurrently, so that it is not necessary to switch away from and back to a stimulus material. However, a child may pass a series item that falls beyond an established ceiling. In this case, the series item should not be included in the total raw score, but it should be qualitatively noted. Series items are specified as such, and the additional items contained in the series are specified on the far left side of each page of the record form. The record form is quite colorful, with each color corresponding to a specific scale. The colors not only are aesthetically pleasing but also function to separate the Cognitive, Language, and Motor scales for the examiner who may need to switch between scales while administering the complete test. Item materials and scoring criteria are specified in the record form, but examiners should closely reference the administration manual. The administration manual provides clear guidelines for item instructions, stimulus layout, child positioning, and so on. The format for referencing the administration manual in this edition is similar to that in the BSID-II, but pictures of the necessary item materials are not provided with the corresponding items in the administration section of the manual. Therefore, examiners must be able to differentiate the proper stimuli without the aid of a picture included with the administration procedures. The manual does, however, provide a page with pictures of the test items and their corresponding names, so that an examiner can familiarize himself or herself with the names of the stimulus materials prior to administering the test. One notable improvement in the Bayley-III administration manual is that it is ring bound, which allows examiners to remain on a desired page without concern that the manual might accidentally close. Although the length of the administration section may seem daunting, the instructions to the examiner are necessary for proper administration of this measure, and the apparent complexity reduces with increased familiarity with and experience in administering the Bayley-III to infants and toddlers of various ages.

A variety of scores across scales and subtests are available. Raw scores from the Cognitive scale, which does not contain separate subtests, can be converted to a scaled score (M = 10, SD = 3), which can then additionally be converted to a composite score equivalent (M = 100, SD = 15). Scaled scores are available for the Receptive and Expressive Communication subtests of the Language scale, which when combined form the Language scale composite score (M = 100, SD = 15). The same procedure holds for the Fine and Gross Motor subtests of the Motor scale. Across all three of these primary domains, the normative sample is divided into 10-day increments (e.g., 2 months 6 days through 2 months 15 days). Raw scores for Cognitive, Language, and Motor subtests translate to scaled scores based on 10-day increments up to age 5 months 16 days, at which point norms are based on 1-month intervals (e.g., 5 months 16 days to 6 months 15 days, 35 months 16 days to 36 months 15 days). The highest two age ranges are formed on the basis of 3-month intervals (36 months 16 days to 39 months 15 days). Thus, depending on the age of the child, normative scaled scores are derived on the basis of 10-day, 1-month, or 3-month intervals. Percentile ranks, confidence intervals (90% and 95% levels), growth score equivalents, and developmental age scores in months and days are available. The scoring for the Social-Emotional Scale is straightforward. The summed raw score is converted to a scaled score (M = 10, SD = 3), which can additionally be converted to a composite score equivalent (M = 100, SD = 15). The normative sample for the Social-Emotional domain is divided into nine age categories (by months: 0-3, 4-5, 6-9, 10-14, 15-18, 19-24, 25-30, and 31-40). A Sensory Processing score can also be calculated. The administration manual provides additional guidance regarding conducting supplemental analyses within this scale. The Adaptive Behavior scale follows the scoring criteria of the ABAS-II. Raw scores for each of the 10 skill areas are converted to scaled scores (M = 10, SD = 3). From these scaled scores, a General Adaptive Composite (GAC) score (M = 100, SD = 15) can be obtained. Additional composite scores are available for a Conceptual Adaptive domain (Communication, Functional Pre-Academics, and Self-Direction skill areas), Social Adaptive domain (Leisure and Social skill areas), and Practical Adaptive domain (Community Use, Home Living, Health and Safety, and Self-Care skill areas). The normative sample for the Adaptive Behavior scale is in 1-month increments for children aged 11 months and younger, 2-month increments for children aged 13

months to 23 months, and 3-month increments for children aged 24 months to 42 months. Percentile ranks and confidence intervals (90% and 95% levels) are available to assist in interpretation.

2.0: PROBLEM STATEMENT

Iron deficiency ranked ninth among 26 risk factors included in the global burden of disease and accounted for 841,000 deaths and 35 million disability adjusted life years lost[38]. Iron deficiency is a very common disorder in low birth weight as compared to normal weight babies[39 40 41]. Low birth weight is very common globally[3] and also in Tanzania[4]. Iron has been shown to be involved in neurodevelopment[42]. Neurodevelopment globally is important and contribute significantly on the Global Burden of diseases[42]. Iron deficiency is common and is of public health importance. Preterm and low birth weight is also common and is main cause of morbidity and mortality.

Therefore, reaffirming the role of iron deficiency in low birth weight in relation to the neurodevelopment outcomes will emphasize the need to address the problem.

3.0: RATIONALE OF THE STUDY

Since in Tanzania low birth weight is high and also the iron deficiency is high then there is a need to do a study to look for the influence of iron deficiency on the neurodevelopment of infant born with low birth weight. Studying the magnitude of this problem will set the basis for appropriate intervention. Knowledge of the predictors of iron deficiency will facilitate its diagnosis, treatment and targeted interventions and improved neurodevelopment

3.1: The study alternative hypothesis.

The iron deficiency is associated with poor neurodevelopment in low birth weight infants.

4.0 OBJECTIVES

4.1 Broad objective: To assess neurodevelopment in low birth weight infants and correlate with iron status.

4.2 Specific objectives:

4.2.1. To determine cognitive, language and motor development of low birth weight infants by age and sex.

4.2.2. To determine the proportion of infants who have poor neurodevelopment

4.2.3. To determine the proportion of low birth weight infants who have iron deficiency.

4.2.4. To determine cognitive, language, and motor development of low birth weight infants in relation to their iron status

4.2.5. To determine the correlation between neurodevelopment of low birth weight infants with maternal age, nutritional status, iron supplementation status, birth weight and gestational age

5.0 METHODOLOGY

5.1 Study design.

Hospital based descriptive cross-sectional study from August 2009 to January 2010.

5.2 Study area

This study was done at Muhimbili National Hospital (MNH) post-natal clinics. MNH is a teaching hospital located in Dar es Salaam, the largest commercial city of Tanzania. At Muhimbili National Hospital in the year 2008, there were 2557(33%) admissions due to prematurity/low birth weight out of 7675 total neonatal admissions. Preterm delivery accounts for 46% of the total neonatal death at MNH. High risk post-natal clinic provide follow up services for neonates who were admitted in the neonatal ward for various reasons. Neonates from outside the MNH who do not have indication for admission to the neonatal unit are also seen at this clinic. High risk post-natal clinic has daily attendance of approximately 72 infants and among them low birth weight infants are 58%. At MNH, in response to the national policy, LBW infants are usually started on iron supplementation at six weeks and it is given for the first twelve months of life.

5.3 Study population

All babies 1-12 months of age, born with low birth weight that were attending the high risk post-natal clinics at MNH.

5.4 Inclusion criteria

All babies who were born with low birth weight (birth weight of <2.5kg) attending high risk postnatal clinic

A low birth weight infant who's Parent/s has agreed to participate after written informed consent given.

5.5 Exclusion criteria

Infants born with any severe congenital abnormalities

Infants who had meningitis, severe birth asphyxia or convulsion at birth

Infants with acute illness at the time of consultation e.g. if infant had fever, convulsion, or any other symptom suggesting acute inflammation.

Infants whose caretaker could not provide details in the structured questionnaire

5.6: Sample size

Sample size for this study was calculated using the formula for cross-sectional study;

$n= z2\ p\ (1-p)/\ \varepsilon 2$

Where n =expected minimum sample size

Z = standard normal deviate, corresponding to 95% confidence; 1.96

ε = maximum likely error taken as 6%

p= Expected proportion of poor Neurodevelopment among iron deficiency premature infants= 51.45%[43] Hence, minimum sample size calculated was 270 premature infants.

5.7 Sampling procedure

All children meeting the inclusion criteria were consecutively enrolled into the study after parents had given an informed written consent until the sample size was reached. The enrolment was done by identifying low birth weight infants using the RCH-1 card. The neurodevelopment was assessed and the questionnaire administered. Children were enrolled into the study every Monday and Friday, between 9 am and 3 pm complying with the planned post-natal high risk clinic days at MNH. Only consecutive 5 children were taken because of the time taken to administer the Bayley scale of infant and toddler development. A thorough history and physical examination was done to all babies to provide the routine follow up as well as assure inclusion and exclusion criteria.

5.8: Definition of terms

Preterm delivery is live born infants delivered before 37 weeks from the 1st day of the last normal menstrual period.[1]

Low birth weight is when a baby has a birth weight of <2500g.[2]

Iron deficiency anemia was defined as hemoglobin level of <11g/dl, low ferritin levels (≤ 12ug/)[30]

Iron deficiency was defined as serum ferritin levels of ≤12ug/dl.[30]

Acute illness was defined as an illness that starts very rapidly and is of short duration and was considered if infant presented with fever, convulsion, cough, diarrhea, skin lesions and unable to breastfeed[2]

5.8.1: Data collection:

The investigator assessed all children who fulfilled the inclusion criteria. All the required information was recorded in a standardized structured questionnaire designed for the purpose of the study (appendix I). Demographic characteristics such as age, sex, gestation age, residence, and birth weight were recorded from the child's RCH card no. 1. The gestation age was calculated from the mother's last normal menstrual period and this was confirmed from the mother's card and if mother was not having a card she was then told to bring it on the next planned visit. Thorough physical examination was done to detect any sign of presence of illnesses and the finding was noted in the child follow up card. If any sign of a disease was found the infants was treated accordingly as per protocol of the given disease. Child's weight, occipital frontal circumference (OFC) mid-upper arm circumference (MUAC) and length were measured. Blood samples were taken to determine FBP and Serum Ferritin.

5.8.2: Weight

Weight was taken using a 25 kilogram Salter hanging scale. (Weighing equipment, High Holborn, London, United Kingdom) with a child putting on light clothing and no shoes. Older children who could not fit into the weighing pants of hanging scale were weighted by a standard beam balance (SECA) to the nearest 10 grams. Calibration of the weighing scales to 0 was performed before each session. For accuracy and consistency, the scales were standardized using a known weight of 1kg every day.

5.8.3: Length

Parent/s assisted in removing children's shoes and gently laying the child in supine position on the length board with their heads placed at 90º touching the fixed head piece. The investigator straightened the legs of the child at the knees and ensures that the feet were at right angles (90º) to the sliding foot piece brought into contact to the child's heels. Length was recorded to the nearest 10 millimeters

5.8.4: Occipital frontal circumference (OFC) and mid upper arm circumference (MUAC)

This was measured using a graduated non-elastic measuring tape and recorded to the nearest 10millimeters

All information obtained was entered in a structured questionnaire.

Interpretation of nutritional status

Using "Epi Nut" programme on the "Epi Info" statistical package version 6.01, Weight for

Length Z-score (WHZ) was calculated.

• Z - scores was interpreted as follows:

• Z – Above median -1SD = Normal nutritional status

• Z - Between -1.0SD & -3SD = Moderate wasting

• Z - Below <-3.0SD = Severe wasting

All anthropometric measurements were corrected for gestational age for each infant

5.8.5: Specimen collection

About 3 milliliters of venous blood was taken from the anterior cubital fossa of each child using a sterile syringe and needle after a thorough cleaning of the puncture site with a swab soaked in 70% ethyl alcohol. 2 mls of blood was dispensed in a 5 ml an EDTA purple top vacutainer tube for FBP. The Full Blood Picture consisting of Hemoglobin estimation (Hb), White Blood Cell count (WBC), Red cell indices (mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC), and Haematocrit (Hct) was determined by coulter counter model-s. The hematological indices (WBC, RBC count, MCV, MCH, MCHC and RDW) were performed for the aim of routine management of the infants with deranged hematological indices. The remaining 3 mls of blood was dispensed in a 5 ml plain red top vacutainer tube for serum ferritin. The collected blood samples were kept in the specimen container till the end of the clinic, and they were sent to the laboratory for processing and testing. At laboratory, the serum ferritin was analysed by Electrochemiluminescence immunoassay (ECLIA) on the Elecsys 2010 and cobas e immunoassay analyser machine. Patients were given a date to come to clinic after one month for laboratory results.

The FBP and Serum Ferritin were performed at Central pathology laboratory (CPL) and Special paediatrics laboratory (SPL) of Muhimbili National Hospital respectively.

5.8.6: Data Collection on neurodevelopment

The neurodevelopment of infants from one month until one year of age was assessed by using Bayley Scale of Infant and toddler Development version III (BSID III) (Appendix III). BSID III was administered to the infants and the information on the score of the each component was entered on the recording form. The total raw score of cognitive, language and motor was calculated by summing up of subtest scores in the specified item. Then the cover page of the recording form was completed by calculating the scaled scores, composite scores, percentile rank and confidence interval. The three parts of BSID which are cognitive scale, language

scale, and motor scale, were assessed by the principle investigator after being in training for two weeks on the use of BSID III. Two researches assistant who participated in this study had been trained by Professor David Belly of Health management system (HMS) and were involved in a large clinical trial setting. They had considerable experience in the administration of the BSID III in the assessment of neurodevelopment. I performed an internal quality control in ten infants selected randomly to make sure that there was no investigator bias and these were well matched. The assessor made age adjustment for the prematurity for every infant and was used for all calculation in the study.

Bayley scale of infant and toddler development III have five components which are cognitive, language, motor, social-emotional and adaptive behavior. This study looked at three components of neurodevelopment which are cognitive, language and motor due to the fact that these have been postulated to be more affected by iron deficiency than the social-emotional and adaptive behavior.

5.8.8; Interpretation of neurodevelopment status

The percentile rank of ≥85 was considered as normal neurodevelopment status

The percentile rank of 70-84 was considered as impaired neurodevelopment status

The percentile rank of <70 was considered as significant impaired neurodevelopment status

5.9: Data analysis plan:

Data were cleaned, entered, and analyzed using EPI Info version 6.01 (CDC, Atlanta, USA) and the Statistical Package for Social Scientists (SPSS) version 15 (Chicago, II). Demographic characteristics of current age, sex, gestation age at birth, maternal age and anthropometric measurement was summarized using frequency distribution and cross tabulation tables. Chi-square test was used to asses association between neurodevelopment to current age, gestational age, maternal age and nutritional status of the infant. And p-value < 0.05 was considered significant. The association between neurodevelopment of the infant and the iron status was also assessed by Chi-square and p-value of < 0.05 was considered significant. Correlation

between individual neurodevelopment components (motor, cognitive, language) were assessed by cross tabulation tables and then using Chi-square test. Multiple regression analysis modal was performed to assess the relationships found between neurodevelopment and nutritional status, gestation age, current age, maternal age and sex of infant. The associations were presented as odds ratio (OR) together with 95% confidence interval (CI) and was considered significant if the corresponding 95% CI did not include one.

5.10: Ethical clearance and Ethical consideration

Ethical clearance to conduct research was obtained from the Higher Degree Research and Publication Committee of the Muhimbili University of Health and Allied Sciences, Dar es salaam, Tanzania. Permission to conduct the study at the postnatal clinic was obtained from MNH.

Written informed consent to participate in the study was obtained from parents prior to enrolment in the study. A complete description of the aims of the study, and assurance of confidentiality of any information given was provided to parents in order to facilitate informed choice. The Investigator provided any other requested additional information to parents.

6.0. RESULTS

During the study period, 270 children were studied of whom 124 (45.9%) were males and 146 (54.1%) were females providing a male to female ratio of 0.8:1. Most of the children, 153 (56.5%) were in the age group of 1-4 months and the mean age was 4 months. Seventy four percent of infants studied had gestation age ≤34 weeks and 66.3% weighed between 1.5-2.5 kg. Most (65.2%) infants had moderate to severe wasting by weight for height Z-score. Majority of infants 169(62.6%) were not on iron supplementation at the time of the study. (Table 1)

Table 1**;** Demographic basic characteristics of the study population (n=270)

Variable	n	%
Sex		
Male	124	45.9
female	146	54.1
Age of infant(months)		
1-4	153	56.7
5-8	93	34.4
9-12	24	8.9
Nutritional status (Z score)		
Normal	94	34.8
Moderate/severe wasting	176	65.2
Gestational age(weeks)		
28-30	79	29.3
31-34	121	44.8
35-38	70	25.9
Birth weight (Kg)		
0.8-1	15	5.6
1.01-1.49	76	28.1
1.50-2.50	179	66.3
Iron supplementation		
Given	101	37.4
Not given	169	62.6

Cognitive, language and motor development was significantly associated with age (p=0.0001). Expression of poor cognitive, language and motor development is noted with increasing age. While sex had no significant association with cognitive development, (p=0.213) it is significantly associated with language development (p=0.05) and motor development (p=0.006). Females expressed more poor language and motor development scores than males. (Table 2)

Of 270 infants, 243 (90%) had poor cognitive development, 160(59.3%) had poor language development and 238(88.1%) had poor motor development. (Table 2)

Table 2; Cognitive, language and motor development in relation to age and sex

variable	Number of babies with Poor cognitive score			Number of babies with Poor language score		Number of babies with Poor motor score	
	Total	N (%)	p-value	N (%)	p-value	N (%)	p-value
Sex							
Male	124	110(85.7)		64(51.6)		106(85.5)	
Female	146	133(98.9)	0.213	96(65.8)	0.05	132(90.4)	0.006
Total	270	243(90)		160(59.3)		238(88.1)	
Age (months)							
1-4	153	144(94.1)	0.0001	76(48.7)	0.0001	123(90.4)	0.0001
5-8	93	75(80.6)		66(71.0)		92(98.9)	
9-12	24	24(100)		18(75.0)		23(95.9)	

Infants with iron deficiency and significant poor cognitive percentile score were 37% compared with 18% of iron deficient infants with normal cognitive percentile scores, p=0.007. Infants with iron deficiency and significant poor language percentile score were 35% compared with 31% of iron deficient infants with normal language percentile scores, p=0.04 Motor development was not significantly different among iron sufficient and iron deficient infants (p=0.94) (Table 3a)

Table 3a: Association between infant's iron status and cognitive, language and motor percentile scores

Iron status	Neurodevelopment percentile scores								
	Cognitive[a]			Language[b]			Motor[c]		
	<70	70-84	>84	<70	70-84	>84	<70	70-84	>84
	Number of Infants (%)								
ID	65(37)	22(34)	5(18)	35(35)	23(40)	34(31)	62(34)	19(35)	11(35)
Non ID	112(63)	43(66)	23(82)	66(65)	35(60)	77(69)	122(66)	36(65)	20(65)

[a]p=0.007, [b]p=0.04, [c]p=0.94, ID= Iron deficient infants

Ninety two infants (34.1%) had iron deficiency. Most infants (86%) who had anemia had significantly (p=0.001) low ferritin levels as compared with non anemic infants. About 55.4% of infants that received iron supplementation had anaemia where as 73.2% of babies that did not receive iron supplementations were anaemic. (p= 0.004) (Table 3b)

Table 3b: Anemic infants in relation to their serum ferritin and iron supplementation status

Number of infants with characteristic	Anemic infants(179)	Non anemic infants(91)		
	Number (%)	Number (%)	Total	p-value
Serum ferritin				
Low	79(86.2)	13 (13.8)	92(34.1)	
Normal	100(56.2)	78(43.8)	178(65.9)	0.0001
Total	179(66.3)	91(33.7)	270(100)	
Iron supplementation status				
Given	56(55.4)	45(44.6)	101(37.4)	0.004
Not given	123(73.2)	46(26.8)	169(62.6)	
Total	179(66.3)	91(33.7)	270(100)	

Median serum ferritin levels in poor cognitive developed infants was statistically significantly low (10.9µg/dl) as compared with normally cognitive developed infants who had 80.6µg/dl (p=0.0007). In poorly language developed infants the median serum ferritin levels was 10.5µg/dl compared to 59.4µg/dl in normal language developed infants (p=0.00004). Poor motor developed infants had median serum ferritin level of 11.9µ/dl and normal motor developed infants had median serum ferritin of 9.7µ/dl. (p=0.34). The median hemoglobin levels of infants who had poor cognitive, language, and motor development was not significantly different from the median haemoglobin level of normally developed infants. The Median OFC for infants with percentile sore ≤85 in cognitive, language and motor development was 2 percentile and this was statistically significant (p=0.00008). The proportion of infants who had microcephaly was 36.4%. (Table 4)

Table 4: Cognitive, language and motor development in relation to serum ferritin levels Hemoglobin and occipital frontal circumference (OFC).

	Cognitive development		Language development		Motor development	
	percentile score		percentile score		percentile score	
	≥85	≤84	≥85	≤84	≥85	≤84
Characteristics	Median(R)	Median(R)	Median(R)	Median(R)	Median(R)	Median(R)
Serum ferritin(µg/L)	80.6(120)	10.9(221)[a]	59.4(220)	10.5(147)[a]	9.7(118.0)	11.9(221)[b]
Hemoglobin (g/dl)	10.95(13)	10.3(14)[b]	10.8(14)	10.2(14)[b]	10.7(9.1)	10.3(18)
OFC(percentile score)*	98(12.3)	2(4)[a]	98(10)	2(3)[a]	98(11)	2(2)[a]

[a]Differences are significant with p<<0.05
[b]Differences are not statistically significant
*Prevalence of microcephaly was 36.4%.

The gestation age was not correlated with poor cognitive development with OR of 0.62 and 95% CI of 0.23 to 1.71. Low maternal age was significantly correlated with poor cognitive development (OR =0.08 and 95% CI 0f 0.01 to 0.62. Nutritional status (Wasting) was not significantly correlated with poor cognitive development with OR of 0.48 and 95% CI of 0.21 to 1.09. Iron supplementation was significantly correlated with normal cognitive development and non iron supplemented infants had poor cognitive development (0R of 0.17 and 95% CI of 0.07 to 0.43). Infants with 0.8-1.49kg of birth weight had more poor cognitive score as compared to 1.50-2.50kg of birth weight infants (p-value of 0.026). Mother's education had no significant association with cognitive development of the infant. (Table 5a)

Table 5(a): Correlation between Cognitive development and maternal age, gestational age, birth weight, infant's nutritional and iron supplementation status and mother's education

variable	Infants with Poor cognitive score				
	Total	N (%)	p-value	OR	95% CI
Gestational age(weeks)					
28-34	200	178(88.9)			
35-38	70	65(92.8)	0.821	0.62	0.23-1.71
Maternal age(years)					
16-35	256	230(89.8)			
35-46	14	13(92.8)	0.892	0.08	0.01-0.62
Nutritional status(Z)					
Normal	94	79(83.0)			
wasted	176	163(90.9)	0.006	0.48	0.21-1.09
Iron supplementation					
Given	101	81(80.2)			
Not given	169	162(95.8)	0.006	0.17	0.07-0.43
Birth weight(kg)					
0.8-1.49	91	87(97.8)			
1.50-2.50	179	156(87.4)	0.026	3.21	1.07-9.57
Mother education					
Non/Primary school	200	182(91.0)			
Secondary/Post-secondary	70	60(85.7)	0.3	0.59	0.26-1.36

Table 5(b); Correlation between Language development in relation to maternal age, gestational age, birth weight, infant's nutritional and iron supplementation status and mother education.

variable	Infants with Poor language score				
	Total	N (%)	p-value	OR	95% CI
Gestational age(weeks)					
28-34	200	126(62.8)			
35-38	70	34(42.5)	0.041	1.8	1.04-3.14
Maternal age(years)					
16-35	276	156(61.0)			
35-46	14	4(29.2)	0.194	3.90	1.19-12.77
Nutritional status(Z-score)	94	38(40.4)			
Normal	176	122(74.4)	0.001	0.3	0.18-0.51
wasted					
Iron supplementation					
Given	101	46(45.6)			
Not given	169	114(67.4)	0.001	0.4	0.24-0.67
Birth weight(kg)					
0.8-1.49	91	62(85.5)			
1.50-2.50	179	98(54.9)	0.016	1.77	1.04-3.0
Mother education					
Non/primary school	200	119(59.5)			
Secondary/post-secondary school.	70	40(57.1)	0.83	0.91	0.52-1.57

Lower gestation age is correlated with poor language development (OR, 1.80, 95% CI of 1.04 to 3.12). Lower maternal age was correlated with poor language development (OR, 3.90, 95% CI of 1.19 to 12.77). Nutritional status (wasting) was significantly correlated with poor language development (OR, 0.30, 95% CI of 0.18 to 0.51). Iron supplementation was significantly correlated with normal language development and non iron supplemented infants had poor language development (OR, 0.40, 95% CI of 0.24 to 0.67). Birth weight of the infant was significantly correlated with poor language development where the lower birth weight infant had poor language development (OR, 1.77, 95% CI of 1.04 to 3). Mother education had no significant association with the language development (Table 5b)

Gestation age at birth was not significantly correlated with poor motor development (OR, 0.83 and 95% CI of 0.41 to 1.69). Advanced maternal age was significantly associated with poor motor development (OR, 2.29, 95% CI of 1.18 to 4.5). Nutritional status of the baby was significantly correlated with poor motor development where the wasted infants were found to have poor motor development (OR, 0.45, 95% CI of 0.22 to 0.94). Non iron supplemented infants had poor motor development as compared with iron supplemented infants. (OR, 0.23, (95% CI of 0.10 to 0.50). Birth weight was not significantly correlated with poor motor development (OR, 1.51, 95% CI of 0.61 to 3.71). Mother's education level was not significantly associated with poor motor development (Table 5c)

Table 5(c): Correlation between Motor development and maternal age, gestational age, birth weight, infant's nutritional and iron supplementation status and mother education.

variable	Infants with Poor motor score				
	Total	N (%)	p-value	OR	95% CI
Gestational age(weeks)					
28-34	200	160(90.2)			
35-38	70	58(82.8)	0.568	0.83	0.41-1.69
Maternal age(years)					
16-35	256	224(87.5)			
35-46	14	14(100)	0.719	2.29	1.18-4.5
Nutritional status(Z)					
Normal	94	77(81.9)			
wasted	176	160(9316)	0.001	0.45	0.22-0.94
Iron supplementation					
Given	101	79(78.3)			
Not given	169	159(93.1)	0.0001	0.23	0.1-0.5
Birth weight(kg					
0.8-1.49	91	84(95.4)	0.02		
1.50-2.50	179	154(86.0)		1.51	0.61-3.71
Mother education					
Non/primary school	200	177(88.5)			
Secondary/post-secondary school	70	62(88.6)	0.84	0.99	0.42-2.33

Iron deficiency is significantly related with birth weight as 66.7% of 0.8-1kg infants had iron deficiency as compared with 30.2% of 1.5-2.5kg infants having iron deficiency (p=0.0013).

Also the nutritional status of the baby was related to iron deficiency where as iron deficiency was observed more in the severe and moderate malnutrition than in the normal nourished infant.(p=0.13). The iron supplementation had no significant relation with iron status of the infant (p=0.81). Among the infants born at gestation age of ≤34 weeks 58(47%) had iron deficiency as compared to 34(23%) infants born at gestation age of >34 weeks and this was statistically significant (p=0.0004) (Table 6)

Table 6; Iron status of infants in relation to the birth weight gestational age, iron supplementation and nutritional status

	Infant's Iron status		
Variable	Iron deficient	Normal iron status	p- value
Birth weight (Kg)			
0.8-1.0	10(66.7%)	5(33.3%)	
1.01-1.49	28(50%)	28(50%)	
1.50-2.5	54(30.2%)	125(69.8%)	0.0013
Nutritional status(Z-score)			
Normal	39(42.0%)	54(58%)	
Moderate	41(29.3%)	99(70.7%)	
Severe	12(32.4%)	25(67.6%)	0.13
Iron supplementation status			
Given	33(32.7%)	68(67.3%)	
Not given	59(34.9%)	110(65.1%)	0.81
Gestation age (weeks)			
≤34	58(47%)	66(53%)	0.0004
>34	34(23%)	112(78%)	
Total No of premature infants were 264 (97.8%)			
Total No of LBW infants were 263 (97.4%)			

Table 7: Multiple logistic regression analysis of cognitive, language and motor development in relation to the maternal age, gestational age, birth weight, infant's nutritional and, iron supplementation status and ferritin levels

	Cognitive development			Language development			Motor development		
Variable	S.E	β-Coefficient	p-value	S.E	β-Coefficient	p-value	S.E	ß-Coefficient	p-value
A	0.03	0.066	0.27	0.05	-0.025	0.663	0.03	0.109	0.062
G	0.025	0.083	0.19	0.04	-0.061	0.311	0.03	-0.094	0.129
B	0.032	-0.157	0.01	0.05	-0.055	0.369	0.04	-0.031	0.620
I	0.037	0.239	0.00	0.06	0.206	0.000	0.04	0.237	0.000
T	0.028	0.063	0.29	0.04	0.249	0.000	0.03	0.105	0.078
S	0.001	-0.067	0.31	0.02	-0.028	0.693	0.01	-1.39	0.166
F	0.89	0.274	0.00	0.11	0.16	0.023	0.08	-0.157	0.079

Where "A = Maternal age, G= Gestation age, B= Birth weight, I= Iron supplementation status and T= nutritional status, S=infant's sex, F= ferritin levels, ß-coefficient= ß-Standardized Regression coefficient, S.E-standard Error

In multiple regression analyses birth weight and iron supplementation status were the predictors of poor cognitive development. Iron supplementation and nutritional status of the baby were the predictors of poor language development. Iron supplementation was the only significant predictor of poor motor development. Low serum ferritin levels were statistically significantly associated with poor cognitive and language development scores in the low birth weight infants. Low ferritin levels were not significantly associated with poor motor development scores. (Table 7)

7: DISCUSSION

7.1. Description of the study population

The study population comprises of low birth weight infants aged between 1-12 months attending high risk postnatal clinics at a tertially health facility in Dar es salaam, Tanzania. Babies excluded were those found with acute febrile illness at time of data collection, severe illness at birth like birth asphyxia, meningitis, congenital malformation and severe jaundice. Besides iron status, low birth weight and their correlation to neurodevelopment, other factors which may be correlated were also studied.

The study population had a male to female ratio of 0.8 to 1. This is in contrast to the finding in the study done by Akman M et al and Georgieff MK et al reporting a predominance of males with the male to female ratio of 1.3:1 and 4:1 respectively[44, 45]. The mean age of studied infants in this study was four months while the mean age of studied infants in the study by Georgieff MK et al was 9 months. The difference could be explained by the fact that mothers in our facilities stop to attend the high risk postnatal clinic after the last routine immunization at 9 months. Many previous studies involved older infants and children for example Caravale B involved children 3-4 years[13], 5.3 corrected years in Steinmacher study[46] and 5 years in Goldeberg study[47]. Few studies were performed during infancy, for example the Israel study was done at the age of one month[43].

Most infants (66%) had a birth weight of 1.5-2.5kg with mean of 1.6kg in our study. This show that our study population had infants with higher birth weights compared to other previous Israel and Australian studies which showed the mean birth weight of 1.3kg and 0.959kg respectively[43, 48].

In this study most studied infants were born before 34 weeks of gestation and this compares well with a number of earlier studies[13, 43, 48, 49] in which the gestation age ranged between 28 weeks and 32 weeks. Premature delivery among other things may be a result of the high level of maternal diseases i.e. pregnancy induced hypertension, antepartum hemorrhage, maternal anemia and low social economic status of parents in the study site[50].

About 65% of infants in this study had moderate to severe wasting and this finding have also been observed in studies done by Wiggens RC et al[21], Maureen et al[22] and Dobbing et al[41]. These finding can be explained by the low social economic status of the people in study sites. (Table 1)

7.2. Cognitive, language and motor development

A high prevalence of poor cognitive, motor and language development was observed in the study patients with prevalence of 90%, 88.2% and 59.2% respectively. A high magnitude of cognitive and motor dysfunction is probably a result of the predominance of premature babies (97.8%) in the study patients. Poor neurodevelopment outcome of preterm infants are due to immature organ system not being able to sustain extra uterine tasks, adverse effects of obstetrics and neonatal treatments and genetic factors. Abbort et al studying VLBW and ELBW babies at age 18-22 months presenting with normal head sonographs observed similar finding and attributed the outcome, to prematurity, hospital neonatal care and social economic characteristic[10]. Other studies that looked at babies born at gestational age ≤34 weeks at a later age also showed significant cognitive and motor dysfunction that continue to manifest even during school age period[14, 47, 51, 52]. The prevalence of poor neurodevelopment in these studies was much lower than that observed in this study. Low socioeconomic status is known to affect cognitive and language development of children. Since in our environment low socioeconomic status is the public problem then this may explain the difference of high prevalence of poor cognitive, language and motor development in infants studied in our study.

Likewise low birth weight probably resulting from intrauterine growth retardation was also associated with high rate of poor neurodevelopment which agrees with findings in earlier studies[15, 47]. The other contributing factor for high prevalence of poor neurodevelopment in our study was microcephaly (low percentile score for OFC). The proportion of infants with microcephaly in our study was 36.4%higher than that of 23% observed in the Israel study[43]. In both studies microcephaly contributed to poor neurodevelopment and this has been attributed to the reduced brain mass that is associated with loss of function corresponding to the missing part of the brain. The high percentage of infants not being on iron supplementation may also be the

contributory factor for high prevalence of poor neurodevelopment in our study. The high level of malnutrition in the community may also contribute to the high prevalence of poor neurodevelopment in infants since it have shown in different studies that the high level of malnutrition in a country is also responsible to the high prevalence of poor neurodevelopment[20-22, 41].

7.3. Prevalence of iron deficiency

This study detected a high prevalence of iron deficiency (34.1%), despite the fact that 37% of the infants gave a history of receiving prophylactic iron supplementation as required by the National guidelines. In this study, iron deficiency is significantly related to birth weight as 80.5% of 0.8-1kg infants had iron deficiency compared with 42% of 1.5-2.5 kg infants. (P=0.019). In Australian study the iron deficient infants was more seen in the extreme low birth weight infants[48]. The study done on extreme premature infants aged 23 -24 months in Australia by O'Keeffe MJ et al showed that the prevalence of iron deficiency in premature infants was 21%[48] very much lower than the prevalence of 34.1% found in this study. The presence of maximal iron accretion during the last trimester, particularly after 34 weeks and a high physiological demand during the catch up growth, leads to a high prevalence of iron deficiency in premature babies[53]. Both these studies indirectly confirm that the intrinsic nature of the preterm to become deficient and the role of rigorous supplementation have a role in improvement of iron status in the body and subsequent neurodevelopment of these infants. The difference in prevalence levels however, may be partly due to the differences in the methods used for assessing the iron status whereby the Australian study used Zinc protoporphyrin while our study used serum ferritin. Although most infant studied were below 34 weeks of gestation, 63% of them were not iron supplemented. Iron accretion is maximal during the last trimester, particularly after 34 weeks, therefore lack of supplementation at 4-6 weeks of birth predisposes them to develop anemia[31]. About 48% of infants who received iron supplementation were found to be iron deficient at the time of assessment and 43.8% of infants with history of no iron supplementation were also found to be iron deficient. Previous randomized controlled study indicated that iron supplementation improved iron status of infants and children[46, 54].

The absence of significant difference could be partly attributed to possibly unreliable obtained history of taking iron. May be a question regarding the color of the stool would have helped in identifying poor compliance to iron intake. The lack of significant difference in levels of body iron between iron supplemented and non iron supplemented infants could be due to inadequate drug supplies to infants to last to the next clinic appointment. The absence of iron supplementation in a number of infants indicates a Maternal Child Health Programme failure on this part and calls for agent attention

7.4. Cognitive, language and Motor development in relation to age and sex of infant

This study showed that the more the advanced age of the infant the more poor cognitive, language and motor development is likely to manifest and this is also related to their iron status. This implies that prolonged iron deficiency states has a role to play in neurodevelopment[2]. Breast milk provides biological highly available iron, but does not meet the needs beyond six months of age and complementary foods have significant iron absorption inhibitors[2]. Iron supplemented babies had better neurodevelopment than non iron supplemented babies. Similar observation was noted in a study by Black et al[24] where Bangladeshi babies supplemented with iron and zinc had improved motor development.

This study using the simple correlation figures showed that females had poor cognitive, language and motor development score than males with p= 0.213, 0.05 and 0.006 respectively. These differences in neurodevelopment between the genders were not statistically significant on further analysis using logistic regression, and thus sex cannot be considered a confounding factor (Table 2 and 7). In the study done by Hinz et al[55] showed that the males are more prone to poor neurodevelopment as compared to females. The reason for the difference may not be obvious and these need prospective study to know if gender has an influence on neurodevelopment.

7.5. Cognitive, Language and motor development in relation to iron status

Iron deficient infants had lowered cognitive and language scores than iron sufficient infants. Iron deficiency was not observed to influence motor development scores. Tamura et al also

observed only poor cognitive and language development among iron deficient 5 year old children[56]. Several studies cited earlier have shown that cognitive and language development is significantly poorly developed among iron deficient infants. These studies are variable in the age and their cohorts, but have similar finding[16, 17, 20, 53, 57, 58]

Iron deficiency identified using serum ferritin concentration was not associated with poor motor or language scores at baseline in a study done in Zanzibar[54](p=0.071). Our study however showed that infants with iron deficiency had more language disability than infants with normal iron status and the difference was statistically significant (p=0.009). The difference noted in the two studies might be due to the different methods of assessment of the neurodevelopment used. In the Zanzibar study parents were asked to report on the gross motor and language milestones to assess the neurodevelopment in full term born children where as in current study Bayley scale of infant and toddler development version III was used on premature and low birth weight infant. Similar finding were reported in the Australian study that used similar laboratory method for assessing iron status and clinical method for assessing the neural development (Bayley scale of infant and toddler development version III) for VLBW babies at 12-24 months of age[48]. A study in Israel assessing 3 weeks old newborn observed similar finding on the poor performance of premature infants in terms of reflexes in the iron deficiency group as compared to normal iron status infants[43]. The level of hemoglobin in our study did not appear to influence cognitive, language and motor development. However levels of ferritin were significantly associated with cognitive and language percentile score. Serum ferritin levels below 12µg/dl indicate iron deficiency. The study in Turkey[44] showed that iron deficiency anemia was associated with poor motor development in children assessed at 5 year. However iron deficiency alone did not seen to influence motor development. This implies that the observed motor dysfunction is related to anemia. A causal link between iron deficiency anemia and delays in child development may be mediated by a variety of direct or indirect pathways; the most obvious are associated decreases in hemoglobin concentration and oxygen delivery to tissues in the brain. Alternative theories relate to reductions in cerebral iron concentrations, including hypomyelination and impaired dopaminergic function[59-61]. The

absence of motor dysfunction might imply that infant studied had satisfactory minimum level of hemoglobin.

Animal models may provide a framework for understanding the mechanisms that underline these findings. Iron is required for normal myelinogenesis[19-21]. Similarly studies in human infants have presented clinical evidence of poor myelination. Although I postulate that a delay in myelination is the most likely explanation for my findings, there is no way to confirm that this is the only mechanism. In addition to its role in the production of myelin, iron is involved in the function of neurotransmitters, such as dopamine and serotonin. Earlier rodent studies documented the effect of decrease in brain iron on a variety of dopaminergic functions. More recent studies on iron status and the developing brain showed that perinatal iron deficiency affects dendritic growth and structure, neural metabolic activity, and synaptogenesis[18-21]. It may be hypothesized that these neural processes may also play an important role in determining the effect of low iron on poor neurodevelopment. Further studies with more specific measures of neurodevelopment and potential confounders are needed to confirm the exact nature of the causal relations between low iron status and neurological impairments in premature infants. Further studies are needed to explore the different mechanism that underline iron status in premature infants (blood loss, iron intake, maternal iron status, and iron therapy) and the relevance of these factors to the neurodevelopmental outcomes.

7.6. Predictors of poor cognitive, language and motor development of infants

7.6.1. Iron supplementation and neurodevelopment of low birth weight infants

Iron supplementation has been shown to have impact on the neurodevelopment of premature or low birth weight infants on all cognitive, language and motor development with OR of 0.17(95%CI 0.07-0.43),0.4(95%CI 0.24-0.67) and 0.23(95%CI 0.1-0.5) respectively. The multiple regression analysis confirmed this finding. This shows that the absence of iron supplementation in infant in current study was a predictor of poor cognitive, language and motor development. This finding was observed in previous studies done in Bangladesh[24], Zanzibar[54] and Germany[56]. The Costa Rica[16] study showed that there were no improvements in

cognitive and motor scores in both iron deficient and normal iron status children after 3 months of iron supplementation. The difference in the effects of supplementation of iron to premature or low birth weight infants may be due to delay in starting supplementation because it has been shown[46] that the late enteral iron supplementation is associated with failure to correct the already present neurodysfunction. This has been found in rat studies in which rat brain and human brain have a number of similarities in that both involve considerable postnatal development which make a rat brain a useful model. At birth in both species the blood brain barrier is incomplete[62] and lacks the ability to regulate the transfer of material from the blood to the interstitial fluid of the brain. The blood-brain barrier matures within 7 to 10 days of birth in the rat and may take up to 6 months in the human[62]. Thus the ability to regulate brain iron availability according to need may not be well developed in early life[63] and infant rats and humans may be susceptible to the effects of iron deficiency in early neonatal period. The premature infant with an incomplete blood-brain barrier when subjected to iron deficiency would be most vulnerable. The brain of the developing rat adapt to iron deficiency and repletion by regulating iron availability on a regional basis according to need[63] Whether iron supplementation is able to correct neurodevelopmental effects of iron deficiency may depend upon the point at which iron supplementation occurs relative to the developmental stage of the brain region at the point of supplementation. Studies in rat pups born to iron-deficient dams showed that repletion of iron commencing at post-natal day 4(i.e. before the peak iron demand) was able to correct the effect of iron deficiency on both iron levels and monoamines function in various brain regions. Giving iron around postnatal day 21(i.e. beyond the peak in iron demand) was unable to completely correct for the deficits in monoamine function despite correcting iron levels[64]. There are limited studies on human infants but for those few done have shown some beneficial effect on cognitive and motor development after initiating early iron supplementation at 6 weeks postnatal as compared with late enteral supplementation

7.6.2. Gestation age at birth, maternal age, birth weight and nutritional status of infant

In this study the gestation age at birth of the infants was not statistically significantly associated with cognitive and motor development of the infant. This observation is supported with studies in Italy[51] and USA[10] in which the cognitive and motor score was lower in preterm children as compared to full term children. In the current study, language development was poor at lower gestational age although in multiple regression analysis gestational age was not a significant predictor of poor neurodevelopment. This is similar to the Italy study which indicated that preterm infants had poor language development[51].

Multiple regression analysis indicated that maternal age was not a predictor of poor language and motor development in premature/low birth weight infants. But low maternal age was correlated with poor cognitive development although this effect was not supported by the multiple logistic regressions. In the other studies low maternal age and more advanced age have been associated with poor neurodevelopment for their infants.[15, 47]

The birth weight of the infant was found to be a negative predictor of poor cognitive development but not of language and motor development. These findings are consistent with previous studies where an increase in birth weight was related to better psychomotor development outcomes as measured by the PDI scores of the BSID-II (r=0.54, P=.008)[11, 12, 51, 57, 65]. In contrast, increased birth weight was not significantly related to overall motor impairment (r=0.25, P=.53). These findings indicate that having a lower birth weight and a lower gestational age are related with poor motor outcomes in the first years of development but the biological basis is not clear.

Nutritional status (wasting) was positive predictor of poor language development and was not significantly associated with the cognitive and motor development. The study population was mostly having malnutrition which in the previous studies has shown to have role on the neurodevelopment[47]. Low body protein, fat and carbohydrates act as raw material and building block of the brain through metabolic processes[2].

8. Study limitation

This was cross sectional study, so it was difficult to follow-up the effect of iron deficiency on the causation of poor neurodevelopment of infants over a period of time. Many other iron deficient children may have been lost to follow-up in the high-risk clinic, thus we may be seeing only those who are relatively healthy to return to clinic. Ethical principles would not allow to keep iron deficient children remain so, to see the effects in long-term, since it is standard of care to supplement infants who were born preterm/low birth weight. Longitudinal studies could have been the ideal to study the effects of iron deficiency in infancy and long-term outcomes.

9. Conclusion

There were high prevalence of poor cognitive (90%), language (59.3%) and motor (88.1%) development in the premature and low birth weight infants. About 93% and 69% of babies with iron deficiency had poor cognitive and language development respectively with BSID III score of ≤84%. The iron status of infants was not associated with motor development. The non-iron supplemented infants were found to have poor BSID III score of ≤84 in cognitive and language development but not with motor development. Wasting was a predictor of poor language development but not of poor cognitive and motor development where as the birth weight was a negative predictor of poor cognitive development, maternal age and gestational age was not predictor of cognitive, language and motor development.

10. Recommendation

The recommendations of iron supplementations among preterm infants and low birth weight infants during post-natal period should be emphasized. Where iron supplementation should be started at 6 weeks and all health workers should be aware about this practice and give adequate advice to mothers on importance of daily iron supplementation.

Early neurodevelopmental assessment should be done on all preterm/low birth weight infants to identify neurologically challenged infants and to direct them for further stimulation and supplementation, in order to improve neurodevelopment.

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Appendix I: Questionnaire

1 Registration no: _____

2 Serial No _____

3 Residents _____

4 Date of interview: _____

5 Name: _____

6 Tel no: _____

7 Sex:

A Male

B Female

8. Age of infant (months) _____

9 What was the Apgar score of the baby at birth ----------

10 Mothers age ----------

11 Marital status:

A Single

B Cohabiting

C Married

D Divorced

E Widowed

12 Highest formal education achieved:

A None

B Primary School

C Secondary School

D Post Secondary

13 What was your last normal menstrual period before the pregnancy of this child ----------

14 What was the birth date of your child ----------

15 What was the birth weight of your baby ----------

17 When was your infant started iron supplementation ----------

18 Current body weight of your baby is----------

19 Measure the height---------- occipital frontal circumference---------and mid-upper arm circumference of the baby----------

Appendix II: Laboratory Form

Test	Results
Serum ferritin	
Hb	
MCV	
MCH	
MCHC	
RDW	
RBC	

Appendix III; Bayley recording form for Assessment of neurodevelopment of infants

Subtest Summary Scores

Subtest	Total Raw Score	Scaled Score	Composite Score	Percentile Rank…(__ %)	Conf. Interv
Cognitive (Cog)					
Use Table A.S					
Language (Lang)					
Receptive Communicat					
Expressive Communicat					
Sum					
Motor (Mot)					
Fine Motor (FM)					
Gross Motor (GM)					
Sum					
Social – Emotional					
Use Table A.S					
Adaptive Behavior					
Communication (Com)					
Community Use (CU)					
Functional Pre-Academic					

Calculate Age			
Date Tested			
Date of Birth			
Age	Years x 12		
Age in Month			
And Days			
Adjustment for Prematurity			
Adjusted Ag	Calculate start point according to chart below		

Age	
16 days -1 month 15	A
1 month 16 days-2 months 1	B
2 month 16 days-3 months 15	C
3 month 16 days-4 months 15	D
4 month 16 days-5 months 15	E
5 month 16 days-6 months 15	F
6 month 16 days-8 months 30	G
9 month 0 days-10 months 30	H
11 month 0 days-13 months 15	I
13 month 16 days-16 months 15	J
16 month 16 days-19 months 15	K
19 month 16 days-22 months 15	L
22 month 16 days-25 months 15	M
25 month 16 days-28 months 15	N
28month 16 days-32 months 15	O
33 month 0 days-38 months 30	P
39 month0days-42 months 15 days	Q

Appendix IV: Consent Form (English)

Consent to participate in a study of Neurodevelopment of infants born premature/ low birth weight in relation to their iron status in Dar es Salaam, Tanzania.

Introduction

Greetings madam/ Sir,

My Name is Dr Albion Kasasa I am working at the Muhimbili National Hospital and I am involved in a research on neurodevelopment in relation to iron status of infants born premature/ low birth weight in our hospital. In a few minutes, I will tell you about this research and will ask for your consent to participate in this research. I will be happy to respond in case you will have any questions.

Purpose of the study

We are recruiting all infants who were born premature/ low birth weight who are attending MNH – High risk post-natal clinic. From those we shall recruit we want to know how their neurodevelopment is and how it is related to the iron status of the infant

What Participation Involves

Those willing to participate will be interviewed on some questions related to the research then physical examination will be done. Furthermore venopuncture will be performed where 5 mls of blood will be taken for analysis. You will also be treated for other conditions that need treatment as normally done. Before we begin this process the individual must sign a form to indicate their willingness.

Confidentiality

Your name will only appear on one form to create an Identification number. Only the created number will be used in the laboratory forms and in the computer where all the information will be stored.

All information we collect from you will be confidential and will only be used for the purpose of this research and better care and treatment. No one else other than the people involved in the research and health personnel involved in your care will have access to this information.

Benefits and Risks[47]

You will benefits from the study by knowing whether your infant has well or poor neurodevelopment which is related to iron status. Also you will be able to get medical advice anytime you need during the period of the study by communicating with the doctor involved in this research. We hope that the information we shall learn from this research will be useful in terms of offering better care and treatment to your infant.

Apart from benefits infant will feel some little pain when the needle pierces when drawing blood samples.

Voluntary Participation & Rights to Withdraw

Your participation is voluntary and you have the right to discontinue from participating in our study at any time. However your decision may be, it will not affect in any way your rights to care and treatment.

In case of Injury

We do not anticipate any harm as a result of taking part in this research. However, should physical injury occur, we shall provide you with treatment according to the current standard of care in Tanzania.

Contact persons

If you have questions about this study or please do not hesitate to contact:

Prof. E Lyamuya; Director; Research and publication committee; Muhimbili University of Health and Allied sciences (MUHAS); Department of microbiology and immunology; P.O. Box 65001 Dar es Salaam **OR** in case of any information about your rights as a participant in this study please contact

1. The Principal Investigator: Dr. Albion Kasasa, Department of Pediatrics & Child Health

Tel: +255 786881438, +255 759389956, E-mail: albion.kasasa@gmail.com.

2. Prof. Karim P. Manji, Department of Pediatrics and Child Health

3. Dr. Festus M. Kalokola, Department of Department of Pediatrics and Child Health

I _____

Have understood the above information and my questions have been answered to my satisfaction. I agree to take part in this research.

Name of the participant: _____

Signature of the participant: _____

Name of the Witness (if participant cant read)_____

Signature of the Witness: _____

Date of signed consent: _____

Appendix V: Consent form: (Swahili version)

Fomu ya ridhaa katika utafiti wa jinsi madini ya chuma yanavyohusiana na maendeleo ya mtoto katika mfumo wa fahamu katika Hospitali ya taifa ya Muhimbili.

Utambulisho

Habari za saa hizi,

Jina langu ni **Dk Albion Kasasa** nafanya kazi katika hospitali ya taifa ya Muhimbili na pia ni mtafiti katika utafiti wa namna ukosefu wa madini ya chuma yanaweza kuleta maendeleo duni katika mfumo wa Fahamu kwa watoto waliozaliwa bila tarehe zao za kuzaliwa hazijatimia.Sasa nitakupa maelezo ya kina kuhusu utafiti huu na kisha nitakuomba ridhaa yako ya kushiriki katika utafiti huu. Endapo utakuwa na maswali yoyote nitafurahi kukujibu.

Malengo ya utafiti.

Utafiti huu unafanyika kwa watoto waliochini ya umri wa mwaka mmoja hapa katika hospitali ya taifa ya Muhimbili. Tunafanya utafiti kujua ni kwa jinsi gani ukosefu wa madini ya chuma yanapelekea kutokuwa na maendeleo mazuri ya mfumo huo wa Fahamu.

Ushiriki unahusisha nini

Watakaokubali kushiriki tutawahoji maswali kadhaa, pamoja kujadiliana kwa kina jinsi wanavyowapatia watoto wao chakula na pia tutawapima na kuwachukua damu kwa ajili ya kuangalia jinsi gani madini ya chuma yana husika na makuzi na maendeleo ya mfumo wa Fahamu.

Usiri

Taarifa zote utakazotupa ni siri na zitatumika tu kwa ajili ya utafiti huu na kuboresha huduma ya afya kwa mtoto. Hakuna mtu mwingine zaidi ya wanaohusika wa utafiti huu ,atakayesoma/kupata maelezo yako.

Faida

Faida utakayopata katika utafiti huu ni kujua kama maendeleo duni ya mfumo wa fahamu unaletwa na ukosefu wa madini ya chuma kwa mtoto wako. Tunatarajia tutakachojifunza katika utafiti huu utaboresha huduma ya afya katika maeneo yetu.

Uhuru wa kushiriki na haki ya kujitoa

Ushiriki wako katika utafiti huu ni kwa hiari kabisa na pia unayo haki ya kukubali kushiriki au kukataa kushiriki katika utafiti huu. Uamuzi wako wa kushiriki katika utafiti huu ama la, hautaathiri hata kidogo haki yako ya kupata huduma unayostahili kuipata.

Kukitokea madhara.

Hatutarajii madhara yoyote katika kushiriki kwako kwenye utafiti huu, lakini endapo utapata madhara yeyote kutokana na ushiriki wako tutakupa huduma zote za matibabu ya afya kama inavyotakiwa kwa kiwango cha Tanzania.

Nani wa kuwasiliana naye.

Iwapo utakuwa na swali lolote kuhusu utafiti huu , unaweza kuwasiliana na

Prof. E. Lyamuya: Mkuu wa tafiti, Chuo kikuu Cha Afya na Sayansi za tiba Muhimbili; Idara ya microbiolojia; S.L.P 65001 Dar es Salaam.

AU Kukiwa na tatizo lolote wasiliana na wafuatao:

Dr.Albion Kasasa Idara ya Magonjwa ya watoto na afya ya mtoto (Tel 0786881438)

Prof. Karim P. Manji, Idara ya Magonjwa ya Watoto na Afya ya Mtoto.

Dr. Festus M. Kalokola Idara ya Magonjwa ya watoto na Afya ya watoto

Mimi _____

Nimeelewa maelezo yaliyoandikwa hapo juu na kuridhika na majibu niliyopewa kwa maswali yangu yote. Ninakubali kushiriki katika utafiti huu.

Jina la mshiriki: _____

Sahihi ya mshiriki: _____

Tarehe ya kusaini ridhaa: _____