ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF NURSING AND MIDWIFERY

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Magnitude and Associated factors of Lactose intolerance among malnourished under five Children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

By: Brhane Gebremariam (BSc)

Advisors: Mistire Wolde (MSc, PhD)
Abebe Edao (BSc, MSc)
Tigist Bacha (MD, Emergency Pediatrician)

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Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Brhane Gebremariam entitled "Magnitude and associated factors of lactose intolerance among malnourished under five children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia" submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviations

AOR	Adjusted odds ratio
B.C	Before Christ
CHO	Carbohydrate
CI	Confidence interval
COR	Crude odds ratio
EBF	Exclusive breast feeding
EC	Enzyme commission
F- 100	Milk formula 100
F-75	Milk formula 75
g	Gram
HBT	Hydrogen breath test
LD	Lactase deficiency
LI	Lactose intolerance
LM	Lactose malabsorption
LPH	Lactase-phlorizin hydrolase
MAM	Moderate acute malnutrition
mg/dl	Milligram per deciliter
pH	Potential of Hydrogen
PPM	Parts per million
SAM	Severe acute malnutrition
SD	Standard deviation
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

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Abstract

Background: Lactose intolerance (LI) is a pathological condition characterized by the inability to digest a sugar, lactose, due to absence or insufficient activity of lactase enzyme (β-galactosidase). The prevalence of LI varies mainly with age, ethnicity, dose of lactose administered, and the laboratory method used for its diagnosis. Currently, LI treatment is based on empirical diagnosis. Laboratory diagnostic procedures for LI are poorly practiced in Ethiopia. Thus, it is difficult to discuss exactly about LI situation in Ethiopia.

Objective: To determine the magnitude and associated factors of lactose intolerance among malnourished under five children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia from March – July, 2018.

Method: A cross-sectional study was conducted among malnourished under five children admitted in pediatric unit, Yekatit 12 Hospital Medical College. By using convenient sampling technique structured questionnaire was administered to gather information on the socio-demographic characteristics of study participants and associated risk factors of LI. Moreover, fresh stool sample was collected from the study participants to measure stool pH by use of pH papers, reducing substances by use of Benedict's solution and microscopy examination for intestinal parasites. Data analysis was done using the Statistical Package for Social Sciences (SPSS 21) software.

Results: The study included 169 malnourished under five children. Among those 90 (53.3%) were male with median age of 12 months. The magnitude of LI was 18.3%. Among the study participants highest numbers of LI cases were in age group of less than 12 month 17(10.1%) followed by 13-24 month 13(7.7%). The factors that show significant association with lactose intolerance on bi-variate logistic analysis were family history of lactose intolerance (P=0.043) and diarrhea (P=0.000). In addition; problem after taking milk (P=0.007), type of therapeutic milk formula (P=0.000) and frequency of stool/24hr (P=0.023) were found to be the independent predictor factors of lactose intolerance in the study population.

Conclusion: The magnitude of LI was high in the study setting. Thus it necessary to give more attention on the proper diagnosis of LI. In addition, similar large scale studies at molecular level is required to strengthen the present findings about LI in Ethiopia.

Key words: lactose intolerance; lactase deficiency; carbohydrate malabsorption

1. Introduction

1.1 Background

Lactose intolerance (LI) was first described by Hippocrates around 400 (B.C) years. However, LI has been recognized and diagnosed as medically important disease only in the past 50 years (1, 2). LI is a pathological condition characterized by the inability to digest a sugar, lactose due to absence or insufficient activity of lactase enzyme (β-galactosidase) (3, 4). It could also be described as an intestinal mucosa disorder that incapacitates the digestion of lactose due to the deficiency of lactase enzyme. Lactose, is the unique sugar in the milk of mammals. Lactose, the major carbohydrate found in milk and dairy products, is a disaccharide composed of two simple sugars, glucose and galactose joined by a glycosidic linkage as β-galactose 1, 4 glucose (5-7).

The inability to digest lactose, lactose maldigestion, occurs when the concentration of lactase enzyme is reduced in the brush border of the small bowel mucosa. Lactase activity is highest at birth and declines after weaning (4). Lactose malabsorption (LM) or hypolactasia is the most common type of carbohydrate malabsorption and is caused by low lactase levels. Lactose intolerance occurs when the malabsorption causes symptoms (i.e. diarrhea, abdominal discomfort, flatulence, and bloating). Gastrointestinal symptoms after lactose ingestion are known as lactose intolerance (6, 8, 9).

Lactase deficiency (LD) is virtually responsible for lactose malabsorption. LM is indicated when a part of ingested lactose is not hydrolyzed in the small bowel and reaches the colon (6). It is the physiologic problem that manifests as lactose intolerance and is attributable to an imbalance between the amount of ingested lactose and the capacity for lactase to hydrolyze the disaccharide (5). A blood glucose concentration increasing less than 20 mg/dl, after ingestion of a (50 to 100 g) dose of lactose is evidence of LI (10, 11).

There are different laboratory diagnostic methods for LI including determination lactase activity of a jejunal biopsy, lactose tolerance test, hydrogen breath test (HBT), analysis of faecal pH and reducing substances and genetic studies (9, 12-14).

1.2 Statement of the problem

Lactose intolerance is a common medical problem, significantly impacts the lives of affected individuals and has limited treatment option (15). Worldwide, 75% of population loses the ability to digest lactose (16). But this prevalence of LI is strongly linked to age, ethnicity, use of dairy products in the diet and method used for its diagnosis. In populations with a predominance of dairy foods in the diet, particularly northern European people as few as 2% of the population has primary lactase deficiency is common among adults. In contrast, the prevalence of primary lactase deficiency is 50% to 80% in Hispanic people, 60% to 80% in black and Ashkenazi Jewish people, and almost 100% in Asian and American Indian people (5, 9, 12, 17). In the United States, the prevalence is 15% among whites, 53% among Mexican Americans and 80% in the Black population. The prevalence of primary lactase deficiency is above 50% in Africa (18).

Incidence of secondary lactose intolerance is variable, depending on its underlying etiology. Up to 50% of infants with acute diarrhea have transient lactose intolerance (19). Secondary LD which result as a consequence of injury to the small bowel mucosal brush border due to viral or non-viral intestinal infection is more common in children particularly those in developing countries. In addition diarrhea is more pronounced in children with secondary LD. Thus secondary LI is noticed as major risk factors for the incidence of persistent diarrhea, in the children between age of 1 month and 5 years (20, 21). Moreover, LI is also a risk factor for colorectal cancer since it negatively effects functioning of the intestines by causing acidification of the lumen, osmotic balance disturbance, and an alteration in intestinal bacteria composition (22).

Carbohydrate malabsorption is prevalent in children with severe acute malnutrition (SAM) and LI in particular. Diarrhea in children with SAM greatly increases the risk of mortality, and lactose intolerance can induce osmotic diarrhea, loss of electrolytes, particularly sodium and potassium, as well as dehydration and cause failure of treatment. Hence LI is a relatively common cause of persistent diarrhea in children with SAM (23-25).

SAM and diarrhoea are major causes of childhood morbidity and mortality in the developing world. Globally, it is estimated that there are nearly 20 million children who are severely acutely malnourished. Most of them live in South Asia and in Sub-Saharan Africa. SAM contributes to 1 million child death every year (26). The current WHO guidelines on the management of SAM and diarrhoea in children with malnutrition recommend the using F-75 of therapeutic milks formula

that is low in protein and fat but relatively high in carbohydrate (lactose), as the first therapeutic feed. Their use in the setting of malnutrition with diarrhoea could be delaying recovery. As a consequence, it is during this stabilization phase that mortality is highest. In line to this a meta-analysis on randomized controlled trials comparing lactose-free versus lactose containing formula after acute gastroenteritis indicated that 22% (95% CI 18-27%) of children who consumed lactose had therapeutic failure compared to 12% (95% CI 9% - 15%) in children who did not (27-29).

Gastro intestinal symptoms caused due to LI condition induced by milk and milk products malabsorption, indirectly interfere with calcium dietary intake. Thus LI has been considered as risk factor for low bone mineral density, osteoporosis and depression. Symptom of LI can affect patients physically, psychologically and socially (30-33). More than one third of all study subjects had gastrointestinal symptoms after lactose ingestion (30). Even though lactose may be a beneficial nutrient for undernourished children; research is needed to define the balance between beneficial and detrimental effects of lactose in undernourished children at different ages and with different degrees of diarrhea and intestinal integrity (27, 34).

Currently in Ethiopia LI treatment is based on empirical diagnosis. Treatment based diagnostic approach is being utilized to diagnose weather children are lactose intolerant or lactose tolerant. This is done by observing for adverse and poor response in the children who are taking therapeutic milk formula. Laboratory diagnostic procedures for LI are poorly practiced in Ethiopia. Furthermore, recently there is no study conducted on the magnitude and associated factors of lactose intolerance among malnourished under five children in Ethiopia. Thus, it is difficult to discuss exactly about LI situation in Ethiopia. Therefore, the aim of this study is to determine the magnitude and associated factors of LI among under five malnourished under five children.

1.3 Significance of the study

The data captured from this study is expected;

- To help patients to acquire immediate and best possible care according the laboratory test results.
- To support clinicians and other concerned health care providers to modify the guide line or treatment approaches of malnourished children with intolerance to lactose and other therapeutic milk formulas.
- Policy makers can access to an updated information on the current magnitude of LI in the study setting and this can help them to plan shot term and long term policies and strategies which can enable intervening LI early as much as possible.
- The result of this research can be used as reference for performing further researches and surveillance.

2. Literature review

2.1 Lactose and Lactase Enzyme

Lactose (Fig.1) is a disaccharide formed by glucose and galactose linked via β-1~4 glycosidic bond which is hydrolyzed by lactase. Lactase is a unique enzyme in its formation, location and enzymatic activity (1, 2). Lactase-phlorizin hydrolase (LPH) (E.C. 3.2.1.108) is a disaccharidase produced in the intestine of mammalian animals and more especially located in the microvilli membrane of epithelial cells in the small intestine (6, 12). It is formed as a 1927 amino acid protein, and then processed, leaving a final protein of 1059 amino acids as a dimer of 320 kDa (1, 2). Lactase is involved in the hydrolysis of lactose to monosaccharides glucose and galactose. It is a large glycoprotein with two active sites that can catalyze the hydrolysis of a variety of β-glucosides (i.e., phlorizin) and β-galactosides including lactose. Lactase is encoded by a single gene (LCT) of approximatively 50 kb located on chromosome number two (6).

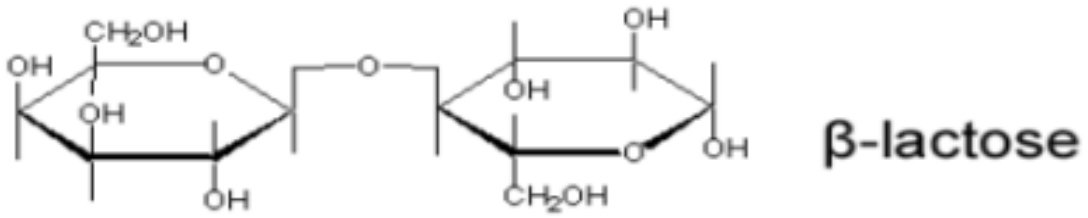

Figure 1: The chemical structure of β-lactose (Source: Adapted from Campbell et al, 2005)

2.2 Lactase deficiency

Lactase deficiency can be genetic (primary lactase deficiency) or disease-related (secondary lactase deficiency). Primary lactase deficiency has two forms. Congenital lactase deficiency is a rare and most severe autosomal recessive disorder affecting new-born. Lactase non-persistence, the most encountered form, is characterized by a down-regulation or gradual reduction of lactase activity in intestinal cells after weaning. This adult-type hypolactasia is an enzyme deficiency determined by single nucleotide polymorphisms. The age of occurrence and the prevalence of lactase deficiency depends on ethnic origin. Secondary lactase deficiency is caused by diseases or treatments injuring the intestinal mucosa (Crohn's disease, celiac disease, chronic intestinal inflammation, cancer chemotherapy, inflammatory bowel disease, gastroenteritis, acute diarrheal

disease, acute infections and severe malnutrition (5, 6, 9). This form is only temporary as lactase activity reappears once the epithelium is healed (13). Developmental (neonatal) lactase deficiency is the other type which is caused due to the immature gastrointestinal tract, in neonates born before 34 weeks' gestation (5).

2.3 Lactose intolerance: Mechanisms, clinical symptoms

After the hydrolysis of lactose by LPH, glucose and galactose are transported across the epithelial cell membranes into the enterocytes and then into the bloodstream via active transport by a sodium-dependent galactose transporter. They finally reach the liver, when they enter the pathways of intermediary metabolism and serve as an energy source (6).

In case of LD, ingested lactose is not degraded in the small intestine and passes into the colon, where it serves as a source of energy for the abundant microflora. Among the hundreds of colonic bacteria, some have the ability to metabolize lactose. Bacterial β-galactosidases catalyze the same chemical reactions as lactase but differ from lactase in structure, enzymatic properties, and regulation. When bacterial β-galactosidases release glucose and galactose, intestinal bacteria convert them in to a variety of products, including short-chain fatty acids (butyric acid, propionic acid, acetic acid, lactic acid) and gases (hydrogen, carbon dioxide, methane). Production of volatile fatty acids and gases (H_2, CO_2, and CH_4), lead to flatulence, abdominal pain and abdominal cramping. The fatty acids lower the fecal pH (1, 5, 6).

The presence of undigested lactose increases the osmotic load in the digestive tract. Because of the high hydraulic permeability of colonic mucosa, the gut cannot maintain a good osmotic gradient between blood and lumen so that water moves from blood to lumen to render luminal contents isotonic. Depending on the amount of lactose present in the colon; fluid, water, and electrolytes inflow in to the lumen can cause loose stool and notable diarrhoea. In addition to this the lactic acid produced by the microorganisms is osmotically active and pull water into the intestines resulting in diarrhea. In a few cases, gastrointestinal motility is decreased and subjects can present with constipation possibly as a consequence of methane production (1, 5, 6).

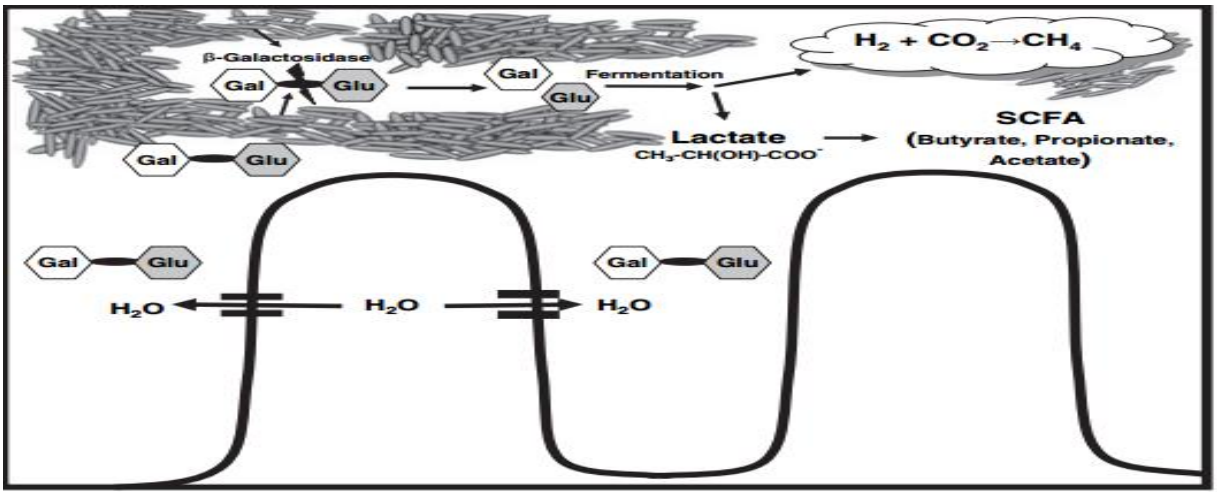

Figure 2: Lactose digestion in case of lactase deficiency (Source: Adapted from Lomer et al, 2008)

Signs and symptoms of lactose intolerance are similar to any other specific enzyme deficiency. They include: abdominal pain, bloating in the abdomen, flatulence, diarrhea, intestinal noises, and particularly in the young, vomiting, constipation sometimes as result of methane production (1, 8, 35). Aside from intestinal symptoms, several systemic symptoms occur such as headaches, loss of concentration, various allergies, dizziness,muscle and joint pain (1).

2.4 Factors influencing lactose tolerance

The symptoms resulting from LM are influenced by: (a) the amount of the lactose load; (b) the rate of gastric emptying; (c) the ingestion of food with the lactose, especially meals with higher fat content or higher osmolality which slow gastric emptying (e.g., chocolate milk); (d) the residual lactase activity, being lower in heterozygotes; (e) the dilution of the lactose load by gastric and small bowel fluid; (f) the rate of contact with the mucosal surface; (g) the sensitivity of the small bowel to distension caused by fluid secretion in response to the osmotic load of unabsorbed lactose; (h) the ability of the colonic flora to ferment unabsorbed lactose, (i) the individual sensitivity to the gaseous distension. Other factors include age, gender, eating habits, genetics, environment, and cultural patterns (11, 36, 37).

2.5 Prevalence and associated factors of Lactose intolerance

Worldwide, 70% of the population LPH activity decreases below a critical threshold which is the most frequent cause of enzyme deficiency (12).

A systematic review done by Harvey L et al and his colleagues on literature published between January 1995-June 2015 related to prevalence, cause and diagnosis of lactose intolerance in children aged 1–5 years reveal that prevalence of primary LI range between 0-17.9% while prevalence of secondary LI ranges from 0-19%. The most common method used to diagnose LI was Hydrogen breath test (38).

A study done on breath tests among Chilean pediatric population with suspected lactose intolerance which included 194 patients aged 1 to17 years of age; shows that 102 (53%) were presented with lactose malabsorption, and 53(27%) were lactose intolerant. The frequency of lactose intolerance varied from 7.1 to 45.4%, and it occurred more frequently at older ages. The most common reported symptoms were abdominal pain, bloating and rumbling. An increase in the frequency of intolerance with age were observed (39).

Another study carried out in Mexican in 2013 on prevalence of lactose malabsorption in children: importance of measuring methane. A total of 138 children (4-17 years old were enrolled and the study reveal the expired air methane production prevalence was 47.8% (29.7% of the children produced methane and hydrogen, whereas 18.1% produced methane only). When measuring only exhaled hydrogen in expired air (H2>=20 ppm), prevalence of LM was 31% (40).

A study done on the prevalence of lactase deficiency and lactose intolerance in Chinese children of different ages, of the total 1168 healthy subjects (between 3 and 13 years) recruited from schools, reveal that the prevalence of LI among Chinese children was 12.2% at age 3-5 years, 33.1% at age 7-8 years, and 30.5% at age 11-13 years respectively (41). Another retrospective study done in Malaysia on Hospitalization of childhood rotavirus infection from Kuala Lumpur in 2003, which include 271 cases under five year age show that 5.2% of the total cases was with lactose intolerance (42).

A prospective cohort study conducted in Tbilisi, Georgia between October 2014 and September 2015 on primary lactase deficiency among malnourished children with persistent diarrhea aged 3–24 months. The study included 78 malnourished children with persistent diarrhea; out of that the prevalence of primary LD among the study children was 41.0%. Four children had Rotavirus infection in lactose intolerant group (24).

A descriptive study was conducted on 70 children under five years of age with persistent diarrhoea at Tamil Nadu, India in 2017 shows secondary lactose intolerance 18 (25.71%) was noticed as

major risk factors in the study population. Secondary lactose intolerance was high in the age group between 6 month and 1 year (21). Another study which was conducted in Indian including 54 adult patients with Giardia lamblia infection and 54 adult controls to detect lactose maldigestion using the lactose HBT indicated that 74% patients with Giardia lamblia and 44.4% controls showed lactose maldigestion ($P<0.01$). This study showed that the frequency of lactose maldigestion was significantly higher in adult Indians suffering from Giardia lamblia infection compared to healthy individuals (43).

A study carried out in Poland in the year 2010–2013 on prevalence of lactose malabsorption and lactose intolerance in pediatric patients with selected gastrointestinal diseases. HBT was conducted in 387 pediatric patients. The prevalence of lactose malabsorption was observed in 37.08% of patients with gastrointestinal diseases. They also reported that lactose malabsorption with LI was found in 27.16% of participants. Abdominal pain, bloating, distention or nausea were the frequent symptoms seen in all subjects (30). Another study done by Szajewska H et al in 1997 on carbohydrate intolerance after acute gastroenteritis in 107 less than 3 years old with mean age of 12.5 months Polish children shows that 14/107 (13.08%) was diagnosed with CHO intolerance (diagnostic criteria: > 0.5% reducing substances and pH less than 5.5) out of them 12 (11.2%) of the patients were having lactose intolerance. They also reported that the most important predisposing factor was Rota virus (44).

A descriptive cross-sectional study was done at Iraq in 2012 on carbohydrate malabsorption in acute diarrhea in 100 patients between 2-36 months of age. Fifty six of them were males with mean ± SD (10.25±13.78) months. The frequency of carbohydrate malabsorption using stool pH and reducing substance was 41% and was highest among infants below one year. There was no significant association between baseline and diarrhea characteristics of the study population and carbohydrate malabsorption except vomiting (45).

A cross sectional study conducted at Samsun, Turkey in 2016 on lactose and fructose intolerance in children with chronic abdominal pain revealed that out of total 86 patients, carbohydrate intolerance was observed in 27 patients; 14 (16.3%) had LI, 11 (12.8%) had fructose intolerance and 2 (2.3%) patients had combined lactose and fructose intolerance (31).

A prospective observational study done in South Africa in children aged 6 months to 5 years old on lactose malabsorption and diarrhoea in children with SAM from December 2012 to November

2013. Out of 81 total participants 59% had stool positive for reducing substances (≥0.5g %); which mean they were with lactose malabsorption. After multivariate analysis, age of <12 months was the only factor found to significantly predict positive reducing substances (p=0.046) (46).

A descriptive cross-sectional study done in Uganda between October 2006 and February 2007 on lactose intolerance among 196 severely malnourished children (3-60 months of age) with diarrhoea admitted to the nutrition unit. Fifty (25.5%) of whom had evidence of lactose intolerance (stool reducing substance ≥ 1 + [0.5%] and stool pH < 5.5). The prevalence was highest among the children in the age group 3-12 months (68.0%). Oedematous malnutrition (p= 0.032), perianal skin erosion (p = 0.044), high mean stool frequency (p = < 0.001) and having ≥2 diarrhoea episodes in the previous 3 months (p = 0.007) were the independent predictors of lactose intolerance. They also reported young age of 3-12 months; lack of up to-date immunization; persistent diarrhoea and vomiting were the factors significantly associated with lactose intolerance on bi-variate analysis (23).

A cross-sectional study carried out in Qena Governorate, Egypt in 2013 on prevalence of lactose intolerance in primary school children aged 6-12 years revealed that 74% of the participants in the study were intolerant to lactose. However, only 56.8% of lactose-intolerant children had positive clinical history of abdominal pain, abdominal distension or diarrheal attacks following ingestion of milk or dairy products. Lactose intolerance was 58% at 6-7 years of age and increased to 90% by the age of 11-12 (47).

A case study approach conducted in Ethiopia in 2015 on the occurrence of lactose intolerance among Ethiopians, using questionnaire survey and document analysis reveal that out of 188 households/individuals surveyed, and 7.45% of the respondents reported that they don't consume milk but consume fermented milk ('Ergo'). The majority reported symptoms of lactose intolerance were (71.4%) get vomiting upon consuming milk and (28.6%) feel abdominal pain (48).

2.6 Conceptual frame work

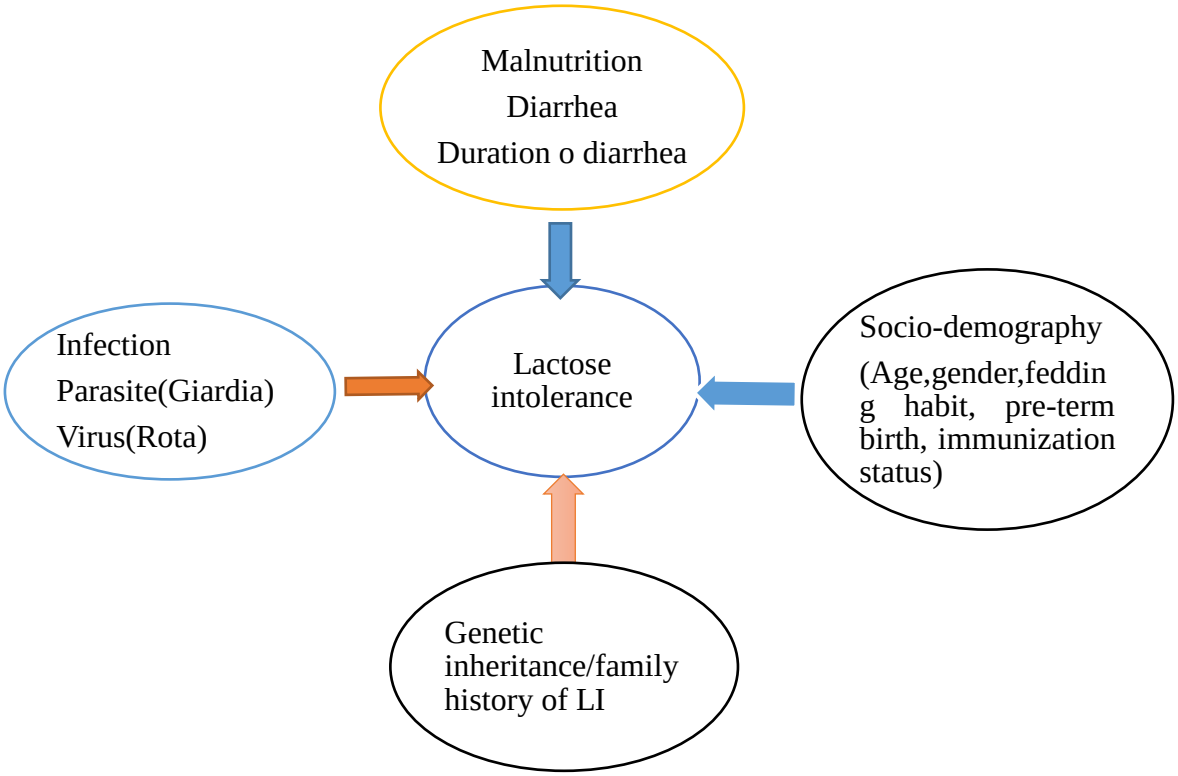

Figure 3: Conceptual frame work

3. Objective

3.1 General objective

To determine the magnitude and associated factor of lactose intolerance among malnourished under five children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia from March – July, 2018

3.2 Specific objectives

- To determine the magnitude of lactose intolerance among malnourished under five children
- To assess factors associated with lactose intolerance among malnourished under five children

4. Hypothesis

- The magnitude of lactose intolerance among malnourished under five children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia is not different from reported Africa.

5. Materials and methods

5.1 Study area

This study was conducted in pediatric unit of Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia. Addis Ababa lies at an altitude of 7,546 feet (2,300 meters) above sea level, located at 9°1′48″N 38°44′24″E Coordinates: 9°1′48″N 38°44′24″E. Based on 2007 census conducted central statistical agency of Ethiopia Addis Ababa has a total population of 2,739,551 (49). Yekatit 12 Hospital Medical College is a public hospital found in Addis Ababa city established in 1915. Yekatit 12 Hospital Medical College provides a health care service at an out-patient and in-patient level as a referral hospital centre for health centers and hospitals in Addis Ababa as well as different regions of the country. It is one of the center serving as a nutrition therapy. The Pediatric unit has 8 beds for the admission of malnourished under five children. The hospital is using the Federal Ministry of Health guide line for the management of severe acute malnutrition adapted from the United Nations Children's Fund (50).

5.2 Study design and Period

A cross-sectional study was conducted from March - July 2018

5.3 Population

5.3.1 Source Population

All children who visit the pediatric unit of Yekatit 12 Hospital Medical College during the study period

5.3.2 Study Population

All malnourished under five children admitted to pediatric unit of Yekatit 12 Hospital Medical College and who were taking therapeutic milk formula during the study period.

5.4 Inclusion and Exclusion Criteria

5.4.1 Inclusion Criteria

- Age (≤5 years)
- Children with malnutrition
- All participant who was on therapeutic milk formulas during the study period

5.4.2 Exclusion criteria

- Children with diabetes
- Children on proton pump inhibitor, laxative use

5.5 Study Variables

5.5.1 Dependent Variable

- Magnitude of lactose intolerance

5.5.2 Independent Variables

- Socio demographic characteristics (Age, gender, pre-term birth)
- Feeding practice
- Type of malnutrition
- Family history of LI
- Problem after milk taken
- Immunization status
- Intestinal parasites

5.6 Measurement and Data collection

5.6.1 Sample Size Calculation

The sample size of the study was determined by using the prevalence (11.2%) of prior study on Carbohydrate intolerance after acute gastroenteritis in Polish children (44). The sample size was calculated by using the formula for sample size determination for single proportion.

$$n = (1.96 /d)^2 p (1-p)$$

Where:-

1.96 = Z score at 95% confidence interval

d= the level of confidence is 5%

P (proportion) = 0.112 (for previous prevalence)

n= desired sample size

$n= (1.96/0.05)^2 X 0.112 (1-0.112)$ n = 153

Non response rate (10%) = 153 x 0.1 = 15.3

Final sample = 153+15.3 n= 169

5.6.2 Sampling Method

Convenient sampling technique was used to select the study participant

5.6.3 Data Collection Procedure

After getting approval and permission from Yekatit 12 Hospital Medical College, a structured questionnaire was used to gather information of the sociodemographic characteristics and associated factors with LI. Trained nurse staff collected the demographic data and instructed the family of the children how to collect the stool. A fresh stool sample was collected 24 hours after they start a standard lactose-based therapeutic milk formula. Then the stool sample was transported from the pediatric ward to the laboratory. Laboratory tests was done in fresh stool of the participants with measurements of stool pH and reducing substances. Stool pH was tested using pH papers, while presence of reducing substance was tested by use of Benedict's solution. Stool microscopy was also performed to detect intestinal parasite (*G.lamblia and E.histolytica).*

5.6.4 Laboratory Analysis

5.6.4.1 Stool reducing substance

Principle

Benedict qualitative reagent contains cupric ion complexes to citrate in alkaline solution. Reducing substances in the stool sample convert cupric to cuprous ions forming red cuprous oxide the degree of reduction corresponding to the concentration of reducing substance present (51). The test will be considered positive for lactose intolerance, if stool reducing substances are ≥0.5g% [Refer to annex-VII]

5.6.4.2 Fecal pH

Principle

After dipping the pH paper into small quantity of the stool sample, color of the pH paper will be changed depending the acidic content of the stool. If the pH result is less than or equal to 5.5 it indicates positive for lactose intolerance. [Refer to annex-VII]

5.6.4.3 Direct stool wet mount

Principle

A small stool sample mixed with drop of normal saline on microscope slide was made. Finally it was examined under microscope using 10x and 40x objective for ova/parasite of intestinal parasite (*G.lamblia and E.histolytica*). [Refer to annex-VII]

5.7 Data Quality Assurance

The questionnaires was translated to local language Amharic and back to English. The questionnaire was pre-tested on potential respondents at Yekatit 12 Hospital Medical College study participant visited the pediatric unit, to check for any ambiguity at time of interview. Any unclear question was revised appropriately to avoid collecting unreliable information. The quality of stool was checked by the principal investigator after collection. All data collected was crosschecked for completeness, accuracy and cleaned before analysis. Quality assurance was maintained in pre analytical, analytical and post analytical stages.

Pre- analytical

- Protocol for stool sample collection, transportation and processing was strictly followed according to the standard operating procedures.
- The quality of stool specimen was checked by the principal investigator after collection.
- All reagents and pH paper were checked for their expiry date.
- Microscopic slides and cover glasses were checked for cleanliness.
- Detail orientation was also given for clinical data and specimen collectors

Analytical

- Quality control was run for both stool pH and stool reducing substance.

Post-analytical

- Result of stool pH and reducing substance was recorded and interpreted carefully.

5.8 Data Analysis and Interpretation

Data was coded, double entered and analyzed using the Statistical Package for Social Sciences (SPSS 21) software. Categorical variables was summarized as frequencies and percentage, while continuous variables as median. In the bi-variate analysis, odds ratios, 95% confidence interval (CI), and chi-square test or Fisher's exact test were used to measure the strength of association between the factors considered and the dependent variable. Multivariate analysis using logistic regression was used to determine the factors that were independently associated with lactose intolerance. Risk factors with p-values below 0.2 on bi-variate analysis were included in logistic

regression analysis to identify independent risk factors. P-value < 0.05 was considered for statistical significance. Result was summarized in texts, tables and bar graph.

5.9 Ethical Consideration

Ethical clearance was taken from the Department of research and ethics review committee of Medical laboratory science department, College of Health Sciences, Addis Ababa University. Ethical clearance was taken from Addis Ababa health bureau research and ethics review committee. In addition letter of permission was taken from Yekatit 12 Hospital Medical College. Prior to data collection, written informed consent was obtained from each parents/caregiver of the study participants after explaining the purpose of the study. Confidentiality of the data was maintained by coding of samples and privacy of the respondents was maintained. The result of the laboratory test was communicated to the treating physician/nurse and they could modify treatment of the participant if they deemed necessary.

5.10 Dissemination of Result

The result of the study will be first presented at Department of Medical laboratory Sciences, College of Health Sciences, Addis Ababa University, and will be submitted to department of Medical laboratory science which could be used as reference. The result will be presented to concerned bodies, conferences, seminars and different professional association annual meeting. The result of the study will also be submitted to Addis Ababa Health bureau. In addition, a copy of the final material will be given to pediatric unit of Yekatit 12 Hospital Medical College, Addis Ababa University and different concerned stakeholders. The final paper will be published on peer reviewed journal.

5.11 Operational definition

Lactose malabsorption: indicates that a sizable fraction of a dosage of lactose is not absorbed in the small bowel and thus is delivered to the colon (10).

Lactase deficiency: is defined as markedly reduced brush-border lactase activity relative to the activity observed in infants (36).

Lactose intolerance: is considered if fecal reducing substances are ≥0.5g% and stool pH less than 5.5 (51).

Malnutrition: refers to deficiencies, excesses or imbalance in a person's intake of energy and/or nutrient (52).

6. Work flow

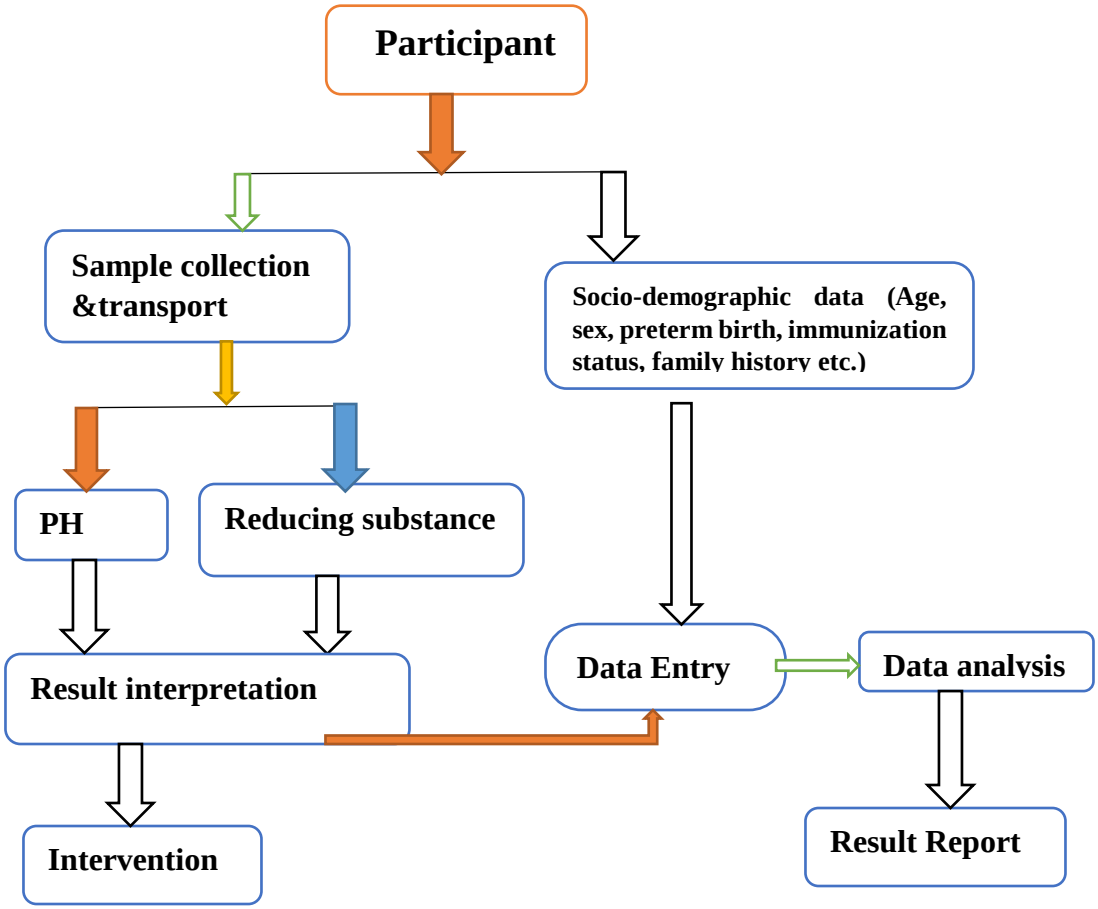

Figure 4: Work flow

7. Results

7.1 Socio- Demographic Characteristics

A total of 169 malnourished under five children admitted in pediatric unit of Yekatit 12 Hospital Medical College were enrolled in the present study. Among them 90(53.3%) were male with median age of 12 months. Moreover, highest numbers of cases were occurring in age group of less than 12 month 91(53.8%) followed by 13-24 month 56(33.1%) age group (Table 1).

Table 1: Socio-demographic characteristics of malnourished under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variables		**Frequency**	**Percent (%)**
Sex	Male	90	53.3
	Female	79	46.7
Age	less than 12 month	91	53.8
	13-24 month	56	33.1
	25-36 month	15	8.9
	37-48month	2	1.2
	49-60mnth	5	3.0
Immunization status	Complete	132	78.1
	Incomplete	37	21.9
Pre-term birth	Yes	39	23.1
	No	130	76.9
Family history of LI	Yes	5	3.0
	No	164	97.0

7.2 Clinical sign-symptom and stool characteristics

Among the study participants 70(41.4%) had diarrhea; of which 30(42.9%) had persistent diarrhea. Among the study participants 51(30.2%) had watery stool, 14(8.3%) had perianal skin erosion and 15(8.9%) had frequency of stool ⁄24 hour ≥5 times (Table 2). *E.histolytica* was found only in 1 patient while *Giardia lamblia* was founded in 8(4.7%) out of the study participants.

Table 2: Clinical symptoms and stool characteristics of malnourished under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variables		Frequency	Percent
Diarrhea	Yes	70	41.4
	No	99	58.6
Duration of diarrhea$^{\beta}$	<2 week	40	57.1
	≥2week	30	42.9
Vomiting	Yes	70	41.4
	No	99	58.6
Stool consistency	Watery/loose	51	30.2
	Semi formed	53	31.4
	Bloody	2	1.2
	Normal/Formed	63	37.3
Frequency of stool in 24hr	One time	20	11.8
	Two times	39	23.1
	Three times	49	29.0
	Four times	46	27.2
	≥5 times	15	8.9
Perianal skin erosion	Yes	14	8.3
	No	155	91.7
Level of consciousness	Conscious	153	90.5
	Unconscious	16	9.5
Giardia lamblia	Yes	8	4.7
	No	161	95.3

β= only children with diarrhea were considered; hence they do not add up to 169, (70/169).

7.3 Feeding pattern and malnutrition type of study participants

Among the study participants 156(92.3%) had SAM, 131(77.5%) were on F-75 therapeutic milk formula and 15(8.9%) out of the study participants had experienced problem with cow milk (Table 3).

Table 3: Feeding practices of malnourished under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variables		Frequency	Percent
Type of malnutrition	SAM	156	92.3
	MAM	13	7.7
Still breast feeding	Yes	95	56.2
	No	74	43.8
Duration EBF	< 4month	48	28.4
	≥4month	121	71.6
Therapeutic milk taken	F75	131	77.5
	F100	38	22.5
Problem with cow milk	Yes	15	8.9
	No	154	91.1

SAM: Severe acute malnutrition, MAM: Moderate acute malnutrition, EBF: exclusive breast feeding

7.4 Magnitude of lactose intolerance of study participants

Lactose intolerance is considered if fecal reducing substances are ≥0.5g% and stool pH less than 5.5. The overall magnitude of lactose intolerance is found to be 18.3% by these criteria (Table 4).

Table 4: Magnitude of lactose intolerance of malnourished under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variable		Frequency	Percent
Lactose intolerance	Yes	31	18.3
	No	138	81.7
	Total	169	100

7.5 Factors Associated with lactose intolerance

7.5.1 Association between diarrhoea characteristics and lactose intolerance

The present study demonstrated that diarrhea and frequency of stool in 24hr show significant association with lactose intolerance while duration of diarrhea, perianal skin erosion, *Giardia lamblia* and vomiting does not show a significant association with lactose intolerance (Table 5).

Table 5: Association between diarrhoea characteristics and lactose intolerance of malnourished under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variables		Lactose intolerant N=31(%)	Lactose tolerant N=138(%)	COR	95% CI	P-value
Diarrhea	Yes	22 (71.0)	48 (34.8)	4.58	1.95-10.73	0.000*
	No	9 (29.0)	90 (65.2)			
Duration of diarrhea$^{\beta}$	≥14days	13 (59.1)	17 (35.4)	2.63	0.94-7.42	0.063
	<14days	9 (40.9)	31 (64.6)			
Frequency of stool/24hr	≥4times	21 (67.7)	40 (29.0)	5.15	2.23-11.89	0.000*
	<4 times	10 (32.3)	98 (71.0)			
Perianal erosion	Yes	5 (16.1)	9 (6.5)	2.76	0.85-8.89	0.139$^{\Psi}$
	No	26 (83.9)	129 (93.5)			
Vomiting	Yes	15 (48.4)	55 (39.9)	1.42	0.65-3.09	0.384
	No	16 (51.6)	83 (60.1)			
Giardia lamblia	Yes	1 (3.2)	7 (5.1)	0.62	0.07-5.26	1.000$^{\Psi}$
	No	30 (96.8	131 (94.9)			

*P-value significant (< 0.05), COR = crude odd's ratio, CI = 95% confidence interval, Ψ=Fisher's exact test, β= only children with diarrhea were considered; hence they do not add up to 169

7.5.2 Association between baseline characteristics and lactose intolerance

Sex, age, preterm birth and immunization status does not show significant association with LI but family history of LI show a significant association with the lactose intolerance (Table 6). There is a gradual decrease in the magnitude of LI among the age groups (Fig.5).

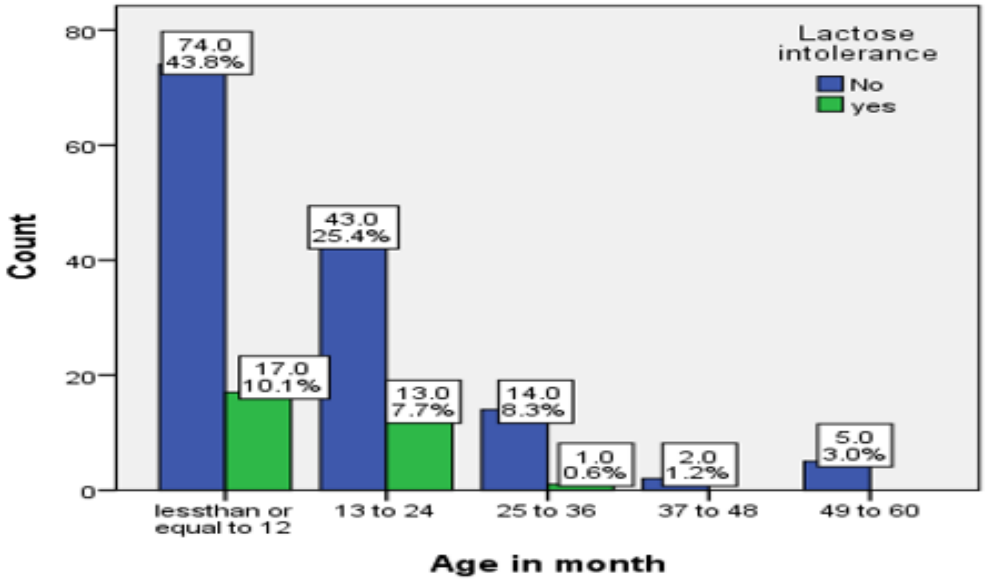

Figure 5: Age distribution by lactose tolerance of malnourished under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Table 6: Association between baseline characteristics and lactose intolerance among under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variables		Lactose intolerant N=31(%)	Lactose tolerant N=138(%)	COR	95% CI	P-value
Sex	Male	18 (58.1)	72 (52.2)	1.27	0.58-2.79	0.553
	Female	13 (41.9)	66 (48.8)			
Age in month	≤12	17 (54.8)	74 (53.6)	1.05	0.48-2.29	0.902
	13-60	14 (45.2)	64 (46.4)			
Preterm birth	Yes	11 (35.5)	28 (20.3)	2.16	0.93-5.03	0.074
	No	20 (64.5)	110 (79.7)			
Immunization status	Incomplete	8 (25.8)	29 (21.1)	1.31	0.53-3.22	0.560
	Complete	23 (74.2)	109 (78.9)			
Family history of LI	Yes	3 (9.7)	2 (1.5)	7.29	1.16-45.63	0.043 Ψ
	No	28 (90.3)	136 (98.5)			

COR = crude odd's ratio, CI = 95% confidence interval, Ψ=Fisher's Exact Test

7.5.3 Association between feeding practices and lactose intolerance

In the present study problem after taking cow milk (P=0.008) and therapeutic milk formula (P=0.004) shows a significant association with lactose intolerance (Table 7).

Table 7: Association between feeding practices and lactose intolerance among under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variable		Lactose intolerant N=31(%)	Lactose tolerant N=138(%)	COR	95% CI	P-value
Breast feeding $^{\Delta}$	No	17 (54.8)	51 (39.9)	1.91	0.87-4.20	0.107
	Yes	14 (45.2)	80 (61.1)			
Duration of EBF	<4month	13 (41.9)	35 (25.4)	2.13	0.95-4.78	0.064
	≥4month	18 (58.1)	103 (74.6)			
Problem with cow milk	Yes	7 (22.6)	8 (5.8)	4.74	1.57-14.29	0.008$^{\Psi}$
	No	24 (77.4)	130 (94.2)			
Therapeutic milk formula	F75	18 (58.1)	113 (81.9)	0.31	0.13-0.71	0.004*
	F100	13 (41.9)	25 (18.1)			
Type of malnutrition	SAM	28 (90.3)	128 (92.8)	0.73	0.19 -2.82	0.708 $^{\Psi}$
	MAM	3 (9.7)	10 (7.2)			

*P-value significant (< 0.05), COR = crude odd's ratio, CI = 95% confidence interval, Ψ=Fisher's Exact Test, Δ= only children in the breastfeeding age range (1-36 months) were considered; hence they do not add up to 169, EBF= Exclusive breast feeding, SAM=Severe acute malnutrition, MAM= Moderate acute malnutrition

7.5.4 Factors independently predicting lactose intolerance

Factors for the multivariate logistic regression were chosen because they have been shown to be associated with LI and/or they had a P-value of <0.2 on bivariate logistic regression analysis. Accordingly, as shown in Table 8 after multivariate logistic regression analysis; high stool frequency in 24 hour, problem after taking cow milk and type of therapeutic milk formula were the independent predictors of lactose intolerance. The respective AOR and CI are 3.80 [1.26-11.48, P=0.023], 8.14 [1.78-37.12, P =0.007] and 0.13 [0.04-0.40, P =0.000].

Table 8: Multivariate logistic regression analysis for factors independently predicting lactose intolerance among malnourished under five children, admitted in pediatric unit of Yekatit 12 Hospital Medical College from March – July, 2018.

Variables		AOR	95% CI	P-value
Problem after taking cow milk	Yes	8.14	(1.78-37.12)	0.007*
	No			
Therapeutic milk formula	F-75	0.13	0.04-0.40	0.000*
	F-100			
Frequency of stool/24hr	≥4times	3.80	1.26-11.48	0.023*
	<4times			
Diarrhea	Yes	1.41	0.40-4.92	0.593
	No			
Duration of diarrhea	≥14 days	3.23	0.84-12.41	0.085
	<14 days			
Perianal skin erosion	Yes	2.85	0.52-15.65	0.227
	No			
Preterm birth	Yes	2.36	0.76-7.35	0.139
	No			

* P-value significant (< 0.05), AOR = Adjusted odd's ratio, CI = 95% confidence interval

8. Discussion

Lactose intolerance is the inability to digest lactose, a disaccharide found in milk and to a lesser extent in milk derived dairy products (16). In the present study the overall magnitude of lactose intolerance is 18.3%. This result is comparable to a study done in Turkey (16.3%) (31). This is lower than a study done in Uganda (25.5%) (23), India (25.71%) (21), Iraq (41%) (45), South Africa (59%) (46) and Georgia (41.0%) (24). In contrast it is slightly higher than a study done in Poland (11.2%) (44). This difference might be due to the difference in the diagnostic method used, sample size, study populations. In this study most of study population were malnourished child without diarrhea (58.6%); while most of the other studies included malnourished child with diarrhea. In addition other difference could be due to difference in study design. This study utilized cross-sectional study design while a prospective cohort study was done in Georgia (24).

In this study 8(25.8%) subjects with lactose intolerance had incomplete immunization status. This finding is nearly similar to study done in Iraq 9 (21.95%) (45), but lower compared to a study done in Uganda 39(39.8%) (23). Inline to this, immunization status had not significant association (P=0.560) with LI which is similar with a study done in in Iraq (P= 0.44) and disagree; with a study done in Uganda (P< 0.001) (23). This might be explained due to difference in sample size, increased awareness towards immunization utilization, difference in health policy towards immunization utilization of study area.

In the present study there is no significant association (P= 0.553) between sex and lactose intolerance. This result is similar to study done in Uganda (P= 0.545) (23), Iraq (P= 0.67) (45). The lack of association with gender probably indicates that if the clinical condition is genetically determined, it is not linked to the sex chromosomes.

Lactose intolerance was seen in 15 (48.4%) children who had vomiting; a finding lower than study done in Iraq (75.6%) (45), and in Uganda (70.0%) (23). In this study there is no significant association (P=0.384) between lactose intolerance and vomiting. This finding contrast with study done in Uganda (P= 0.027) (23), Iraq (P=0.01) (45) and Tbilisi, Georgia (P=0.0011) (24). This might be due to difference in the diagnostic method, study design and study population. In the present study preterm birth (P= 0.074) had not significant association with LI while family history of LI (P= 0.043) showed significant association with LI.

Out of the study population 28.4% had an EBF duration of less than 4 month; which was lower compared to study in Uganda (35.7%) (23), and Iraq which 72% (45). There is no significant

association between EBF (P= 0.064) and LI which is agree with study done in Iraq (P=0.50) (45) but it differ to study done in Uganda (P=0.015) (23). Evidence of lactose intolerance seen in 22.6% study participant who had problem after taking cow milk. In addition in the current study a significant association was demonstrated (P=0.008) between problem after milk taken with LI. This finding is comparable to study done in Tbilisi, Georgia (P= 0.0076) (24); though the result disagree with a study done in Uganda (50.0%; P=0.175) (23). This could be explained due to difference in the sample size and study population.

In the present study most of the lactose intolerant children (58.1%) were on F-75 as compared to 41.9% children who were in F-100 therapeutic milk formula. This outcome accords to a study done in Uganda in which most of the lactose intolerant children (82%) were on F-75 as compared to 18% children who were in F-100 therapeutic milk formula (23). In line to this there is significant association (P= 0.004) between LI and therapeutic milk formula; a finding which contrast to study done Uganda (P=0.938) (23). This might be due to difference in the study population i.e. there is no equal distribution of study participants among the therapeutic milk formula feeding.

Most children (54.8 %) with lactose intolerance were children less than 12 months of age, which is consistent with study done in Uganda (68%) (23), Iraq (73.17%) (45), South Africa (69%) (46), and Tbilisi, Georgia (42%) (24). In line to this, there is a gradual decrease in the magnitude of LI from 10.1% in the age group less than 12 months, 7.7% in the 13-24 month age groups, 0.6% in the age group 25 -36 month and with zero both in the 37-48 and in the 49-60 months age groups; a result consistent to a finding in Uganda (23). This could be attributed to the higher susceptibility of intestine of children less than 12 month of age; which could be aggravated by the diarrhoea and malnutrition. On bivariate analysis age in months had not significant association with LI (P=0.902) a finding similar to a study done in Iraq (P=0.58) (45); but inconsistent with a study done in Uganda (P=0.018) (23) and in South Africa (P=0.047) (46). This could be due to the difference in study subjects in which most of the study participants in the present study were children less than 12 months of age i.e. there was not normal distribution between the age group of study subjects.

Children with LI had higher mean stool frequency (≥4motions in 24 hour period; P= 0.000), a finding consistent with a study in Uganda (23), South Africa (46) and Tbilisi, Georgia (24). This is expected as unabsorbed lactose would remain in the colonic lumen and lead to osmotic diarrhoea. Furthermore, undigested lactose may attract fluid, water and electrolytes in to the lumen that the colon cannot handle (5, 6).

Five children (16.1%; P= 0.139) with lactose intolerance had presented perianal skin erosion. There is not significant association between perianal skin erosion and lactose intolerance; a finding similar to study done in Iraq (39.02%; P=0.20) (45), South Africa (71%; P=0.264) (46). This report contrasts to a finding in Uganda that was statistically significant (70.0%); P < 0.001) between perianal skin erosion and LI (23). This difference might be due to the difference in study populations as perianal skin erosion is more related to frequency of diarrhea episodes per day; in this study only more than half study subject without diarrhea were enrolled.

Seventy (41.4%) of the study participants had diarrhoea; of those 42.9% had a diarrheal duration of ≥14days. This seems to strengthen the fact that diarrhea contributes to intestinal mucosal damage associated with variable degrees of malabsorption (5). Among the study population who had diarrhea 31.4% were lactose intolerant (P=0.000) which is lower compared to a study done in South Africa 59% (46), Iraq 41% (45); but higher to a finding in Uganda 25.5% (23). This might be difference in the study population.

In the present study LI was seen more frequently in the children who had persistent diarrhea (43.3%) compared to patients with acute diarrhea (22.5%); this contrasts with a study in South Africa in which duration of diarrhoea was found to be shorter in children with lactose malabsorption than in those without (46). The finding is comparable to study done in Uganda in which LI was seen more frequently in children with persistent diarrhea (34.2%) as compared to (20.0%) with acute diarrhea (23). This confirms the observation that the lactase enzyme is localized to the tips of the intestinal villi, a factor of clinical importance when considering the effect of diarrheal illness on the ability to tolerate lactose. Persistent diarrhoea also results in a more prolonged and extensive damage of the intestinal mucosa and the immature epithelial cells that replace these are often lactase deficient, leading to secondary lactase deficiency and lactose malabsorption (5, 35).

At multivariate logistic regression analysis, problem after taking milk (P=0.007), type of therapeutic milk formula (P=0.000) and high stool frequency in 24 hour (P=0.023), were the independent predictors of lactose intolerance in malnourished children. In a study done in Uganda (23), high mean stool frequency (P=< 0.001) was among the factors which independently predict lactose intolerance while in a study conducted in South Africa age of <12 months (P=0.046) was the only factor found to significantly predict lactose malabsorption.

9. Limitation and strength

9.1 Strength

This research will provide updated information on the current magnitude of lactose intolerance in the study setting.

9.2 Limitation

In the present study hydrogen breathe test which is gold standard test was not done because of its in availability in our country and expensive cost. The Benedict's test relies on a colour change which leads the interpretation of the colour to somewhat subjective but this observer bias was minimized by only one researcher conducting the laboratory test. The study was conducted in one hospital and the sample size was small. This could have resulted in decreased variability in the study results. Associated factors with lactose intolerance could be evident if the study was done in larger study sample size. Culture for bacterial infection and stool antigenic test for Rota virus was not done.

10. Conclusion

The overall magnitude of lactose intolerance which is 18.3% among malnourished under five children in this study setting is high. On bivariate analysis; diarrhea, frequency of stool/24hr, family history of LI, problem with cow milk and therapeutic milk formula show a significant association with LI. In the present study LI was found to be common among malnourished under five children with diarrhea duration of ≥14days i.e. persistent diarrhea. According to the multivariate logistic regression analysis; high stool frequency in 24 hour, problem after taking milk and type of therapeutic milk formula were found to be the independent predictor factors of lactose of lactose intolerance.

11. Recommendation

- Hospitals or health centers is better to consider providing lactose free diet for babies whose response is poor on the therapeutic milk formula.
- Lactose intolerance should be considered and routine screening by stool pH and reducing substance would be good if it is done in these hospitals or health centers who provide treatment for malnourished children.
- Availability of other tests like hydrogen breath testing and genotyping study for detection of lactose intolerance in the hospital could better.
- A research with different study design, larger sample size and different variable deserves in this field.
- Malnourished under five children with higher frequency of stool in 24hr and having problem after taking milk need a due care and attention by health care providers.
- This study highlights the need for a future studies on investigating the impact of the use of the lactose free feeds on the recovery of children with malnutrition whose stool is positive for reducing substances.

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ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF NURSING AND MIDWIFERY

DEPARTEMENT OF MEDICAL LABORATORY SCIENCE

13. Annexes

Annex-I: Participant information sheet

Principal Investigator: Birhane Gebremariam

Title of the project: Magnitude and associated factors of lactose intolerance among malnourished under five children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

Institution: Addis Ababa University, College of Health Sciences, School of Nursing and Midwifery, Department of Medical Laboratory Science

Introduction and Invitation to participate

Hello. My name is Birhane Gebremariam, I am a master's student of Addis Ababa University College of Health Sciences, School of Nursing and Midwifery, Department of Medical Laboratory Science and I am doing a research project for my degree. I would like to ask for some of your time to explain the research that I am doing and to ask for you and your child's help to do this research. Research is the way that we find information that can help us to answer certain questions.
Please feel free to ask any questions during our discussion.

Aim of this study?

This study is not part of the normal care that your child will receive in hospital, but it is being done to learn more information that will help us to treat children like yours. There are many children in Ethiopia who are suffering from lactose intolerance (which is when the body does not absorb the sugar found in breast milk, cow's milk and most formulas) but its magnitude is not known at recent. We are trying to identify some of the factors associated with lactose intolerance. Only children under the age of 5who are admitted at this hospital are invited to participate in this study.

What is involved in the study?

If you agree to take part in the study, one of the investigators or a health worker will give you verbal or written information about the study and you will be given the consent form to sign, the physician or health professional will ask you some questions about your general health and perform

a complete medical examination and assess whether you qualify to participate in the study. I would then need to take a little bit of your child's stool.

What are the risks of this study?

Participating in this project will not cause more discomfort than is required you could go through for routine examination. If anything unexpected does occur as a result of this procedure, I will take the responsibility of making sure that it is fixed as best as possible.

What will be benefits of this study?

The result of the laboratory finding will be communicated to your physician for use in the management of the disease. The information that we collect may be used to help other children who have a similar condition to your child. You will not be provided with any direct incentives for your participation in the research.

What are your rights in this study?

You have full right to withdraw from participating in this study at any time before and after consent without explaining the reason. Your decision will not affect your right to get health service you are supposed to get otherwise.

Confidentiality of participant information

All information about the patients will be kept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name will be locked inside a box and it will not be revealed to anyone except your physician and the principal investigator.

If you have any question please contact the following:

Principal Investigator: Mr. Birhane Gebremariam ((BSc, MSc candidate)

Phone: +251-914798197 Email: birhane1982@gmail.com

Thanks for your participation!!!

Code No._____

Annex II. Consent form for parents/guardians (English version)

Principal investigator: Birhane Gebremariam

Advisors: 1) Mistire Wolde (MSc, PhD) 2) Abebe Edao (MSc) 3) Tigist Bacha (MD, Emergency Pediatrician)

Funded by: Addis Ababa University

Reviewed: By Department research ethics committee of department of Medical Laboratory Science, School of Nursing and Midwifery, Addis Ababa University

Research title: Magnitude and associated factors of lactose intolerance among malnourished under five children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

Parent or legal guardian declaration section:

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. **I voluntarily consent that my child participates** in this study provided he/she gives assent.

To give his/her stool ☐

To collect her/his stool and be a participant in this study and understand that I have the right to withdraw my child from the study at any time. ☐

Name of participant: _____ Address _____

Date: ____ /____ /____ Signature: _____

Name of interviewer: _____ Date: _____/____/___Signature: _____

Name Principal Investigator: _____ Date: ___/___/___Signature:_____

Department of Medical Laboratory Science Research and Ethics Committee Office Telephone: +251-112755170

Thanks for your participation!!!

Annex-III: የተሳታፊዎች መረጃ መስጫ ሰነድ (Amharic version)

የአጥኚው ስም: ብርሃነ ገብረማርያም

የተቋሙ ስም: አዲስ አበባ ዩኒቨርስቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል

የጥናቱ ኣርእስት: የላክቶዝ ስኳር በሰውነት ውስጥ ያለመፈጨት ችግር፣ ስርጭትና ተዛማጅ ነገሮችን ከኣምስት ኣመት በታች በሆኑ ህፃናት መካከል ማጥናት

<u>መግቢያ</u>

ብርሃነ ገብረማርያም እባላለሁ። በ አዲስ አበባ ዩኒቨርስቲ የህክምና ፋኩልቲ የህክምና ላቦራቶሪ ሳይንስ የማስተርስ ዲግሪ ተማሪ ነኝ፡፡ በኣሁኑ ሰኣት ጥናት/ምርምር እያደረኩ ነው። ለዚህ ጥናት የሚሆን ትንሽ ጥያቄ ስላለኝ ልጃችሁን ወይም እርስዎ እንድጠይቅ እና ግዜ እንድትሰጡኝ እጠይቃለሁ። ጥያቄ ካልዎት በማንኛውም ሰኣት መጠየቅ ይቻላል::

<u>የጥናቱ ዓላማ:</u> የላክቶዝ ስኳር በሰዉነት ዉስጥ ያለመፈጨት ችግር፣ ያለው ስርጭትና ተዛማጅ ችግሮች ከኣምስት ኣመት በታች በሆኑ ህፃናት ጥናት ማካሄድ ነው፡፡ የእርስዎ ልጅ በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ በኋላ በመጠይቁ ይተባበሩናል፡፡ ትንሽ ሰገራ ከልጆ እንድንወስድ ይፈቅዱልናል:: በተጨማሪ ከጤናዎ ጋር የተያያዝ መረጃ ለማግኘት የሚረዳ የተዘጋጀ መጠይቅ እንዲጠየቁ ትንሽ ግዜ ይተባበሩናል፡፡

<u>ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት</u>: ጥናቱ በልጆ ሆነ በራስዎ ላይ የሚያመጣዉ ጉዳት ሆነ ለጥናቱ የሚያጠፉት ብዙ ጊዜ ኣይኖርም።

<u>ከጥናቱ የሚያገኙት ጥቅም</u>፡ በጥናቱ በመሳተፎ ምንም ኣይነት የገንዝብ ክፍያ ባይኖረውም ከጥናቱ የሚገኘዉ ዉጤት በማወቅ ልጆ ተጠቃሚ ይሆናል፡፡

<u>ስለ ጥናቱ ሚስጥራዊነቱ</u>: የተሰጠውን ማንኛውም መረጃ ሁሉ ሚስጥራዊነቱ የተጠበቀ ነው፡፡ የእርሶ ስም በዚህ መጠይቅ ላይ ኣይጠቀስም፡፡

<u>ከጥናቱ ስለ ማቋረጥ:</u> በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ ስለዚህ መሳተፍ ከጀመሩ በኋላም ቢሆን ማቋረጥ፣ መመለስ የማይፈልጉት ጥያቄ ካለ ይለፈኝ ማለት መብትዎ ነው፡፡ በጥናቱ መሳተፍ ወይም ኣለመሳተፍ በሚያገኙት ኣገልግሎት ላይ ምንም ዓይነት ጥቅምም ሆነ ጉዳት ኣይኖረውም፡፡

በዚህ ጥናት ላይ ያለዎትን ጥያቄ የሚከተሉት ኣድራሻ በማንኛውም መጠቀም ይችላሉ።

የኣጥኚው ስም: ኣቶ ብሃነ ገብረማሪያም ተንቀሳቃሽ ስልክ: +251914798197 Email: birhane1982@gmail.com

የህክምና ላቦራቶሪ ትምህርት ክፍል የምርምርና ሥነምግባር ቢሮ ስልክ ቁጥር: +251 11 275 5170

ኮድ:_____

Annex-IV: የወላጅ ወይም ጠባቂ የስምምነት ፈቃድ (Amharic version)

የአጥኝው ስም: ኣቶ ብርሃነ ገብረማሪያም

ኣማካሪዎች: 1) ዶ/ር ሚስጥረ ወልዴ 2) ኣበበ ኢዳኦ 3) ዶ/ር ትእግስት ባቻ

ስፖንሰር ያደረገው ድርጅት: አዲስ አበባ ዩኒቨርስቲ

ፍቃድ ሰጪ: የህክምና ላቦራቶሪ ትምህርት ክፍል የምርምርና ሥነምግባር ቢሮ

የጥናቱ ርእስ: የየላክቶዝ ስኳር በሰውነት ውስጥ ያለመፈጨት ችግር፣ ያለው ስርጭትና ተዛማጅ ነገሮች ከኣምስት ኣመት በታች በሆኑ ህፃናት መካከል ማጥናት

<u>የወላጅ ወይም ጠባቂ የስምምነት ፈቃድ</u>

ከላይ የተፃፈዉ መረጃ ኣንቢቤዋለሁ ወይም ተነቦልኛል:: የልጄ መሳተፍ ፣ኣለመሳተፍ፣ ከጀመረ በኋላ ማቋረጥ ወይም መመለስ የማልፈልገው ጥያቄ ካለ ይለፈኝ ማለት እንደሚቻል፡ በጥናቱ መሳተፍ ኣለመሳተፍ በማገኘው ኣገልግሎት ላይ ምንም ኣይነት ጥቅምም ሆነ ጉዳት እንደሌለው እና በውጤቱ መሰረት ህኪም ጋር እንደሚያገናኙኝ በመሳተፌም ምንም ኣይነት ክፍያ እንደማይሰጠኝ እና እኔም እንደማልከፍል ተገልፆልኛል፡፡

የጥናቱ ዓላማ ግልፅ ስለሆነልኝ ልጄ በዚህ ጥናት እንዲሳተፍ እና የሚያስፈልገዉን ናሙና ሰገራ እና ደም እንዲሰጥ ተስማሚቻለዉ:: ፊርማዬንም እንደሚከተለው ኣስቀምጫለሁ::

የወላጅ/ ጠባቂ ስም: _____ ቀን _____ /_____/_____ ፌርማ:_____

የጠያቂ ስም: _____ ቀን _____ /_____/_____ ፌርማ: _____

የአጥኚው ስም: _____ ቀን _____ /_____/_____ ፌርማ: _____

ስለ መልካም ትብብርዎ ኣመሰግናለሁ!!

Code No._____

Annex-V: Questionnaire (English version)

Questionnaire for "Magnitude and associated factors of lactose intolerance among malnourished under five children in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia"

Questionnaire Number: ☐

Data Collector's Name: _____ Date: ___/____/___ Signature:_____

A. **Socio-demographic information**

1) Age (in months) _____

2) Sex a) Male ☐ b) Female ☐

3) Weight (Kg): _____

4) Height (M): _____

5) BMI _____

B) Gastrointestinal symptoms

1) Diarrhea a) Yes ☐ b) No ☐

1.1 Duration of diarrhea a) <2 week ☐ b) >2 week ☐

2) Vomiting a) Yes ☐ b) No ☐

C. Stool characteristics

1) Stool consistency a) Watery/Loose ☐ b) bloody diarrhoea ☐

c) Greasy; foul smelling ☐ d) Normal ☐

2) Frequency of stool in 24 hrs. ☐

D. Other symptoms

1) Level of consciousness: a) Conscious ☐ b) Unconscious ☐ c) other ☐

2) Perianal skin erosion a) Yes ☐ b) No ☐

E. Malnutrition status

1) Type a) SAM ☐ b) MAM ☐ c) Other ☐

F. Still Breast feeding a) Yes ☐ b) No ☐

G. Duration of exclusive breast feeding a) < 4 months ☐ b) ≥4 months ☐

H. Did he/she take cow milk a) Yes ☐ b) No ☐ if yes is there any problem after he/she take cow milk a) Yes ☐ b) No ☐

I. Type of therapeutic milk taken a) F75 ☐ b) F100 ☐

J. Family history of LI a) Yes ☐ b) No ☐

K. Pre-term birth a) Yes ☐ b) No ☐

L. Immunization status: a**)** Complete ☐ b) Incomplete ☐

Laboratory result

A) Fecal PH: _____

B) Fecal reducing substances: _____

C) Stool microscopy: _____

ኮድ:_____

Annex-VI: መጠይቅ (Amharic version)

“የላክቶዝ ስኳር በሰውነት ውስጥ ያለመፈጨት ችግር፣ ያለው ስርጭትና ተዛማጅ ነገሮች ከአምስት አመት በታች በሆኑ ህፃናት መካከል ማጥናት በ የካቲት 12 ሆስፒታል ሜዲካል ኮሌጅ ፣ አዲስ አባባ፣ ኢትዮጵያ”

የመጠይቅ ቁጥር: ☐

የጠያቂ ስም: _____ ቀን: ___/____/___ ፌርማ: _____

ሀ. የህፃኑ አካላዊ ማህበራዊ እና ኢኮኖሚያዊ ዝርዝር

1. ዕድሜ (በወር) _____
2. ፆታ ሀ) ወንድ ☐ ለ) ሴት ☐
3. ቁመት(ሜትር):_____
4. ክብደት(ኪ.ግ):_____
5. Body Mass index:_____

ለ) የሆድ ስሜቶች

1. ተቅማጥ ሀ) አዎ ☐ ለ) አይደለም ☐ አዎ ካሉ ለስንት ግዜ?

   ሀ) <2 ሳምንት ☐ ለ) >2 ሳምንት ☐

2. ማስታወክ/መትፋት ሀ) አዎ ☐ ለ) አይደለም ☐

ሐ) የሰገራ ባህሪያት

1. አይነት ሀ) ደም የተቀላቀለ ☐ ለ) ውሃ ያዘለ ☐ ሐ) ቅባት ያለው እና መጥፎ ሽታ ያለው ☐
2. ስንት ግዜ ወደ ሽንት ቤት ይሄዳሉ(ድግግሞች በ24 ሰአት) ☐

መ) ሌሎች ስሜቶች

1. የልጁ ንቃተ ህሊና ደረጃ: ሀ) ንቁ ህሊና ያለው ☐ ለ) ላእምሮውን የሳተ ☐ ሐ) ሌላ ☐
2. የፊንጢጣ መቁሰል ሀ) አዎ ☐ ለ) አይደለም ☐

ሠ) የተመጣጠነ ምግብ እጥረት

1. ኣይነት ሀ) **SAM** ☐ ለ) **MAM** ☐ ሐ) ሌሎች ኣይነት ☐

ረ) የእናት ጡት ይጠባል ሀ) ኣዎ ☐ ለ) ኣይደለም ☐

ሰ) **የእናት ጡት ብቻ ለስንት ወር ጠብተዋል?** ሀ) <4 ወር ☐ ለ) ≥4 ወር ☐

ሸ) የላም ወተት ይወስዳል ሀ) ኣዎ ☐ ለ) ኣይደለም ☐

ከወሰደ በኃላ የሚፈጠር ሽግር ኣለ? ሀ) ኣዎ ☐ ለ) ኣይደለም ☐

ቀ) የወሰዱት የወተት/ምግብ ኣይነት ሀ) F75 ☐ ለ) F100 ☐

በ) ከቤተሰብ የተወረሰ ወተት ያለ መዉሰድ ችግር ኣለ? ሀ) ኣዎ ☐ ለ) ኣይደለም ☐

ተ) ልጁ የተወለደዉ ካልተለመደዉ የእርግዝና ግዜ ነዉ? ሀ) ኣዎ ☐ ለ) ኣይደለም ☐

ቸ) የክትባት ሁኔታ ሀ) ወስደዋል ☐ ለ) ኣልወሰደም ☐

Annex-VII: Standard operating procedure (SOPs)

1. Fecal reducing substance test

Clinical significance: It is used to evaluate the body's ability to digest carbohydrates, or to absorb nutrients from food and drinks. Testing for fecal reducing substances detects congenital disaccharidase deficiencies as well as enzyme deficiencies due to nonspecific mucosal injury. It measures unabsorbed sugars in stool.

Requirements

- Readymade Benedict's qualitative reagent from Abron chemicals, India (CAT No. CH-143) pack volume of 500ml
- Water bath
- Distilled water
- Test tube
- Pasture pipet
- Stool cup

Specimen Collection and Processing

Specimen Type: Random stool (loose stool)

Volume: 3 g or 3 mL stool (Minimum: 2 g or 2 mL stool)

Collection: Random stool which is not mixed with urine will be collected in a clean, leak-proof container. Urine will interfere with the results.

Transport/Storage: Specimen must be received in the laboratory within 1 hour of collection, unless frozen. Delay may cause falsely low results. Freeze specimen if testing is delayed.

Principle

Benedict qualitative reagent contains cupric ion complexes to citrate in alkaline solution. Reducing substances convert cupric to cuprous ions, forming yellow cuprous hydroxide or red cuprous oxide.

Quality controls

Negative control: 0.2 ml distilled water

Positive control: 0.2ml glucose solution

Reagent composition

- Sodium carbonate (100g)
- Tri-sodium citrate dehydrate (173g)
- Copper (II) sulphate. $5H_2O$ (17.3g)
- 1000mL distilled water.

Procedure

1. Take three or more (depending on the number of tests) heat-resistant glass tubes and label as follows:

POS – Positive control NEG – Negative control 1, 2, etc. – Patients' tests

2. Dispense 2.5 ml of Benedict's reagent into each tube. A plastic 2.5 ml syringe or graduated plastic bulb pipette can be used to dispense the reagent. It is not necessary to use a calibrated pipette.

3. Add to each tube as follows:

Tube

POS .....0.2 ml glucose control solution

NEG..... .0.2 ml of distilled

1, 2, etc..... 8 drop of fresh patient's stool

Mix the contents of each tube.

4. Place the tubes in a heat-block set at 100 °C or in a container of boiling water for exactly 5 minutes.

5. Remove the tubes and examine the solution in each tube for precipitate and change of colour.

Report the sugar concentration as follows:

Appearance of solution	Sugar concentration
Blue, clear or cloudy	Nil
Green, no precipitate	Trace
Green, with precipitate	about 0.5 g%
Brown and cloudy	about 1.0 g%
Orange and cloudy	about 1.5 g%
Red and cloudy	2.0 g% or more

Note:

Controls: The negative control (tube NEG) should show the nil reaction. The positive control (tube POS) should show the reaction equivalent to 1.0% of reducing substance.

Interpretation

Normal: 0.25%g

Suspicious: 0.25-0.5%g

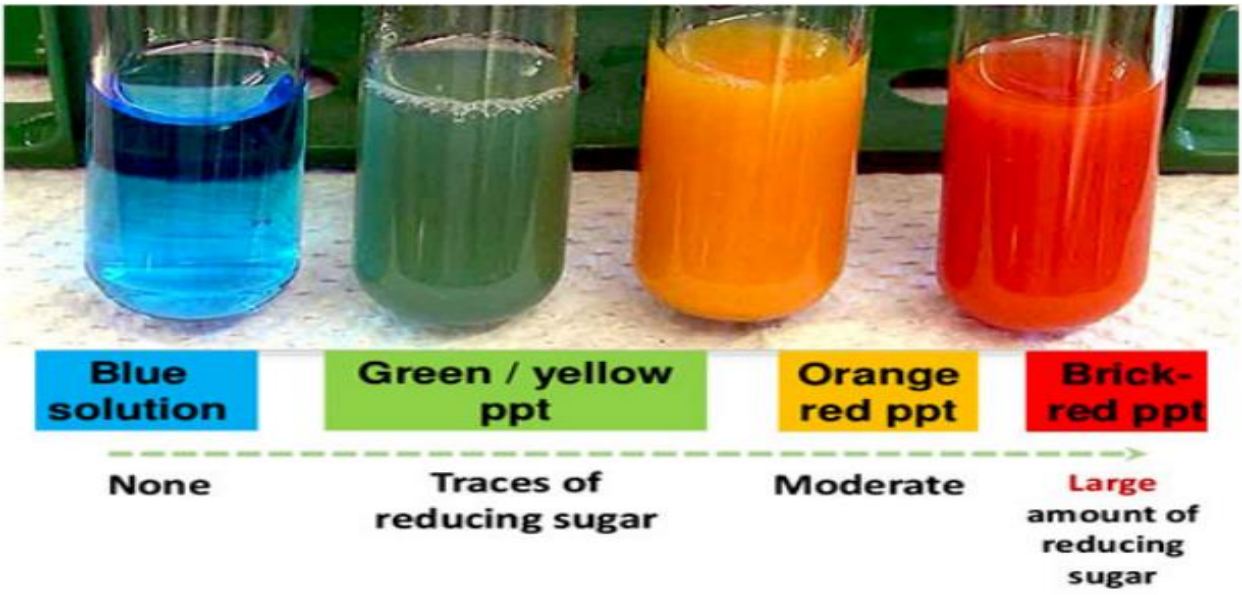
Abnormal: > 0.5%g (positive lactose intolerance)

Interferences

Substances which also reduce Benedict's reagent include other sugars present in stool such as glucose, galactose, fructose, and pentose. False positive reactions may also be obtained if certain drugs are present, e.g. salicylates, penicillin, streptomycin, isoniazid, and p-aminosalicyclic acid.

Benedict's test colour chart

(With corresponding level of Reducing Substances)



2) Fecal pH determination

Clinical significance: pH determination of fecal is most valuable in assessing cases of diarrhea in children caused due to lactose malabsorption. Measurement of fecal pH is useful when rapid diagnosis of carbohydrate malabsorption is important.

Requirement

- pH paper (Universal pH 1-14 from effective laboratory supplies, India)
- Specimen Type: Fresh random liquid stool
- Container: Plastic leak proof container
- Volume: 1 gram or 1 mL stool

Principle

The basic principle of the pH paper is to measure the concentration of hydrogen ions. Acids dissolve in water forming positively charged hydrogen ions (H+). The greater this concentration of hydrogen ions, the stronger the acid.

Quality control: HCL to check acidity and KOH to check alkalinity.

Procedure

1. Collect a fresh and well mixed random stool sample
2. Dip the pH paper in small quantity of the stool material
3. Observe for color change
4. Compare with the color chart and record the pH

Interpretation

Normal stool pH: between 7 and 8 Positive lactose intolerance: pH $\leq$ 5.5

Sample rejection: specimen contaminated with urine; specimen on outside of container

3) Stool Microscopic examination

Materials and reagents

- Microscope
- Microscope slides
- Coverslips
- Wooden applicators
- Pencils
- Normal saline

Procedure

1. Place a drop of physiological saline (0.85%w/v) in the center of the microscope slide.
2. With an applicator stick, pick up a small portion of the feces and put on the drop of saline.
3. Mix the feces with the drops to form homogeneous suspensions.
4. Cover each drop with a cover slip by holding the cover slip
5. Examine the saline preparations using the 10X and 40X objective for motile forms, cyst of intestinal protozoa and for any ova or larva of helminths.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Brhane Gebremariam (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Mistire Wolde (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mr. Abebe Edao (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.