"CROSS-SECTIONAL STUDY OF SERUM ZINC LEVELS IN CHILDREN WITH ACUTE GASTROENTERITIS IN A TERTIARY CARE HOSPITAL"

By

DR.AKSHATHA S.

DISSERTATION SUBMITTED TO SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTER, KOLAR, KARNATAKA

In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE

IN

PEDIATRICS

Under the Guidance of

DR. KRISHNAPPA J.
PROFESSOR
DEPARTMENT OF PAEDIATRICS

Under the Co-Guidance of

DR. K.PRABHAVATHI
ASSOCIATE PROFESSOR
DEPARTMENT OF BIOCHEMISTRY

DEPARTMENT OF PAEDIATRICS,
SRI DEVARAJ URS MEDICAL COLLEGE,
TAMAKA, KOLAR-563101

2018- 2021

SRI DEVARAJ URS MEDICAL COLLEGE,

TAMAKA, KOLAR-563101

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled **"CROSS-SECTIONAL STUDYOF SERUMZINC LEVELS IN CHILDREN WITH ACUTEGASTROENTERITIS IN A TERTIARY CARE HOSPITAL"** is a bonafide and genuine research work carried out by me under guidance of **DR. KRISHNAPPA J,** Professor & Head of the Department of Pediatrics, and under the Co-Guidance of **DR. K.PRABHAVATHI,** Associate Professor, Department of Biochemistry, Sri Devaraj Urs Medical College, Tamaka, Kolar.

Date: **DR. AKSHATHA S.**

Place: Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION, TAMAKA, KOLAR, KARNATAKA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation/thesis entitled **"CROSS-SECTIONAL STUDYOF SERUMZINC LEVELS IN CHILDREN WITH ACUTEGASTROENTERITIS IN A TERTIARY CARE HOSPITAL"** is a bonafide and genuine research work carried out by **DR. AKSHATHA S.** in partial fulfilment of the requirement for the degree of **DOCTOR OF MEDICINE** in **PEDIATRICS.**

Date :

Place :

DR. KRISHNAPPA J MBBS, MD
Professor,
Department of Pediatrics,
Sri Devaraj Urs Medical College,
Tamaka, Kolar.

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION, TAMAKA, KOLAR, KARNATAKA

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation/thesis entitled **"CROSS-SECTIONAL STUDYOF SERUMZINC LEVELS IN CHILDREN WITH ACUTEGASTROENTERITIS IN A TERTIARY CARE HOSPITAL"** is a bonafide and genuine research work carried out by **DR AKSHATHA.** in partial fulfilment of the requirement for the degree of **DOCTOR OF MEDICINE** in **PEDIATRICS.**

Date :

Place :

DR. K.PRABHAVATHI MBBS, MD
Associate Professor,
Department of Biochemistry,
Sri Devaraj Urs Medical College,
Tamaka, Kolar.

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTER, TAMAKA, KOLAR, KARNATAKA

ENDORSEMENT BY THE HOD, PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation/thesis entitled **"CROSS-SECTIONAL STUDYOF SERUMZINC LEVELS IN CHILDREN WITH ACUTEGASTROENTERITIS IN A TERTIARY CARE HOSPITAL"** is a bonafide and genuine research work carried out by **DR AKSHATHA S** in partial fulfilment of the requirement for the degree of **DOCTOR OF MEDICINE** in **PEDIATRICS.**

DR. SUDHA REDDY. V. R.
Professor & HOD
Department of Pediatrics,
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:
Place: Kolar

DR. P. N. SREERAMULU.
Principal,
Sri Devaraj Urs Medical College
Tamaka, Kolar

Date:
Place: Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTER, TAMAKA, KOLAR, KARNATAKA

ETHICAL COMMITTEE CERTIFICATE

This is to certify that the Ethical committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved **DR.AKSHATHA S** Post-Graduate student in the Department of Pediatrics at Sri Devaraj Urs Medical College, Kolar to take up the Dissertation work entitled **"CROSS-SECTIONAL STUDYOF SERUMZINC LEVELS IN CHILDREN WITH ACUTEGASTROENTERITIS IN A TERTIARY CARE HOSPITAL"** to be submitted to the SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR, KARNATAKA.

Date:

Place: Kolar

Member Secretary

Sri Devaraj Urs Medical College & Research Center,

Tamaka, Kolar–563101

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION

TAMAKA, KOLAR, KARNATAKA

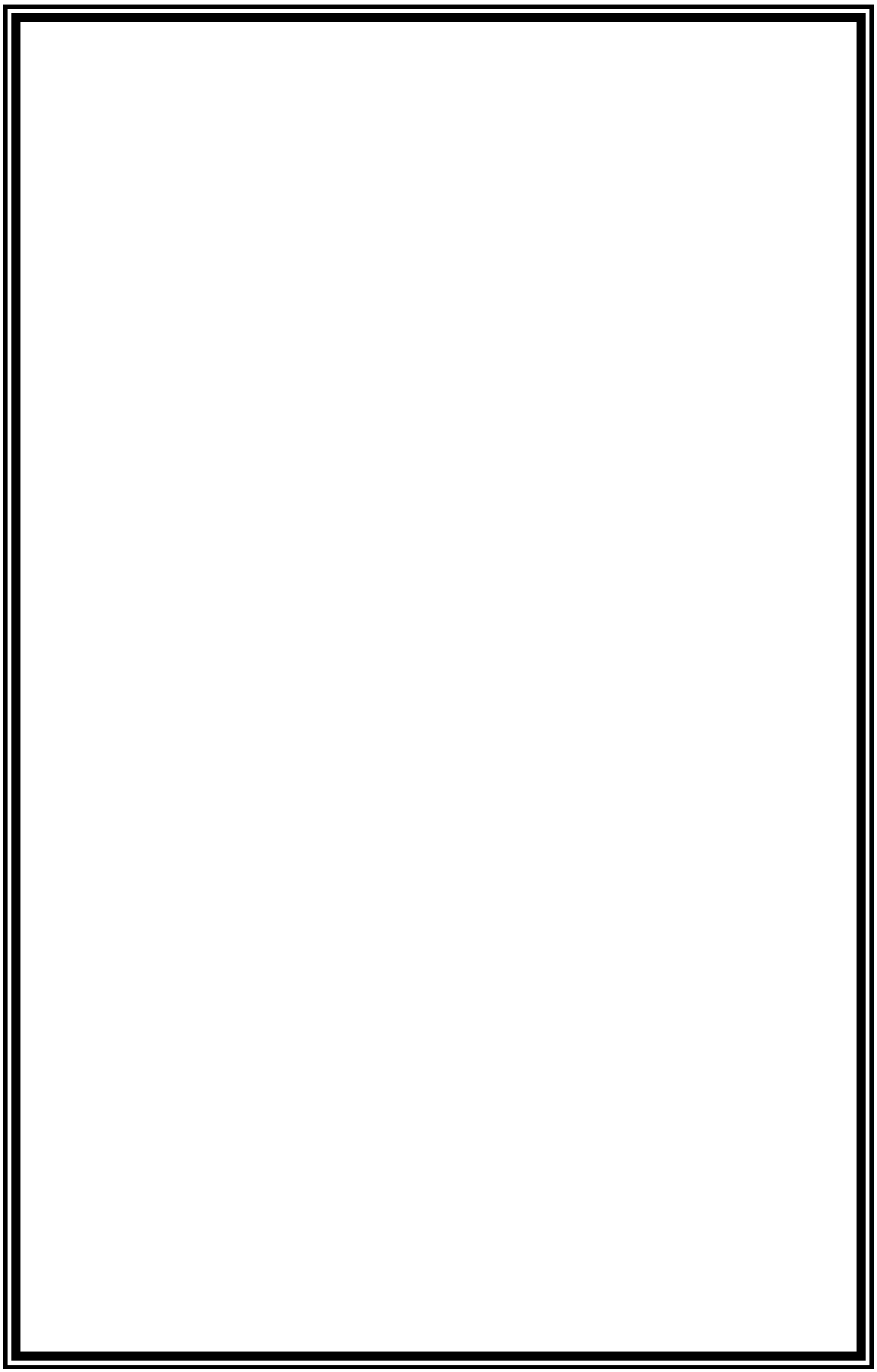
COPY RIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Sri Devaraj Urs Academy of Higher Education and Research Center, Kolar, Karnataka shall have the rights to preserve, use and disseminate this dissertation/thesis in print or electronic format for academic /research purpose.

Date:

Place: Kolar **DR. AKSHATHA S**



ACKNOWLEDGEMENT

First and fore most I thank the ***Almighty*** *for giving me with endless blessings and giving me the strength both mentally and physically during my post-graduation and to make this dissertation possible.*

I wish to express my heart full indebtedness and owe a deep sense of gratitude to my mentor and guide, ***Dr. KRISHNAPPA J*** *Professor, Department of Paediatrics for being very helpful throughout the study and offered his valuable guidance and support to fully understand and complete this study. I am sincerely thankful for encouraging me to the highest peak, paying close and continuous attention towards me to finish all tasks and also providing his kind support, valuable suggestions, immense patience and great care. His precious advice on both the dissertation as well as the path of my career has been priceless. Without his constant supervision and advice, completion of this dissertation would have been impossible. I express my deep sense of gratitude to my senior Professors,* ***Dr Sudha Reddy V R, Dr KNV Prasad, Dr BeereGowda Y C, Dr Bhanuchand P,*** *for their advice and encouragement throughout the study.*

My heartfelt thanks to Assistant Professors ***Dr Narendra, DrJamesDaniel,DrShivaraja, Dr Srikanth, Dr Manasa, Dr Priyantha, Dr Naveen*** *for their practical tips, invaluable advice and constant encouragement.*

I extend my sincere thanks to my seniors Dr. Naveen, Dr.Yasar, Dr. Raksha, whose knowledge has guided and inculcated in me a sense of confidence. I am thankful for their valuable guidance and helping me with my dissertation.

I am very indebted to Dr.Shibhani, Dr. Neha, Dr. Rashmi, Dr Divya, Dr Aradhna, for the constant motivation, support and encouragement throughout the years

I express my sincere thanks to my colleagues and friends, Dr.Chinthana, Dr.Srinadh, Dr.Sampath Goud, Dr. Pradeep Goud, for their cooperation and help in carrying out this study.

I thank my juniors Dr. Vidya, Dr. Niranjan, Dr. Rajitha, Dr. Sanjana, Dr.Pravallika and all budding first-year juniors for their constant support and help in the study

I express my profound gratitude to my beloved parents ***MR. SONNAPPA. N*** *and* ***MRS. SAROJA*** *whose love, blessings and sacrifices made me the person I am today. Also, my gratitude goes to my loving sister and brother Archana, Ashwini, Mohan, Vishwanath for always being there to help me in all ways possible for them.*

Heartfelt thanks to all the staff nurses who are our pillars of support. Special thanks to all ICU, Ward staff for their help and support throughout my study. Last but not least, I extend my gratitude towards all the patientswho agreed to participate in this study; without their precious support, it would not be possible to conduct this research.

Date : **DR. AKSHATHA S**

Place : Bangalore

ABSTRACT

BACKGROUND: Acute gastroenteritis is one of the most important causes of childhood mortality and morbidity. The annual death rate in the world due to diarrhea in under-five children is 1.5-2.5 million. Deaths due to diarrhea in India is estimated at about 3.4lakh per year. In the year 1992, WHO and UNICEF recommended the use of zinc along with ORS for the treatment of diarrhea. Zinc is a ubiquitous trace element essential for mucosal integrity, skeletal growth and gut immunity and absorption of sodium and water. Zinc is absorbed in the small intestine; zinc absorption is inhibited by dietary fiber and phytates present in cereals and legumes. A balanced diet is essential to maintain normal zinc levels. Zinc also plays a role with protease and polymerase as a cofactor and helps in many cellular functions like wound healing and intestinal epithelial cell regeneration

In developing countries, the incidence of children suffering from acute gastroenteritis is worsening due to illiteracy, malnutrition and infection. Zinc losses increase significantly during an episode of diarrhea in infants and are associated with negative zinc balance. There is a mutual dependence seen between diarrhea and zinc deficiency. As zinc deficiency causes diarrhea, diarrhea leads to zinc deficiency.

OBJECTIVES:

1. To determine serum zinc levels in a patient having acute gastroenteritis attending to tertiary care hospital.
2. To correlate the serum zinc levels with severity of diarrhea, nutritional status in children having acute gastroenteritis.

MATERIAL AND METHODS:

Conducted a cross-sectional study in the Pediatrics deapartment at Sri Devaraj Urs Medical College, Tamaka, Kolar for a period of one year from January 2019 to December 2019.

RESULTS: In total we have recruited 120 children. Age distribution of the study participants was 6 months to 60 months. Around 17.5% of the study population had severe dehydration. The mean serum zinc level identified was 9.71 ± 3.88µmole/L.Statistically significant weak negative correlationwas observed betweenthe level of dehydration and serum zinc levels. The trend of decrease in the levels of mean serum zinc with an increase in the severity of dehydration was statistically significant. The mean "serum zinc level" was higher at 11.47 micro mole/L in no dehydration group compared to severe dehydration with 88 ± 2.65 micro mole/L. The negative correlation betweenthe degree of malnutrition and "serum zinc level" was weak and was statistically significant.

CONCLUSION: The present study estimated the association between the serum zinc level, severity of diarrhea and dehydration status.

ABBREVATIONS

GLOSSARY	ABBREVIATIONS
CBC	Complete blood count
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
EIA	Enzyme immunosorbent assay
HC	Head circumference
HFA	High functioning autism
MUAC	Mid-upper arm circumference
MUAC/HC	Mid-upper arm circumference/ head circumference
RNA	Ribonucleic acid
WFA	Water footprint assessment
WFH	Weight for height
WHO	World health organization

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES/GRAPHS

INTRODUCTION

One of the major cause of pediatric morbidity and mortality around the world is acute gastroenteritis.[1] Acute gastroenteritis is a clinical syndrome often defined by an increase in the frequency of stool (>3 loose /watery stool in 24 hours or number of loose /watery bowel movements that exceed the child usual number of daily bowel movements by two or more) with or without fever, vomiting or abdominal pain.[2]

The most common causes of acute gastroenteritis are infectious agents such as viruses, bacteria and parasites. Rotavirus species are responsible for around 70 to 80% of infectious diarrhea cases. Another 10 to 20 percent of cases are caused by the various bacterial pathogens, and parasitic organisms such as Giardia species accounts for less than 10 percent of cases.[2] Increased stool frequency with alteration of stool consistency is the hallmark of acute gastroenteritis.

In gastroenteritis,diarrhea is one of the important risk factor.[3]The occurrence of diarrhea is high among the children in low and middle-income countries due to child malnutrition, low socioeconomic status and low education of mothers, lack of safe drinking water, inadequate sanitation, poor hygiene, crowding and low maternal age.[3]

Sudden onset of loose/watery stool with or without vomiting, abdominal pain/cramps, mild fever and recent contact with someone with diarrhea or vomiting are the clinical features indicative for acute gastroenteritis.[4]

In developed countries, the main cause of morbidity was identified as acute gastroenteritis.[3] The risk of morbidity and mortality associated with acute gastroenteritis in children is high in developing countries. In acute diarrheapatient's death was caused mainly by the excessive fluid and electrolyte losses which may leads to dehydration and acidosis.

In developing countries, malnutrition, reduced growth and impaired cognitive development are the direct outcomes of gastroenteritis in children.The occurance and severity of diarrhoea and other infection can be increased by malnutrition.

Malnutrition is defined as an acute, subacute or chronic state of nutrition, in which varying degrees of overnutrition or undernutrition with or without inflammatory activity have led to a change in body composition and diminished function.[5]While changing the diarrhea from minor to severe,Children may suffer with loss of appetite, food restriction and malabsorption syndrome are the basic reason for malnutrition.[3]

There is an association identified between zinc deficiency, diarrhea and malnutrition usually in the malnourished children. It can also cause further depletion of zinc.[6]Zinc level can be compromised by frequent episodes of acute diarrhoea. It is due to the increased faecal zinc losesepisode of diarrhoea episode and can also lead to a "negative" zinc balance. Zinc stores are limited; therefore, it can be rapidly depleted by acute intestinal illness.[7]

In a North Central Nigeria study[8], the zinc deficiency (serum zinc levels < 65 µg/dl) was identified in the most of the children with diarrhea as compared to those without diarrhea. Thereby, it indicates that the mean level of serum zinc differ among the bacteria isolated.

In the year 2016, a study[9], was done in 80 children admitted in a pediatric hospital in which the serum zinc level was negatively correlated with the severity of dehydration and duration of hospitalization. A case-control study[10],was carried out in Iran, between July 2012 and January 2013 in which the mean "serum zinc levels" were higher in the patients with acute bloody diarrhea as compared to the control group.

A dose of 10–20 mg zinc/d (dependent on age) for 10–14 d is recommended by "WHO/UNICEF" injoint Statement on Clinical Management of Acute Diarrhea to reduce the duration of diarrhea, its severity and also for the prevention of subsequent episodes.[11] Studies conducted in both the developing and developed countries showed a potential effect of zinc in decreasing the rate of complications and mortality associated with diarrhea.[12, 13]

NEED OF THE STUDY:

Despite great advances in the management of diarrheal diseases, persistent diarrhoea remains a major problem due to its syndromic nature in developing countries. Zinc depletion is one of the major factors that contribute to the detrimental effects of persistent diarrhoea on the human body. There is an association identified between zinc deficiency, increased incidence, severity and duration of childhood diarrhoea. Worldwide, acute diarrhoea is associated with morbidity and mortality in children. Zinc has been studied extensively recently for its potential effect on prevention, control and treatment of acute diarrhoea. Its deficiency is associated with an increased risk of gastrointestinal infections, adverse effects on the structure and function of the gastrointestinal tract, and impaired immune function. The routine treatment plan to all children with diarrhea is to supplement zinc as per WHO protocol, without knowing exact zinc status, therefore this study is undertaken to estimate serum zinc level andseverity of diarrhea . It is valuable in detecting subtle deterioration in clinical condition leading to earlier intervention and reduced morbidity and mortality.

AIMS AND OBJECTIVES

1. To determine serum zinc levels in a patient having acute gastroenteritis attending to tertiary care hospital.

2. To correlate the serum zinc levels with severity of diarrhea, nutritional status in children having acute gastroenteritis.

REVIEW OF LITERATURE

1. Gastroenteritis in children (2 PAGES) –

a) Definition,

"Gastroenteritis" is the inflammation of gastrointestinal tract exemplified in combination with abdominal pain, cramping, nausea, vomiting, diarrhea and dehydration. Gastroenteritis can be acute or chronic gastroenteritis.[3] Chronic gastroenteritis is the most common cause of childhood mortality.[3]

b) Epidemiology- global, India,

In children under 5 years, acute gastroenteritis accounts 3-5 billion cases and nearly 2 million deaths each year worldwide.[14] Around 87% of the "acute gastroenteritis" is caused by a virus in the developed countries. Globally, 40% of the cases of diarrhea are caused by rotavirus in infants. In Asia, Rotavirus account around 145,000 deaths each year with the maximum numbers in India, Pakistan and Indonesia. In Pakistan, 1 in 40 infants experiences a severe episode of rotavirus gastroenteritis per year.[3]Gastroenteritis affects 3 to 5 billion children worldwide each year. It accounts for around 1.5 to 2.5 million deaths per year or 12% of all deaths among children less than 5 years of age.[15]

c) Types of gastroenteritis:

Gastroenteritis can be classified as acute, persistent, chronic or recurrent.[16]

- Acute: It lasts 14 days or fewer than 14 days in duration.
- Persistent: It occurs for more than 14 but fewer than 30 days in duration.
- Chronic: It occurs for more than 30 days in duration.
- Recurrent: Diarrhea that recurs after 7 days without diarrhea.[17]

2. Acute gastroenteritis in children

a) Definition

The AAP defines acute gastroenteritis as diarrheal disease of rapid onset, with or without accompanying symptoms or signs such as nausea, vomiting, fever or abdominal pain.[2]

b) Clinical Presentation

Sudden onset of loose/watery stool with or without vomiting, abdominal pain/cramps, mild fever and recent contact with someone with diarrhoea or vomiting are the clinical features indicative for acute gastroenteritis.[4]Viral gastroenteritis typically presents with the short prodrome, along with a mild fever and vomiting, followed by 1-4 days of nonbloody, watery diarrhea.

c) Causes, Risk Factors and Pathogenesis

Increased stool frequency with alteration of stool consistency is the hallmark of acute gastroenteritis. Viruses, bacteria and parasites are the main causative agents of "acute gastroenteritis". In the developed world, rotavirus species, are responsible for around 70 to 80% of infectious diarrhea cases. Another 10 to 20 percent of cases are caused by the various bacterial pathogens, and parasitic organisms such as Giardia species accounts for less than 10 percent of cases.[2]

Diarrhea is the common risk factor for gastroenteritis.[3]Attendance at day care centers and impoverished living conditions with poor sanitation are the other actors that increase the risk of acute gastroenteritis in children.[2] The occurrence of diarrhea is

high among the children in low and middle-income countries due to child malnutrition, low socioeconomic status and less educated mothers , lack of clean water for drinking, poor hygiene, crowding and low maternal age.[3]

The primary cause of bacterial gastroenteritis is the Campylobacter jejuni in the developed world. Poultry is associated with the majority of these cases. Around 15% of cases in children are caused by bacteria. Salmonella, Shigella, Escherichia coli, and Campylobacter are the most common types of agents. When the food gets contaminated with bacteria and remains at room temperature for several hours, the bacteria can multiply and increase the risk of infection in those who eat the food.

One of the important causes of diarrhoea is the toxigenic Clostridium difficile. It commonly occurs in the elderly population. Whereas, it occurs in infants without developing symptoms. The risk of infections can be increased by acid-suppressing medication. The risk is greater with proton-pump inhibitors as compared to histamine-2 antagonists. Giardia lamblia is the most common protozoa that cause gastroenteritis.

Faecaloral transmission of contaminated food and water and the airborne route is the mode of spread from person to person. The maldigestion of carbohydrates and their accumulation in the intestinal lumen (in the absence of lactase) are induced by the rotavirus infection. Malabsorption of nutrients along with the concomitant inhibition of water reabsorption can lead to a malabsorption component of diarrhoea. A calcium-dependent chloride secretory mechanism is caused by the rotavirus secretion called as an “enterotoxin”, NSP4.The infected epithelial cells can release the rotavirus without destroying them. Diarrhoea can be initiated by the viral attachment and entry

into the epithelial cell without cell death.Numerous cytokines and chemokines are secreted and synthesized by the epithelial cells. They can direct the host immune response and can also regulate cell morphology and function.[18]

Table 1: Causes of acute gastroenteritis in children.[14]

Viruses (~70%)	• Rotaviruses • Noroviruses (Norwalk-like viruses) • Enteric adenoviruses • Caliciviruses • Astroviruses • Enteroviruses
Bacteria (10-20%)	• Campylobacter jejuni • Non-typhoid Salmonella spp • Enteropathogenic Escherichia coli • Shigella spp • Yersinia enterocolitica • Shiga toxin-producing E coli • Salmonella typhi and S paratyphi • Vibrio cholerae
Protozoa (<10%)	• Cryptosporidium • Giardia lamblia • Entamoeba histolytica
Helminths	• Strongyloidesstercoralis

d) Diagnosis, Microbiological profile – main focus with culture and sensitivity

Stool studies for occult blood, white blood cell count and microscopy for protozoa, Clostridium difficile toxin, Giardia lamblia by enzyme immunoassay or bacterial culture are performed if any bacterial or protozoal infection suspected. Serum electrolytes, urea, creatinine, amylase and complete blood count, and conducting abdominal imaging studies can be used to evaluate the patients with low-grade fever, nausea, vomiting, abdominal pain and extreme dehydration.

Stool studies and culture

Inflammatory diarrhoea is indicated by the presence of blood or leukocytes in the stool. Wright stain or methylene blue is used to perform the stool studies. Faecal leukocytes are identified in 80-90% of patients with Salmonella or Shigella infections. Whereas, they are less common in the other infecting organisms' cases, such as Campylobacter and Yersinia.

A stool culture is not preferred until unless an unusual bacterial cause is suspected. Immunocompromised, immunosuppressed patients and those who have recently travelled to remote locations or developing nations are preferred for stool culture. The indications for the stool culture include fever, bloody stools, leukocytes in the stool, pain that resembles with appendicitis (Yersinia), and diarrhoeal illness associated with partially cooked hamburgers.

Bloody stools, stools that test positive for occult blood or leukocytes, prolonged diarrhoea that has not been treated with antibiotics, an immunocompromised host, or for epidemiological purposes, such as cases involving food handlers are the specific indications for stool cultures. Campylobacter, Shigella, Salmonella, Aeromonas, and Yersinia species can be identified by routine stool cultures. The diagnosis of rotavirus infection can be performed by rapid antigen testing of the stool, either by EIA (> 98% sensitivity and specificity) or latex agglutination tests (less sensitive and specific in comparison to EIA). [18]

e) Complications, morbidity, mortality

In developed countries, acute gastroenteritis is one of the important causes of morbidity .[3] The risk of morbidity and mortality associated with acute gastroenteritis in children is high in developing countries

The following are the complications associated with acute gastroenteritis [14]

- Dehydration
- Metabolic acidosis
- Electrolyte disturbance (hypernatremia, hyponatremia, hypokalemia)
- Carbohydrate (lactose, glucose) intolerance
- Susceptibility to reinfection
- Development of food (cow's milk, soy protein) intolerance
- Haemolyticuraemic syndrome
- Iatrogenic complications (due to inappropriate composition or amount of intravenous fluids)
- Death

The cause of death in acute diarrhea is electrolyte losses and excessive fluid leads to 17 dehydration and acidosis. In developing countries, malnutrition, reduced growth and impaired cognitive development are the direct outcomes of gastroenteritis in children. The occurrence of severity of diarrhea and other infections can be increased by malnutrition. The changing of diarrhea from minor to severe, and children suffer from loss of appetite, food restriction and malabsorption syndrome are the basic reason for malnutrition.3

3. Association between malnutrition and acute gastroenteritis in children

Define malnutrition

Malnutrition is defined as an acute, subacute or chronic state of nutrition, in which varying degrees of overnutrition or undernutrition with or without inflammatory activity have led to a change in body composition and diminished function.[5]

Classification:

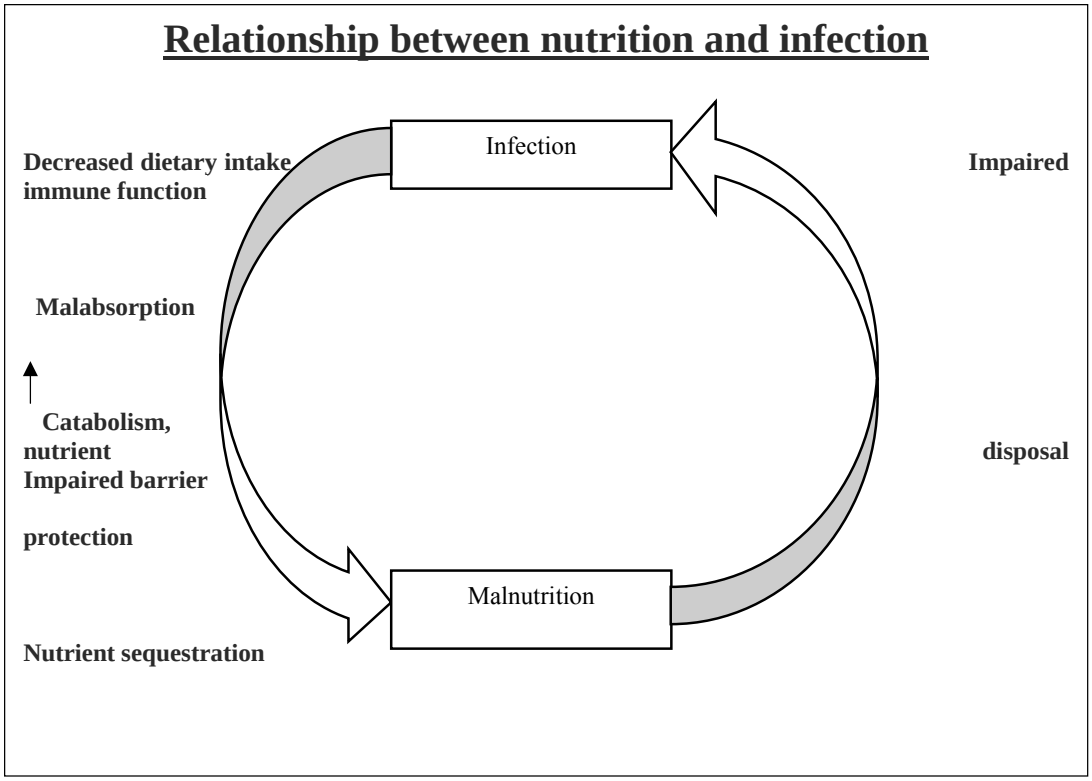
Table 2: Historical malnutrition classification schemes: anthropometry.[19]

Classification	Variable	Grade	Definition
Gomez	Median WFA (%)	Mild (grade I)	75%-90% WFA
		Moderate (grade II)	60%-74% WFA
		Severe (grade II)	<60 WFA
Waterlow (wasting)	Median WFH (%)	Mild	80% - 89%WFH
		Moderate	70%-79% WFH
		Severe	<70% WFH
Waterlow (stunting)	Median HFA (%)	Mild	90%-94% HFA
		Moderate	85%-90%HFA
		Severe	<85%HFA
WHO (wasting)	WFH (Z scores between WFH Medain score)	Moderate	Z score between -2 and -3
		Severe	Z score < -3
WHO (stunting)	HFA (Z scores between WFH Medain score)	Moderate	Z score between -2 and -3
		Severe	Z score < -3
Kanwati and McLaren	MUAC/HC	Mild	<0.31
		Moderate	<0.28
		Severe	<0.25

Pathogenesis of acute gastroenteritis in malnutrition:

Infection can adversely affect the nutritional status in children through the decrease in dietary intake and intestinal absorption, increased catabolism and sequestration of nutrients that are required for tissue synthesis and growth. On the other hand, Due to the negative impact on barrier protection afforded by the skin and mucous membranes and by inducing alterations in host immune function, the malnutrition can predispose to infection.

Figure 1: Relationship between nutrition and diarrhoea.[20]



4. Role of zinc in acute gastroenteritis in children (5PAGES)

Zinc – functions, pharmacokinetics, adverse effects

Zinc, micronutrient which is essential for human health. It acts as a catalyst for more than 300 enzymes. It plays a major role in role in the DNA and RNA synthesis and degradation, protein synthesis, cell-mediated immunity, cell growth and differentiation and gene expression. Inadequate intake or absorption, increased excretion or increased daily needs can lead to zinc deficiency.[21]

Pharmacokinetics of zinc:

Absorption:

The molecular weight of elemental zinc sulfate and zinc sulfate are 287.5 and 65.37. Crude zinc sulfate is also called as white Vitriol. Each gram of zinc sulfate represents 3.5 millimoles of Zn. It is insoluble in alcohol whereas, its solubility is 1 in 0.6 ml of water. Only 20 to 30% of the zinc and its salts are absorbed from the gastrointestinal tract, duodenum and ileum. Re-absorption of the endogenous zinc takes place in the ileum and colon, thereby creating enterohepatic circulation.

Distribution:

zinc is bound to protein metallothionein in the intestines after the absorption. It is widely distributed throughout the body. RBCs, WBCs, muscles, bones, Skin, Kidneys, Liver, Pancreas, retina, and prostate, are the primary sites of storage. The extent of binding to plasma albumin is 60 - 70% whereas, 30 - 40% to alpha 2 macroglobulins or transferring and 1% to amino acids like histidine and cysteine. Peak plasma concentration reaches by around two hours.

Elimination:

Around 90%of the zinc is excreted through feces. Kidney plays a small role in regulating the body Zn content; therefore, traces of zinc are also found in the urine.[22] Growth retardation, delayed puberty, erectile dysfunction, diarrhea, alopecia, glossitis, nail dystrophy, hypogonadism (in males), decreased immunity are the symptoms of zinc deficiency. The adverse effects caused by the zinc supplementation are its metallic taste, nausea, vomiting, abdominal cramping, diarrhea, suppressed immunity, reduced levels of high-density lipoprotein cholesterol, decreased copper stores, urinary tract infection, nephrolithiasis. The diseases associated with the zinc deficiency are the crohn disease, celiac disease, chronic alcoholism, cirrhosis, sickle cell disease, acrodermatitis enteropathica.[23]

Normal zinc levels:

Table 3: Estimated physiological requirements for the absorbed zinc by age group and sex.[21]

Variables age	WHO		Variables age	FNB		IZiNCG	
	References wt. (kg)	Requirement (mg/day)		References wt. (kg)	Requirement (mg/day)	References wt. (kg)	Requirement (mg/day)
6-12 mo	9	0.84	6-12 mo	9	0.84	9	0.84
1-3 year	12	0.83	1-3 year	13	0.74	12	0.53
3-6 year	17	0.97	4-8 year	22	1.20	21	0.83
6-10 year	25	1.12	-	-	-	-	-
10-12 year	35	1.40	8-13 year	40	2.12	38	1.53
12-15 year	48	1.82	-	-	-	-	-
15-18-year M	64	1.97	14-18-year M	64	3.37	64	2.52
15-18-year F	55	1.54	14-18-year F	57	3.02	56	1.98
Pregnancy	-	2.27	Pregnancy*	-	4.1-5.0	-	2.68
Lactation	-	2.89	Lactation*	-	3.8-4.5	-	2.98

** Different stages of pregnancy/lactation. WHO= World Health Organization; FNB=Food and Nutrition Board; IZiNCG=International Zinc Nutrition Consultative Group*

Abnormal levels and reasons:

The reference range of serum zinc is 10.7 - 22.9µmol/L (70-150 µg/dl). The zinc deficiency is indicated by a concentration of below 7 µmol/L (46µg/dl). The optimal range of plasma zinc is 13.8 - 22.9µmol/L (90-150µg/dl).When the concentrations of plasma zinc drop below 9.9µmol/L (65 µg/dl), the clinical signs of zinc deficiency began to occur. Values of less than of "5µmol/L (33 µg/dl)" are associated with loss of the "senses" of taste and smell, abdominal pain, diarrhoea, skin rash and loss of appetite.

Table 4: Possible conditions suggested by serum zinc levels and countermeasures.[24]

Serum zinc level (g/dl)	Possible conditions
300–700	Acute intoxication
160–299	Intoxication or secondary elevation due to excessive intake or hemodialysis
84–159	Normal range
60–83	Zinc deficiency Physiological fluctuation Variation caused by drugs
Below 59	Deficiency
Below 30	Definite deficiency (acrodermatitis enteropathica, Zinc replacement prolonged high-calorie parenteral therapy)

Levels in children with diarrhoea without malnutrition:

Ahmed TM et al[25], in their study of 50 children,mean level of serum zinc in children with malnutrition was 54.48±18.91 ug/dl whereas, it was 72.72±8.21 ug/dl in healthy children. Afolabi O et al[8], conducted a study in 200 participants in which the mean level of serm zinc children with diarrhoea and in controls was identified with 65.3 ±

7.4 μg/dl and 69.0 ± 6.5 μg/dl respectively. Zinc deficiency (serum zinc levels < 65 μg/dl) was identified in 47% of patients while in controls with 32%. Conclusion of the study was mean serum zinc levels differ among the bacteria isolated. Mahyar A et al[10], conducted a case-control study in 60 children in which 74.1 ± 23.7 μg/dL, 169.4 ± 62.7 μg/dL and 190.1 ± 18.0 μg/dL were the mean level of serum in patients with acute bloody diarrhoea, acute watery diarrhoea and the control group.

5. Correlation between zinc levels and severity of diarrhoea in children

Correlation between zinc levels and severity of diarrhoea in general:

Zinc , essential trace element that is required for normal intestinal mucosal integrity, immune function and sodium and water transport.[26] Zinc deficiency is identified in 30% of the world's population.[27]Studies conducted in both the developing and developed countries showed a potential effect of zinc in decreasing the rate of complications and mortality associated with diarrhea.[12, 13]

Zinc level can be compromised by frequent episodes of acute diarrhoea. It is due to the increased faecal zinc loses in the episode of diarrhoea and can also lead to a negative zinc balance. Zinc stores are limited; therefore, it can be rapidly depleted by acute intestinal illness.[7] The risk of zinc deficiency can be increased with a high prevalence of diarrheal episodes.[28]

Eskander AE et al.[9] conducted a study in 80 children in which zinc level was negatively correlated with the severity of the disease measured by VSS. A dose of10–20 mg zinc/d (dependent on age) for 10–14 d is recommended by WHO/UNICEF Joint Statement on Clinical Management of Acute Diarrhea for the reduction of diarrheal duration and its severity also for the prevention of subsequent episodes.[11]

Association between zinc levels and diarrhoea severity in malnourished children:

In developing countries, the predominant host factor associated with diarrhea is the presence of malnutrition. There is an association identified between zinc deficiency, diarrhea and malnutrition usually in the malnourished children. It can also cause further depletion of zinc.[6] Malnutrition can increase the severity and duration of diarrhea.[29-31]

Zinc is essential for the controlled growth of the intestinal mucosa.[32] The transport of water and electrolytes across the intestinal mucosa can be improved with zinc supplementation.Castillo-Duran et al [33], concluded in his study that the daily losses of zinc in the intestinal fluid during acute diarrhea were as high as 159 µg/kg/day as compared with 47 µg in control children.

The risk of developing zinc depletion with an episode of diarrhoea is high in children with marginal nutritional status. The duration and frequency of acute dehydrating diarrhea can be reduced by the supplementation of zinc in malnourished children who have a pre-existing zinc deficiency.[6] Zinc supplementation can improve the immunity in children, thereby can reduce the severity of diarrhoea by limiting growth and multiplication of diarrheal pathogens in the intestine.[34, 35]

Abolurin OO et al.[36], conducted a comparative cross-sectional studyin 500 children. The study purpose was to compare the prevalence of zinc deficiency in children with acute diarrhoea. The study results revealed that 78.8 ± 35.6 µg/dl and 107.3 ± 46.8 µg/dl were the serum zinc level in children with diarrhoea and without diarrhoea. The mean levels of serum zinc was low in diarrhoea patientsthan the controls. The prevalence of zinc deficiency among the patients was 30.4% whereas 12.4% in

controls. There was an association identified between the low social class and higher prevalence of zinc deficiency in children with diarrhoea. This study concluded that routine zinc supplementation should be practiced in the management of diarrhoea.

Afolabi O et al.[8], conducted a study in 200 participants. Thestudy purpose was to compare the prevalence of bacterial etiology in children with acute diarrhoea and also to compare their serum zinc levels. Bacteria were isolated in 73.0% patients whereas, 6.0% in controls. EscherichiacoliKlebsiella spp., Proteus spp.and Pseudomonas aeruginosa were isolated with 39%, 28%, 4% and 2% respectively. Whereas E. coli and Klebsiella spp. were identified with 4% and 2% in controls. The "mean serum zinc level" in patients and in controls was identified with 65.3 ± 7.4 µg/dl and 69.0 ± 6.5 µg/dl, respectively. Zinc deficiency (serum zinc levels < 65 µg/dl) was identified in 47% of patients while in controls with 32%. This study concluded that the "mean serum zinc levels" differ among the bacteria isolated.

Afolabi OF, et al[37], performed a comparative cross-sectional in 200 participants. The study purpose o was to identify the prevalence of viral etiology and to compare the mean levels serum zinc in children with acute diarrhea. The prevalence of viruses was identified in 62.0% of patients. Rotavirus, adenovirus and norovirus were detected with 30%, 21% and 11% respectively. The only virus detected in controls was rotavirus with 2%. The mean value of serum zinc level in the patients and controls were 65.3 ± 7.4 µg/dl and 69.0 ± 6.5µg/dl, respectively. The prevalence of zinc deficiency in the patients and in controls were 47% and 32%. This study concluded that the mean serum zinc levels differ significantly among the viruses isolated.

Bitarakwate E et al[38], conducted a cross-sectional descriptive study.The study purpose was to identify the serum zinc status in children with persistent diarrhoea. The study results revealed that 5.83mol/l and 8.99mol/l were the "mean serum zinc level" in children with persistent diarrhoea and children without diarrhoea. The serum zinc concentration was low among the children with persistent diarrhoea as compared to the children without diarrhoea. The prevalence of zinc deficiency was 47.9% in children with persistent diarrhoea. There was an association identified between hypoproteinemia and hypoproteinemia in children with persistent diarrhoea. This study concluded that the prevalence of zinc deficiency and malnutrition are high in children with persistent diarrhoea.

Dutta P et al[39], performed a double-blind, randomized, placebo-controlled trial in167 subjects. The study aimed to compare the clinical efficacy of supplementation of zinc, zinc plus vitamin A, and zinc plus a combination of micronutrients and vitamins in children with on acute diarrhea. There was a significant reduction identified in the outcome variables in all the supplemented groups as compared with the placebo group. The lowest reduction of outcome variables was identified in group 3 (zinc plus a combination of micronutrients and vitamins) while group 2 (zinc plus vitamin A) was observed with the speedy recovery. This study concluded the clinical benefit of zinc in children with diarrhea.

El-HawaryM et al[40], conducted a study in 160 children. The objective of the study was to evaluate the role of zinc as a risk factor in the development of acute diarrhea in children. Statistically significant difference was not identified in the mean serum zinc level among cases and controls. Hemoglobin level and TLC were statistically

significant difference between cases and controls. Amebiasis was identified in the controls and cases with37.5% and 54.4% respectively. Mean levels of serum zinc was significantly associated with the low serum sodium level. The study concluded the influence of zinc in children with acute diarrhoea.

Eskander Aet al[9], performed a study in 80 children. The purpose of the study was to correlate the level of zinc with the severity of pediatric diarrhoea estimated by Vesikari Scoring System. The study results revealed that 56.2% had zinc deficiency. Serum zinc level was negatively correlated with the severity of dehydration and duration of hospitalization. Zinc therapy should be considered in children with diarrhoea.

Fathima M et al[41], conducted an observational study in 70 children.This study aimed to determine the zinc status and its relation in children with diarrhoea. The prevalence of zinc deficiency was higher in children belonging to 3-4 years age group. The study results revealed that 49% of the children were belonged to the age group of less than one year. Around 67% of the children were from the upper-lower socioeconomic class, and 62.42±23 mg/dL was their mean serum zinc level. This study concluded that 60% of the children with diarrhoea are identified with deficiency of zinc.

Gunes H et al[42], performed a study in 88 patients. The study objective was to identify the serum zinc status in children. The study results revealed that the serum mean levels of serumZn and Ca were lower in the case group as compared to controls. This study concluded that measurements of serum zinc levels should be performed in children.

Habibi R, et al[43], conducted a cross-sectional study in 160 children. The purpose of the study was to determine the baseline serum zinc level of children with gastroenteritis. The history of zinc use was identified in 53.8% of participants, whereas 46.2% had no history of zinc use. Normal zinc level was identified in 65% of the samples in the gastroenteritis group. The study concluded that the serum zinc level should be measured before the administration of zinc in children with gastroenteritis.

Mahyar A et al[10], conducted a case-control study in 60 children. The study aimed to compare serum zinc levels in children with and without acute diarrhoea. The study results revealed that 74.1 ± 23.7 μg/dL, 169.4 ± 62.7 μg/dL and 190.1 ± 18.0 μg/dL were the mean serum zinc levels in the patients with acute bloody diarrhoea, acute watery diarrhoea and the control group. Hypozincemia in children with acute bloody diarrhoea and in those with acute watery diarrhoea was observed with 50% and 12.5% respectively. This study concluded that serum Zn levels are decreased in children with acute bloody diarrhoea.

Patel A et al[44], conducted a meta-analysis study.The purpose of the study was to determine the therapeutic benefits of zinc supplementation in the treatment of acute or persistent diarrhea in children. The study results revealed that there was significant unexplained heterogeneity across the studies for the effect of Zn supplementation in reducing the diarrheal outcomes. The mean duration of diarrhea can be reduced by 19.7% with zinc supplementation. There was no effect on stool frequency or stool output with zinc supplementation and also increased the risk of vomiting. This study

concluded the influence of zinc supplementation for the prevention and treatment of childhood diarrhea.

RerksuppapholS et al[45], performed a cross-sectional study in 50 children. The purpose of the study was to evaluate the serum zinc levels in children admitted with acute diarrhoea. The study results revealed that 24.0 hours was the median duration of diarrhoeabefore admission, and it was 4 times high than the frequency of diarrhoeal episodes in the next 24 hours. Zinc deficiency was identified in 44% of the children, and 69.2±18.5µg/dL was the mean serum zinc concentration at admission. This study concluded a high prevalence of low zinc levels in children with acute diarrhoea.

Sezer RG et al[46], performed a case control study in 203 children. The aim of the study was to determine the zinc deficiency in acute gastroenteritis. Disease type showed a statistically significant relationship with plasma zinc level. Zinc-deficient children were identified with a higher odds ratio for acute gastroenteritis. There was an association identified between low zinc levels and lower weight measurements. This study confirmed the deficiency of zinc in acute gastroenteritis

Taghavi-Ardakani A, et al[47], conducted a cross-sectional study of 105 children. The aim of the study was the determination of the"serum zinc level" in children with diarrhea. The study findingsshowed that 48.6% were male, and the majority of them were aged more than 12 months. The mean value of "serum zinc level" and diarrhea duration more than 3 days were lower in the hospitalized children. The "mean serum zinc level" was lower in children with FTT. The study concluded the role of "serum zinc" in children with diarrhoea.

LACUNAE OF LITERATURE:

Globally, acute diarrhoea is associated with morbidity and mortality are high in under-five children with conflicting reports regarding the therapeutic benefit of zinc across the different causative pathogens. Existing literature suggested that the "micronutrient zinc" plays a pivotal role in childhood diarrhea. However, there are inconsistent reports of its therapeutic benefit across the various causative enteropathogens. There are not many studies about serum zinc levels in children suffering from acute gastroenteritis in India. There was significant unexplained heterogeneity across the studies for the effect of zinc supplementation in reducing important diarrhea outcomes. The role of zinc in the pathogenesis of diarrhoeais controversial.

MATERIALS AND METHOD

Study site: This study was conducted in the Department of Pediatrics at Sri Devaraj Urs Medical College, Tamaka, Kolar

Study population: Children aged between 3months to 60months attending to RLJH, a tertiary care center for treatment of acute gastroenteritis were considered as the study population.

Study design: The current study was a cross-sectional study

Sample size: Sample size is estimated based on serum zinc level and its correlation in acute pediatric diarrhea. Keeping prevalence of 56.2% absolute error of 10%, confidence interval of 95%, precision at 5%, the sample size calculated is 95, with 20% drop out rate, sample size comes to 114.[9]

Sampling method: All the eligible subjects were recruited into the study consecutively by convenient sampling till the sample size is reached.

Study duration: The data collection for the study was done between January 2019 to December 2020 for a period of 1 year.

Inclusion Criteria:

Children aged between 3months to 60months attending to RLJH for treatment of acute gastroenteritis with or without malnutrition.

Exclusion criteria:

1. Patients developed diarrhea after the intake of drugs (drug-induced diarrhea)/ received zinc before attending to hospital.
2. Children having persistent Chronic diarrhea (>14days).
3. Children with lactose intolerance.
4. Children having systemic infections with gastroenteritis (parenteral diarrhea).

Ethical considerations: Study was approved by the institutional ethics committee. Informed written consent was obtained from all the parents/guardian of study participants, and only those participants whose parents/guardian are willing to sign the informed consent were included in the study. The risks and benefits involved in the study and the voluntary nature of participation were explained to the parents/guardian of participants before obtaining consent. Confidentiality of the study participants was maintained.

Data collection tools: All the relevant parameters were documented in a structured study proforma.

Methodology:

This study was started after obtaining ethical clearance from the institutional ethical committee as well as consent from the parents. Children aged between 3months to 60months having acute gastroenteritis attended to RLJH for treatment of acute gastroenteritis are included in the study. The study period was from January 2019 to December 2019.Children with a history of diarrhea are examined clinically as per proforma, and all clinical features were documented. A complete physical examination was performed, and WHO guidelines were used for the assessment of dehydration. The degree of dehydration was classified as No dehydration, some dehydration, and severe dehydration.

Patients were started on treatment as per WHO guidelines, signs of dehydration and duration of diarrhea were recorded in the proforma and monitored for improvement.

3ml of blood was collected and stored at -20 degree centigrade for serum zinc level. Zinc levels were estimated using the caloric metric method with spectrophotometer of the wavelength of 560nm, other routine investigations were CBC, CRP, Serum electrolytes, and Stool examination will be done.

Diarrhea was defined as the passage of unusually loose or watery stools, usually at least three times in a 24hour period. Malnutrition is defined as children whose weight <2 SD and length/height <2 SD.[13]

Table 5: Assessment of malnutrition.[12]

Assessment	Weight for Age	Weight for Height	Mid-Arm Circumference
Moderate Malnutrition	60-75%	70-80%	11.5-12.5%
Severe Malnutrition	<60%	<70%	<11.5%

Table 6: Assessment of Dehydration.[12]

Condition	Well, Alert	Restlessness, Irritable	Lethargic, Unconsciousness
Eyes	Normal	Shrunken	Shrunken
Thirst	Drinks Normally, Not Thirsty	Thirsty, Drink Eagerly	Drinks Poorly, Not Able To Drink
Skin Pinch	Goes Back Quickly	Goes Back Slowly	Goes Back Very Slowly
Assessment	No Dehydration	Some Dehydration	Severe Dehydration

STATISTICAL METHODS:

Zinc level was considered as primary outcome variables. Dehydration and malnutrition were considered as Primary explanatory variable. Age, gender, blood parameter and serum electrolytes and kidney profile were considered as study relevant variables

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables.Data was also represented using appropriate diagrams like a bar diagram, pie diagram.

All Quantitative variables were checked for normal distribution within each category of an explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro- wilk test was also conducted to assess normal distribution. Shapiro wilk test p value of >0.05 was considered as a normal distribution.

For normally distributed Quantitative parameters, the mean values were compared between study groups using an Independent t-test (2 groups) and ANOVA (>2 groups). Association between quantitative explanatory and outcome variables was assessed by calculating the Spearman correlation coefficient ($r_{s)}$. Linear regression analysis was done. Regression coefficient, along with its 95% CI and p values, are presented.

Categorical outcomes were compared between study groups using Chi square test /Fisher's Exact test (If the overall sample size was < 20 or if the expected number in any one of the cells is < 5, Fisher's exact test was used.)

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis.[48]

RESULTS:

120 children included into the final analysis.

Table 7: Distribution of study population according to age group (N=120)

Age	Frequency	Percentages
6-12 months	42	35.00%
13 months to 36 months	31	25.83%
37 months to 60 months	47	39.17%

Among the study population, 42(35%) of them were aged between 6 to 12 month, and 31(25.83%) of them were aged between 13 to 36 months, and 47 (39.17%) of them were aged between 37 to 60 months. (Table 7 and Figure 2)

Figure 2: Bar chart showing the distribution of the study population according to age group(N=120)

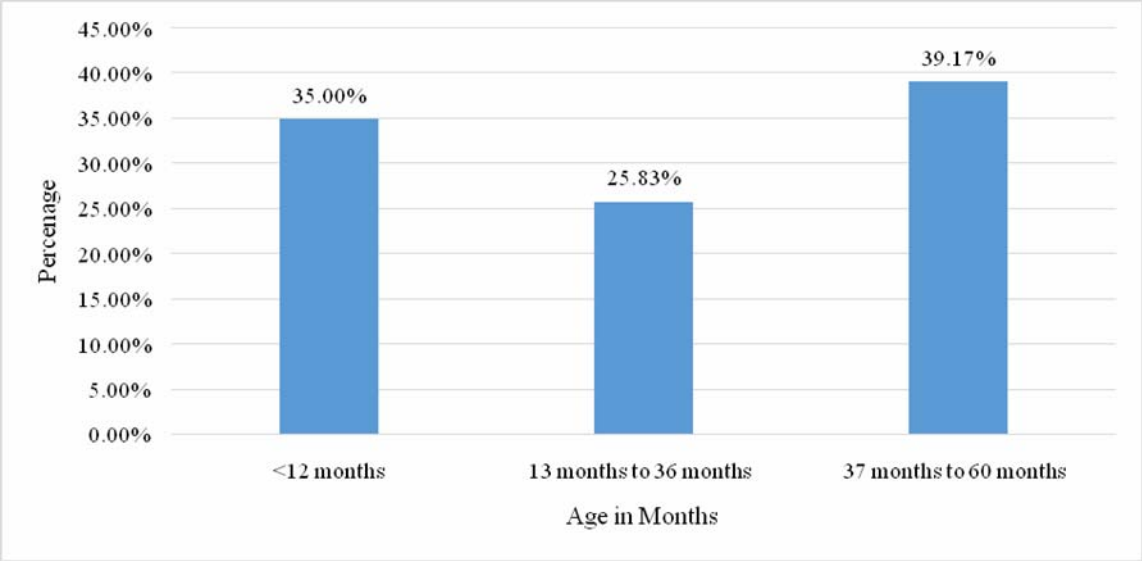


Table 8: Distribution of study population according to gender (N=120)

Gender	Frequency	Percentages
Male	62	51.67%
Female	58	48.33%

Among the study population, 62(51.67%) of the children was male, and 58(48.33%) of the children were female. (Table 8 and Figure 3)

Figure 3: Pie chart showing the distribution of the study population according to gender (N=120)

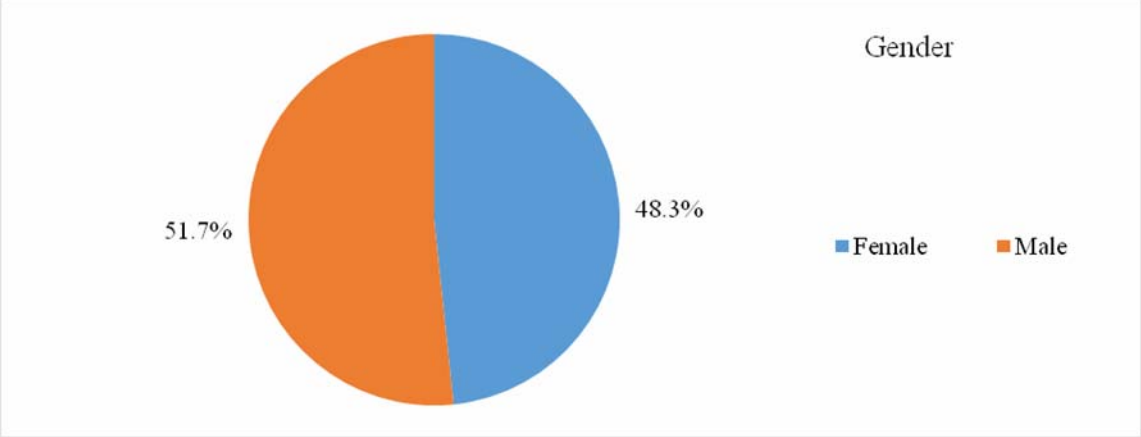


Table 9: Distribution of study population according to the "grades of dehydration" in the study population (N=120)

Level of dehydration	Frequency	Percentage
Severe Dehydration	21	17.5%
Some Dehydration	44	36.7%
No Dehydration	55	45.8%

Among the study population, it was observed that 44 (36.7%) participants had some dehydration,21(17.5%) participants had severe dehydration, and 55(45.8%) had no dehydration. (Table 9 and Figure 4)

Figure 4: Bar diagram showing the distribution of study population according to the grades of dehydration (N=120)

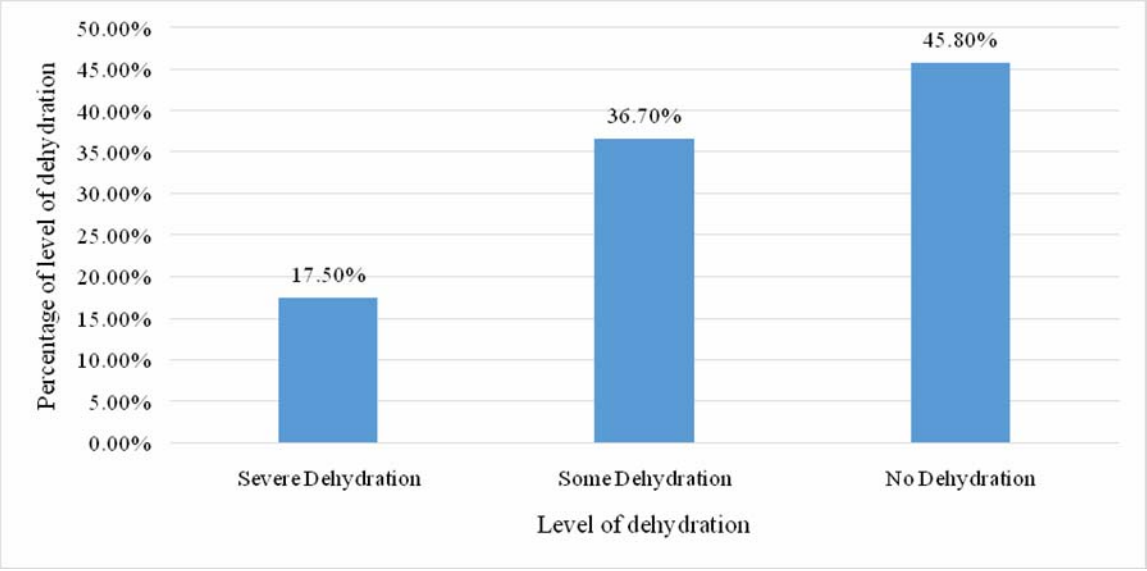


Table 10: Descriptive analysis of the study population according to malnutrition status (N=120)

Degree of malnutrition	Frequency	Percentages
Severe malnutrition(<3SD)	13	10.83%
Moderate malnutrition (<2SD)	6	5.00%
No malnutrition	101	84.17%

Among the study population, 13 (10.83%) participants had severe malnutrition, and 6(5%) participants had moderate malnutrition. (Table 10 and Figure 5)

Figure 5: Pie chart showing descriptive analysis of the study population according to malnutrition status (N=120)

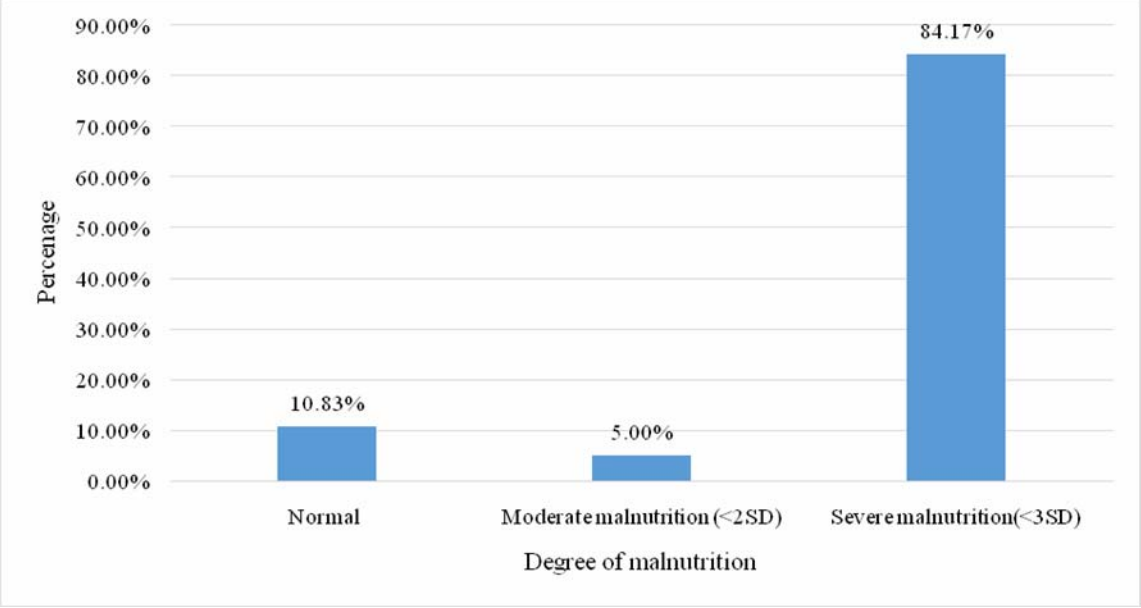


Table 11: Descriptive analysis of blood parameter in study population (N=120)

Parameter	Mean ± SD	Minimum	Maximum	95% C. I	
				Lower	Upper
Hemoglobulin (Gm%)	10.58 ± 2.18	3.30	15.00	10.19	10.98
Red Blood Cell (Mil/Cumm)	4.54 ± 0.63	2.47	5.90	4.43	4.66
Packed Cell Value (%)	31.93 ± 6.15	12.40	47.00	30.82	33.04
White Blood Cell (Th/Cumm)	10.74 ± 3.95	2.62	24.18	10.03	11.45
Platelets (Th/Cumm)	353.48 ± 128.67	117.00	753.00	330.22	376.73

Among the study population, the mean hemoglobulin (gm) was 10.58 ± 2.18; it was 4.54 ± 0.63 for red blood cell (mil/cumm), it was 31.93 ± 6.15 for packed cell value, it was 10.74 ± 3.95 for white blood cell (th/cumm), it was 353.48 ± 128.67 for Platelets (Th/Cumm). (Table 11)

Table 12: Descriptive analysis of serum electrolytes and kidney profile in study population (N=120)

Parameter	Mean ± SD	Minimum	Maximum	95% C.I	
				Lower	Upper
Sodium (Meq/L)	136.37 ± 6.14	122.00	167.00	135.26	137.48
Potassium (Me/L)	4.18 ± 0.86	2.20	6.90	4.02	4.33
Urea (Mg/Dl)	30.02 ± 17.97	4.00	62.00	26.77	33.27
Creatinine (Mg/Dl)	0.42 ± 0.3	0.10	2.90	0.37	0.48

Among the study population, the mean sodium (meq/L) was 136.37 ± 6.14, it was 4.18 ± 0.86 for potassium (me/l), it was 30.02 ± 17.97 for urea (mg/dl), it was 0.42 ± 0.3 for creatinine (Mg/Dl). (Table 12)

Table 13: Descriptive analysis of CRP in the study population (N=120)

CRP	Frequency	Percentages
Positive	35	29.17%
Negative	85	70.83%

Among the study population, 35(29.17%) participants had positive CRP, and 85(70.83%) of them had negative CRP. (Table 13 and Figure 6)

Figure 6: Pie chart of CRP in the study population (N=120)

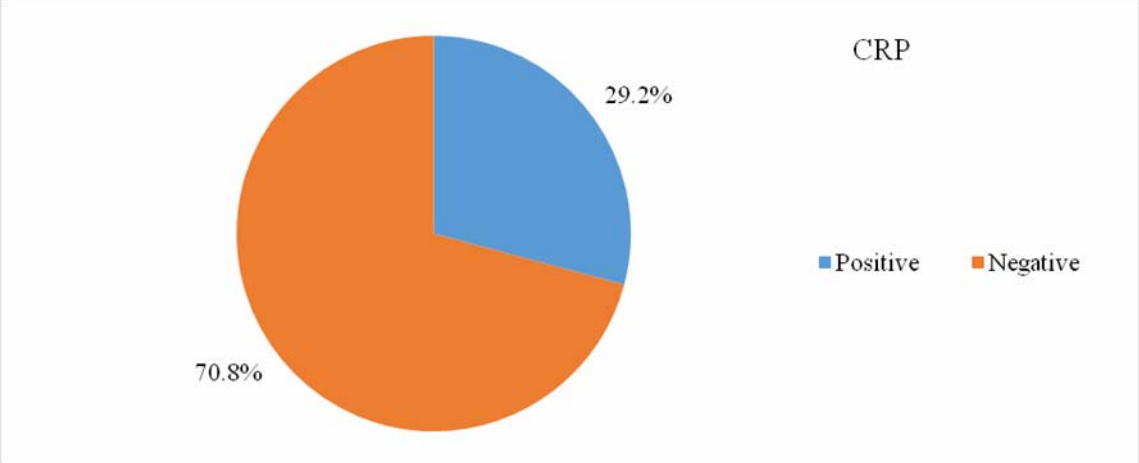


Table 14: Descriptive analysis of serum zinc (micro moles/l) in the study population (N=120)

Parameter	Mean ± SD	Minimum	Maximum	95% C.I	
				Lower	Upper
Serum Zinc (Micro Moles/L)	9.71 ± 3.88	4.00	20.00	8.99	10.40

Among the study population, the mean serum zinc (micro moles/L) was 9.71 ± 3.88 (4 to 20). (Table 14)

Table 15: Comparison of mean of serum zinc (micro moles/l) between gender (N=120)

Gender	SERUM Zn (micro moles/L) (Mean± SD)	Mean difference	P value
Male	9.77 ± 3.54	0.117	0.869
Female	9.65 ± 4.24		

Among the study population, they mean zinc value for male was 9.77 ± 3.54, and it was 9.65 ± 4.24 for female. The mean difference for zinc value between the gender was statistically not significant. (P value 0.869). (Table 15 and Figure 7)

Figure 7: Bar graph showing the mean of serum zinc (micro moles/l) between gender (N=120)

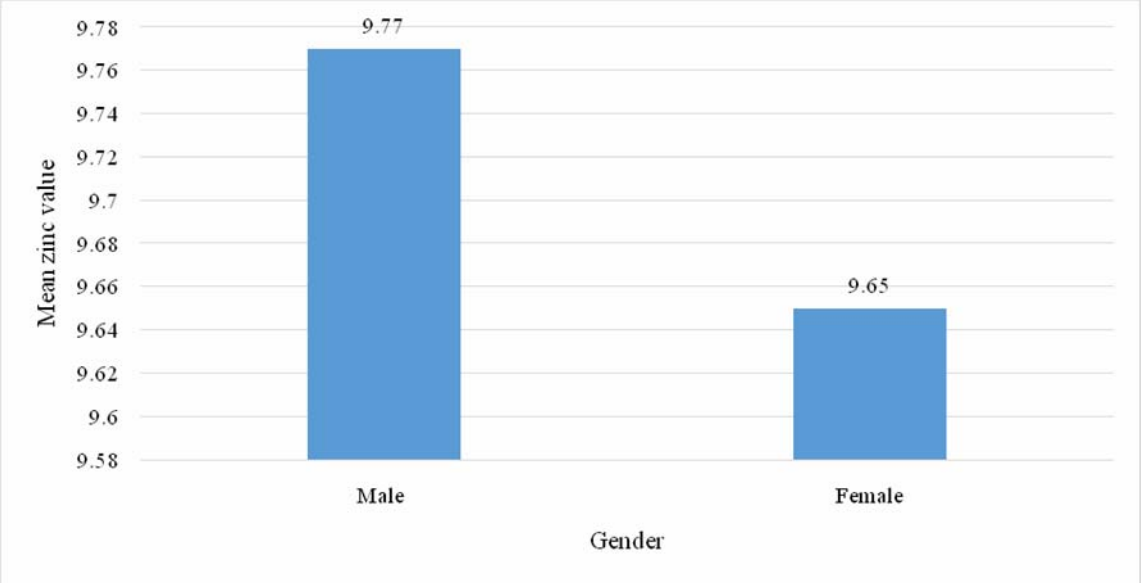


Table 16: Comparison of mean of serum zinc (micro moles/l) across age group

Age group	SERUM ZINC (micro moles/L) (Mean± SD)	P value
6-12 months	10.34 ± 4.10	0.09
13- 36 months	8.42 ± 3.73	
37- 59 months	9.99 ± 3.88	

(N=120)

Among the study population, the mean zinc value for 6-12 months age group was 10.34 ± 4.10,and it was 8.42 ± 3.73 for 13- 36 months. It was 9.99 ± 3.88 for 37- 59 months. The mean difference for zinc value across the age group was statistically not significant. (P value 0.09) (Table 16 and Figure 8)

Figure 8: Bar graph for the mean of serum zinc (micro moles/l) across age in months (N=120)

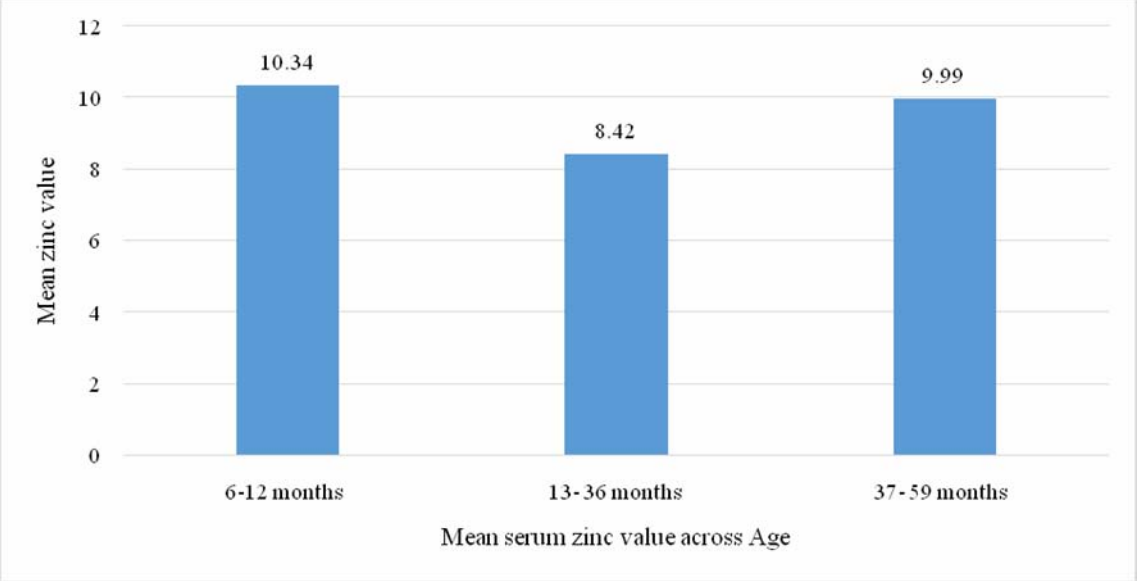


Table 17: Comparison of mean of serum zinc (micro moles/l) across the level of dehydration (N=120)

Level of dehydration	SERUM ZINC (micro moles/L) (Mean± SD)	P value
No dehydration	11.47 ± 3.81	<0.001
Some dehydration	8.86 ± 3.40	
Severe dehydration	6.88 ± 2.65	

Among the study population, the mean zinc value for no dehydration was 11.47 ± 3.81, and it was "8.42 ± 3.73"for some dehydration, it was "9.99 ± 3.88" for severe dehydration. The mean difference for zinc value across the level of dehydration was statistically significant. (P value <0.001). (Table 17 and Figure 9)

Figure 9: Bar graph for the mean of serum zinc (micro moles/l) across the level of dehydration (N=120)

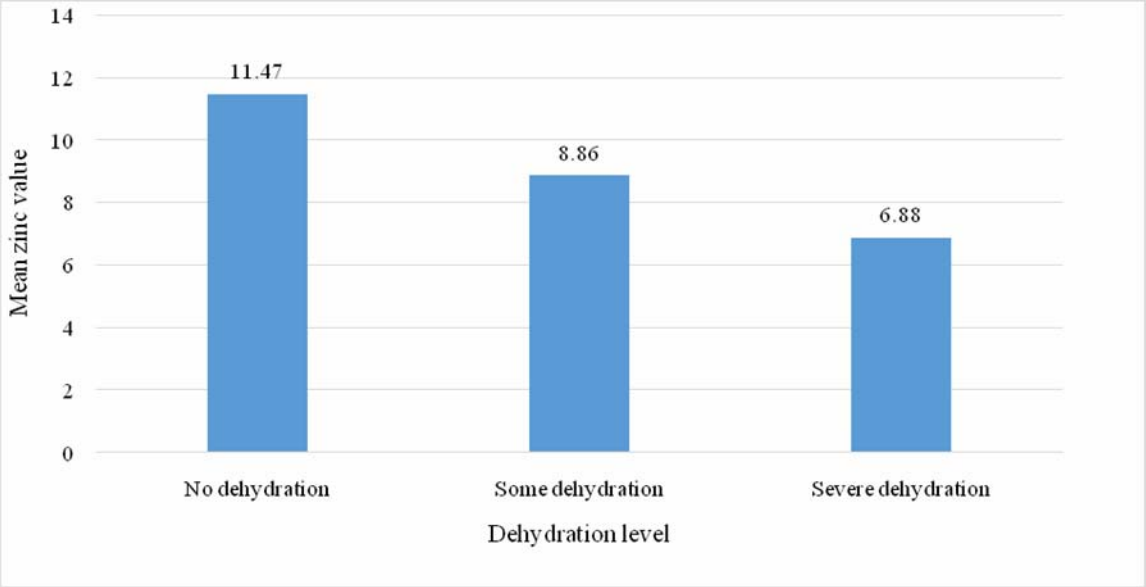


Table 18: Comparison of mean of serum zinc (micro moles/l) across the malnutrition (N=120)

Degree of Malnutrition	Mean	P value
Normal	10.07 ± 3.87	0.051
Moderate malnutrition (<2SD)	7.05 ± 3.05	
Severe malnutrition(<3SD)	8.11 ± 3.62	

Among the study population, the children who had severe malnutrition, the mean serum zinc was 8.11 ± 3.62, the children who had moderate malnutrition, the mean serum zinc was 7.05 ± 3.05, the children who had normal, the mean serum zinc was 10.07 ± 3.87. The mean difference for "serum zinc" between the degree of malnutrition was not statistically significant. (P value 0.051). (Table 18 and Figure 10)

Figure 10: Bar graph for serum zinc (micro moles/l) across the malnutrition (N=120)

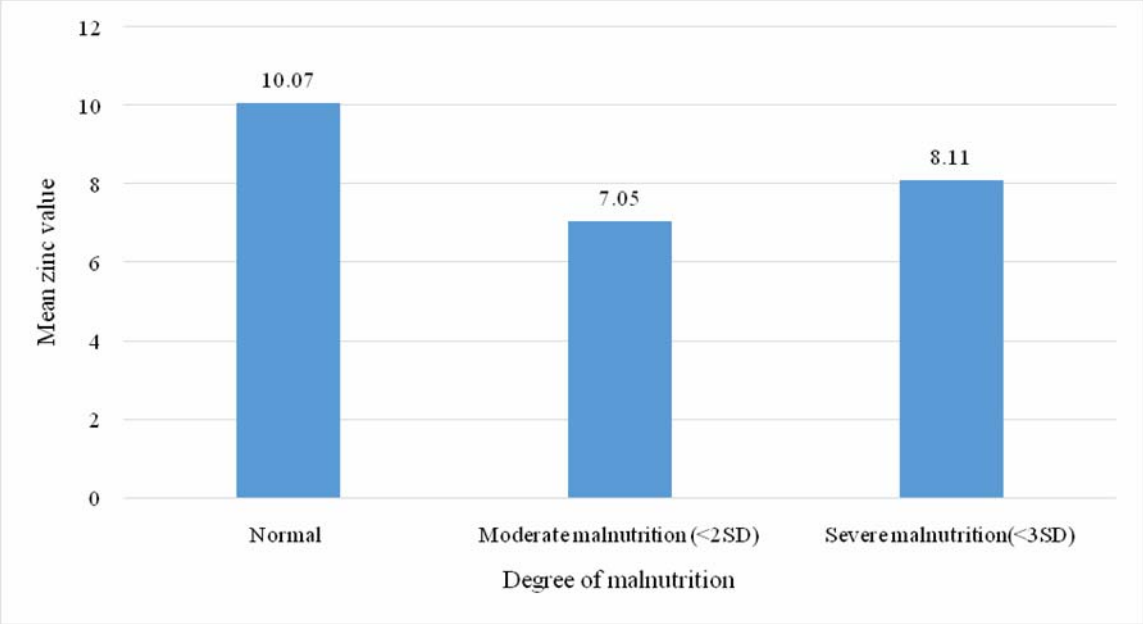


Table 19: Correlation between parameter and serum zinc (micro moles/l)(N=120)

Parameter	Spearman rank correlation (r_s)	P value
Dehydration Vs serum zinc level	-0.467	<0.001
Malnutrition Vs serum zinc level	-0.217	0.017

There was a week negative correlation betweendehydration and serum zinc level (r_s value: -0.467, P value: <0.001). and there was a week negative correlation betweenmalnutrition and serum zinc level (r_s value: -0.217, P value: 0.017). (Table 19)

DISCUSSION

Diarrhoea is a major cause of morbidity and mortality in under-5 children in developing countries. Persistent diarrhea, due to its syndromic nature, is a major problem encountered in under-5 children, despite the advances made in the treatment of diarrhoea over the decades. Zinc deficiency is associated with a higher risk of gastrointestinal infections, adverse effects on the function and structure of the gastrointestinal tract, and also impairment of the immune function. A dose of 10–20 mg zinc/d (dependent on age) for 10–14 d is recommended by "WHO/UNICEF Joint Statement on Clinical Management of Acute Diarrhea"to reduce diarrheal duration and severity and also for the prevention of subsequent episodes.[11] Studies conducted in both the developing and developed countries showed a potential effect of zinc in decreasing the rate of complications and mortality associated with diarrhea.[12, 13]There is an association identified between zinc deficiency, diarrhea and malnutrition usually in the malnourished children. It can also cause further depletion of zinc.[6]Zinc level can be compromised by frequent episodes of acute diarrhoea. Hence the present study was undertaken to estimate serum zinc level &"severity of diarrhea" also the duration of hospitalization. It is essential to detect subtle worsening in the clinical condition of the child so that early interventions can be taken to reduce morbidity and mortality. The routine treatment plan to all children with diarrhea is to supplement zinc as per WHO protocol, without knowing exact zinc status. Therefore, this study was undertaken to estimate "serum zinc level" and severity of diarrhea and duration of hospitalization. The results of the study are discussed under the following headings:

1. Baseline socio-demographic variables.
2. Mean serum Zn level in the study subjects.
3. Blood parameters in the study population.

4. Clinical characteristics of the study subjects.
5. Association between serum Zn levels and socio-demographic characteristics.
6. Association between serum Zn levels and severity of dehydration.
7. Association between serum Zn levels and degree of malnutrition.

BASELINE SOCIO-DEMOGRAPHIC CHARACTERISTICS:

A cross-sectional study was done on 120 children of aged 6 months to 60 months. The baseline characteristics of our study population were similar to that of Fathima M et al [41], Afolabi O et al.[8]Talachian E et al.[49]Habibi R et al[43],and Mahyar A et al.[10]Although most of the studies evaluated the mean zinc levels in diarrhea, there was a subtle difference in objectives across the studies. Some studies compared mean level of serum zinc in children with diarrhea and normal healthy controls[81049], or children with other diseases.[43] The design and objectives of the study by RerksuppapholS et al[45], and Fathima M et al[41], were almost similar to our study. Fathima M et al[41], in their study also evaluated the subjects with Acute Respiratory Infection besides Diarrhoea. The age range of our study population was similar to other studies. In our study ,the majority (39.17%) of the children were in the age group of 3 to 5 years. RerksuppapholS et al[45], in their study observed the mean age to be around 25.8 ± 15 months while Fathima M et al[41], in their study observed that nearly 50% of the participants were had diarrhea were aged less than 1year. This difference could be due to various socio-demographic, cultural and clinical factors affecting the presentation of the children to the hospital. There was almost an equal distribution of boys and girls in the present study, similar to other studies.[4543]Hence the baseline characteristics of our study were comparable to other similar studies.

Table 20: Comparison of baseline characteristics between various studies:

Author	Study period	Sample size (Country)	Age group	Mean age	Gender (Male)	Mean serum zinc levels
Present study	2019 to 2020	120 children with diarrhoea (India)	6 to 60 months	3 – 5 years (39.17%) 6 -12 months (35%)	51.67%	9.71 ± 3.88 µmole/L
Habibi R et al.[43]	2017	80 children with acute gastroenteritis (Iran)	1 to 5 years	-	55.6% (including controls)	86.15 µg/dL
Rerksuppaphol S et al.[45]	2016 to 2017	50 children with acute diarrhoea (Thailand)	6 to 58 months	25.8 ± 15 months	50%	69.2 µg/dL
Fathima M et al.[41]	2016	29 children with Diarrhoea (India)	Below 5 years	-	-	<70 µg/dL – 66% 70-120 µg/dL-34%
Afolabi O et al.[8]	2015 to 2016	100 children with acute diarrhoea (Nigeria)	2 to 59 months	8.7 ± 1.8 months	63%	65.3 ±7.4 µg/dL
Talachian E et al.[49]	2012	25 children with acute infectious diarrhoea (Iran)	6 months to 15 years	2.4 years	64%	102.77 ± 38.3 µg/dL
Mahyar A et al.[10]	2012 - 2013	48 children with acute bloody diarrhoea	3 to 60 months	28.8 ± 15.2 months	62%	74.1 ± 23.7 µg/dL
		12 children with acute watery diarrhoea (Iran)				169.4 ± 62.7 µg/dL

MEAN SERUM ZINC LEVELS:

To prevent morbidity and mortality among children, Zinc is one of the essential "micronutrient". Its deficiency is one of the major causes of diarrhea in children. The reference range of serum zinc is 10.7 - 22.9µmol/L (70-150 µg/dl). The zinc deficiency is indicated by a concentration of below 7 µmol/L (46µg/dl). The optimal range of plasma zinc is 13.8 - 22.9µmol/L (90-150µg/dl).[21] When the concentrations of plasma zinc drop below 9.9µmol/L (65 µg/dl), the clinical signs of zinc deficiency began to occur. Values of less than of 5µmol/L (33 µg/dl) are associated with loss of the senses of taste and smell, abdominal pain, diarrhoea, skin rash and loss of appetite.[21]

In our study, the mean serum zinc was 9.71 ± 3.88µmole/L. The mean serum zinc was 63.5 µg/dL in the present study. (1 µmole/L is 6.538 µg/dL). The mean serum levels in our study was comparable to other studies by RerksuppapholS et al[45], - 69.2 µg/dL, Afolabi O et al[8], - 65.3 ± 7.4µg/dL and Fathima M et al[41], – 66% with <70 µg/dL. Some studies have also reported higher values than our study at 86.15 µg/dL by Habibi R et al [43], 102.77 ± 38.3 µg/dL by Talachian E et al.[49]The serum Zinc level in an individual depends on several factors such as; dietary factors, bio-availability, the physiological needs and also the losses from the body. In developing countries like India, mild to moderate zinc deficiency is common due to the prevalent food habits. The commonly used staple foods in India have low zinc but are rich in phytates, which inhibit the absorption and utilization of zinc. Afolabi O et al[8], in their study also observed there was comparatively lower serum zinc levels in children with acute diarrhoea compared to healthy controls. In the present study we did not compare the levels with controls.Mahyar A et al[10], in their study observed children with acute

bloodydiarrhoea had significantly low "serum zinc levels" in comparison with healthy children. This reduction in serum zinc levels in children with diarrhoea compared to normal children may be related to either the excretion of zinc following acute diarrhoea or acute phase responses against the infections or a combination of both. The mean difference for zinc value across the age group was statistically not significant in the present study.

OTHER BLOOD PARAMETERS IN THE STUDY POPULATION:

In subjects with increased inflammatory markers, there could be decreased absorption of zinc because of causes that impair intestinal absorption of zinc. High zinc intake may also compete for absorption with other nutrients such as iron.The mean hemoglobin was 10.58 ± 2.18 g/dL. The mean WBC count and Platelet were within normal limits. CRP was increased in 29.17% of the subjects.

Table 21: The blood parameters of our study were comparable with that of the study by Rerksuppaphol S et al.[45]

Characteristics (Mean ± S.D.)	**Present study**	**Rerksuppaphol S et al.[45]**	**Afolabi O et al.[8]**
Serum Zinc (µg/dL)	**9.71 ± 3.88 µmoles/L**	69.2 ± 18.5	
Hb level (g/dL)	**10.58 ± 2.18**	11.9 ± 1.4	
Haemotocrit (%)	**31.93 ± 6.15**	36.3 ± 4	
WBC (10^3 cells/ mm^3)	**10.74 ± 3.95**	12.2 ± 4.5	
Platelet count (10^3 cells/ mm^3)	**353.48 ± 128.67**	373.1 ± 110.4	
Sodium (meq/L)	**136.37 ± 6.14**	136 ± 3 mmol/L	
Potassium (meq/L)	**4.18 ± 0.86**	4 ± 6 mmol/L	
Urea (mg/dL)	**30.02 ± 17.97**		
Creatinine (mg/dL)	**0.42 ± 0.3**	0.29 (0.26-0.35) (Median with Interquartile range)	
Serum CRP (mg/dL)	**CRP positive - 29.17% CRP negative - 70.83%**		24.6 ±4

ASSOCIATION BETWEEN "SERUM ZINC LEVELS" AND SOCIODEMOGRAPHIC CHARACTERISTICS:

Bitarakwate E et al[38], in their study observed age and gender were not significant factors affecting the serum zinc levels in children. Similarly, in the present study, there was no significant difference between male and female children with respect to mean serum zinc levels in the present study. There was also no significant difference between the mean serum zinc levels across various age groups. It was higher in the 6 to 12-month age group at 10.34 ± 4.10 micro moles/L, followed by 9.99 ± 3.88 micro moles/L in 3 to 5-year age group. It was lowest at 8.42 ± 3.73 micro moles/L in 1 to 3-year age group. The mean of "serum zinc level" was found to be lowest at 62.42 mg/dL in 3-4 years age group in the study by Fathima M et al.[41] There were no significant differences in term of age and sex between the low and normal serum zinc level groups in the study by Rerksuppaphol S et al.[45]

CLINICAL CHARACTERISTICS OF THE STUDY POPULATION:

Sudden onset of loose/watery stool with or without vomiting, abdominal pain/cramps, mild fever and recent contact with someone with diarrhoea or vomiting are the clinical features indicative for acute gastroenteritis.[4] It may present with or without symptoms of dehydration depending on the water content lost from the body. 45.8% of the children in the present study had no dehydration. 36.7% had some dehydration, while only 17.5% had severe dehydration. RerksuppapholS et al[45], in their study also similarly observed 42% of the children had minimal or no dehydration while 58% had moderate dehydration. Mahyar A et al[10], in their study observed 28.3% of the subjects had mild dehydration, 50% had moderate dehydration, and 21.7% had severe

dehydration. Zinc deficient children are at increased risk of developing diarrheal diseases besides other infections such as respiratory tract infections.

ASSOCIATION BETWEEN "SERUM ZINC LEVELS" AND SEVERITY OF DEHYDRATION:

Zinc deficiency can increase susceptibility to diarrhoea through several mechanisms. But also,diarrheal diseases can reduce the absorption of zinc and also result in loss of zinc. There was a statistically significant weak negative correlation betweenthelevel of dehydration and serum zinc levels (r_s value: -0.467, P value: <0.001) in the present study. But Mahyar A et al[10], in their study observed there was a positive correlation betweenthelevel of dehydration and serum zinc levels (r-value = 0.6 (watery diarrhoea), p = 0.01). But similar to our study, they observed a negative correlation betweenthelevel of dehydration and serum zinc levels in bloody diarrhea. (r-value = -0.24, p=0.4). The trend of decrease in the levels of mean serum zinc with an increase in the severity of dehydration was statistically significant. (p<0.001) in the present study. The mean serum zinc level was higher at 11.47 micro mole/L in No dehydration compared to only 6.88 ± 2.65 micro mole/L in severe dehydration. There was a significant difference of 4.59 micro mole/L between No dehydration and Severe dehydration, 2.61 micro mole/L between No dehydration and Some dehydration respectively. But there are also chances of a transient effect of the diarrhoea on the serum zinc levels in our study.

ASSOCIATION BETWEEN "SERUM ZINC LEVELS" AND DEGREE OF MALNUTRITION:

Zinc deficient children tend to be malnourished or stunted. Because both malnutrition and lower serum zinc levels may share the common sociodemographic and clinical risk profile linked to poverty such as low socioeconomic status, lack of awareness of mothers, lack of safe drinkingwater, poor sanitation and hygiene etc.In the present study,10.83% of children had severe malnutrition, while 5% had moderate malnutrition. There was no statistically significant trend in the difference between the mean serum zinc levels across the various degrees of malnutrition (p=0.051) in the present study. RerksuppapholS et al[45], in their study observed 16% of the children were wasted, and 8% of the children were stunted. The negative correlation betweenthe degree of malnutrition and "serum zinc level" was weak and was statistically significant in the present study. (r_s value: -0.217, P value: 0.017). Bitarakwate E et al[38], in their study observed that 66.7% of the children with persistent diarrhoea were either stunted, wasted or both. They observed a lack of significant correlation between nutritional states and serum zinc levels. It could be due to a cytokine directed internal redistribution of zinc. The relationship between zinc levels in malnourished children and the severity of dehydration cannot be interpreted clearly as a deficiency could be due to the diarrhoea or could be due to the poor nutritional status.

Table 22: Clinical presentation and association with serum zinc level across studies.

Author	Severity of dehydration	Correlation between serum zinc levels and Dehydration severity	p value	Level of malnutrition	Correlation between serum zinc levels and level of malnutrition	p value
Present study	**No – 45.8% Some – 36.7% Severe – 17.5%**	**-0.467**	**<0.001**	**-0.217**	**-0.217**	**0.017**
Rerksuppaphol S et al.[45]	Minimal or No – 42% Moderate – 58%	-	-	Wasting – 16% Stunting – 8%	--	-
Mahyar A et al.[10]	Mild - 28.3% Moderate – 50% Severe – 21.7%	0.6 (watery diarrhoea)	0.01	-	-	-
		-0.24 (bloody diarrhoea)	0.4			

Zinc ,essential micronutrientthat plays a major role in the growth and development of the children. Its deficiency leads to increased infections such as diarrhea resulting in dehydration. The present study sheds light on the serum zinc levels in children presenting with acute diarrhoea in India.

CONCLUSION

Our study concluded that there is an increased prevalence of low zinc levels in children with acute diarrhea. So, based on the findings of our study, many children require zinc administration. The initial administration of zinc can promote the recovery time in such children. Therefore, before the initial administration of zinc, it is necessary to measure the baseline zinc level of the children and then apply the zinc syrup as required.

LIMITATIONS

The present study was only a simple descriptive cross-sectional study. It was a single centre, hospital-based study and hence the external validity of the results are questionable. The sampling was convenient. "The association between serum zinc levels and diarrhea or serum zinc levels and malnutrition cannot be defined as causative because of the cross-sectional nature of the study". It could be bidirectional. We could not also measure serum zinc concentrations after patients had completed their course of treatment because of practical constraints.

RECOMMENDATIONS:

Future multi-centric large-scale community-based studies of analytical or experimental nature can test and confirm the hypothesis. Prospective studies with zinc supplementation involving control groups for comparison can fill the pertaining gaps.

SUMMARY

One of the major causes of pediatric morbidity and mortality around the world is acute gastroenteritis. The most common causes of acute gastroenteritis are infectious agents such as viruses, bacteria and parasites. “Diarrhea is one of the important risk factor associated with gastroenteritis”. Zinc level can be compromised by frequent episodes of acute diarrhoea.

Zinc depletion is one of the major factors that contribute to the detrimental effects of persistent diarrhoea on the human body.The present study had focused on estimation of serum zinc level and “severity of diarrhea” and dehydration status in 120 children aged between 6 months to 60 months. There was almost an equal distribution of boys and girls in the present study with 51.67%. Only 17.5% of the study population had severe dehydration. The mean serum zinc level identified was 9.71 ± 3.88µmole/L.It was highest in 6 to 12-month age group at 10.34 ± 4.10 micro moles/L While, lowest in 1 to 3-year age group at 8.42 ± 3.73 micro moles/L. There was a statistically significant weak negative correlation betweenthe level of dehydration and serum zinc levels. The trend of decrease in the levels of mean serum zinc with an increase in the severity of dehydration was statistically significant. The mean serum zinc level was higher at 11.47 micro mole/L in no dehydration group compared to severe dehydration with 88 ± 2.65 micro mole/L. The negative correlation between the degree of malnutrition and serum zinc level was weak and was statistically significant.

REFERENCES

1. Salami A, Fakih H, Chakkour M, Salloum L, Bahmad HF, Ghssein G. Prevalence, risk factors and seasonal variations of different Enteropathogens in Lebanese hospitalized children with acute gastroenteritis. BMC Pediatrics. 2019;19(1):137.
2. Burkhart DM. Management of acute gastroenteritis in children. Am Fam Physician. 1999;60(9):2555.
3. Mushtaq A, Khan S, Zeb F, Ain Q, Syed A. Risk Factors Associated with Gastroenteritis in Children 2-5 Years of Age Attending Rehman Medical Institute Peshawar. Am J Food Sci Health. 2016;2(5):94-101.
4. Sattar SBA, Singh S. Bacterial Gastroenteritis. [Updated 2020 Aug 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513295/.
5. Kellett J, Kyle G, Itsiopoulos C, Naunton M, Luff N. Malnutrition: The Importance of Identification, Documentation, and Coding in the Acute Care Setting. J Nutr Metab. 2016;2016:9026098.
6. Dutta P, Datta A, Niyogi S, Dutta S, Manna B, Basak M, et al. Impact of zinc supplementation in malnourished children with acute watery diarrhoea. J Trop Pediatr. 2000;46(5):259-63.
7. Castillo-Duran C, Vial P, Uauy R. Trace mineral balance during acute diarrhea in infants. J Pediatr. 1988;113(3):452-7.
8. Afolabi O, Saka A, Ojuawo A, Biliaminu S. Serum zinc levels amongst under-five children with acute diarrhoea and bacterial pathogens. Niger Postgrad Med J. 2018;25(3):131-6.

9. Eskander A, Sherif L, Nabih M, El Baroudy N, Marcos G, Badawy E, et al. Serum Zinc Level and Its Correlation with Vesikari System Scoring in Acute Pediatric Diarrhea. Open Access Maced J Med Sci. 2017;5(5):677-80.
10. Mahyar A, Ayazi P, Chegini V, Sahmani M, Oveisi S, Esmaeily S. Serum Zinc Concentrations in Children with Acute Bloody and Watery Diarrhoea. Sultan Qaboos Univ Med J. 2015;15(4):e512-e6.
11. Wardlaw T, Salama P, Brocklehurst C, Chopra M, Mason E. Diarrhoea: why children are still dying and what can be done. Lancet. 2010;375(9718):870-2.
12. Patel A. Zinc for Acute Diarrhea and Amoxicillin for Pneumonia, Do They Work? : Delivered at the AIIMS, IJP Excellence Award for the year 2013 on 7th September 2014. Indian J Pediatr. 2015;82(8):703-6.
13. Hambidge M, Krebs N. Zinc, diarrhea, and pneumonia. J Pediatr. 1999;135(6):661-4.
14. Elliott EJ. Acute gastroenteritis in children. Bmj. 2007;334(7583):35-40.
15. Chow CM, Leung AK, Hon KL. Acute gastroenteritis: from guidelines to real life. Clin Exp Gastroenterol. 2010;3:97-112.
16. Sattar SBA SS. Bacterial gastroenteritis [Internet]. Treasure Island: StatPearls Publishing; 2020 [cited 2020 Oct 30]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513295/.
17. Hiyoshi H, Tiffany CR, Bronner DN, Bäumler AJ. Typhoidal Salmonella serovars: ecological opportunity and the evolution of a new pathovar. FEMS Microbiol Rev. 2018;42(4):527-41.
18. Revelas A. Acute gastroenteritis among children in the developing world. South Afr J Epidemiol Infect. 2012;27(4):156-62.

19. Mehta N, Corkins M, Lyman B, Malone A, Goday P, Nieman L, et al. Defining Pediatric Malnutrition: A Paradigm Shift Toward Etiology-Related Definitions. J Parenter Enteral Nutr. 2013;37(4):460-81.
20. Brown KH. Diarrhea and Malnutrition. J Nutr. 2003;133(1):328S-32S.
21. Vale SHL, Leite LD, Alves CX, Dantas MMG, Costa JBS, Marchini JS, et al. Zinc pharmacokinetic parameters in the determination of body zinc status in children. Eur J Clin Nutr. 2014;68(2):203-8.
22. Bajait C, Thawani V. Role of zinc in pediatric diarrhea. Indian J Pharmacol. 2011;43(3):232-5.
23. Saper RB, Rash R. Zinc: an essential micronutrient. Am Fam Physician. 2009;79(9):768-72.
24. Yanagisawa H. Zinc deficiency and clinical practice. Japan Med Assoc J. 2004;47(8):359-64.
25. Ahmed TM, Mahmood MT. Serum zinc level in children with malnutrition. J Pediatr Gastroenterol Nutr 1998;27(2):257.
26. Olmez A, Yalçin SS, Yurdakök K, Coskun T. Serum zinc levels in children with acute gastroenteritis. Pediatr Int. 2007;49(3):314-7.
27. Ezzati M, Lopez AD, Rodgers AA, Murray CJ. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors [Internet]. World Health Organization; 2004 [cited 2020 Oct 30]. Available from: https://apps.who.int/iris/handle/10665/42770.
28. Manary MJ, Abrams SA, Griffin IJ, Quimper MM, Shulman RJ, Hamzo MG, et al. Perturbed zinc homeostasis in rural 3-5-y-old Malawian children is associated with abnormalities in intestinal permeability attributed to tropical enteropathy. Pediatr Res. 2010;67(6):671-5.

29. Tomkins A. Nutritional status and severity of diarrhoea among pre-school children in rural Nigeria. The lancet. 1981;317(8225):860-2.
30. Black RE, Brown KH, Becker S. Malnutrition is a determining factor in diarrheal duration, but not incidence, among young children in a longitudinal study in rural Bangladesh. Am J Clin Nutr. 1984;39(1):87-94.
31. Ahmed K, Hassan N. Food consumption, energy and nutrient intake and nutritional status in rural Bangladesh: changes from 1981–1982 to 1995–96. Eur J Clin Nutr. 1983;57:586-94.
32. Roy SK, Tomkins AM. The impact of experimental zinc deficiency on growth, morbidity and ultrastructural development of intestinal tissue. Bangladesh J Nutr. 1989;2(2):1-7.
33. Castillo-Duran C, Vial P, Uauy R. Trace mineral balance during acute diarrhea in infants. J Pediatr. 1988;113(3):452-7.
34. Castillo-Duran C, Heresi G, Fisberg M, Uauy R. Controlled trial of zinc supplementation during recovery from malnutrition: effects on growth and immune function. Am J Clin Nutr. 1987;45(3):602-8.
35. GARAFOLO J, IWATA T, INCEFY G, TWOMEY J, GOOD R, editors. Increased T-lymphocyte function and thymopoietin following zinc repletion in man. Federation Proceedings; 1979: FEDERATION AMER SOC EXP BIOL 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998.
36. Abolurin OO, Oyelami OA, Oseni SB. A comparative study of the prevalence of zinc deficiency among children with acute diarrhoea in SouthWestern Nigeria. Afr Health Sci. 2020;20(1):406-12.

37. Afolabi OF, Saka AO, Ojuawo A, Biliaminu SA. Serum zinc levels of hospitalized children with acute diarrhea differ by the isolated viruses. Int J Health Sci (Qassim). 2019;13(5):4-10.
38. Bitarakwate E, Mworozi E, Kekitiinwa A. Serum zinc status of children with persistent diarrhoea admitted to the diarrhoea management unit of Mulago Hospital, Uganda. Afr Health Sci. 2003;3(2):54-60.
39. Dutta P, Mitra U, Dutta S, Naik TN, Rajendran K, Chatterjee MK. Zinc, vitamin A, and micronutrient supplementation in children with diarrhea: a randomized controlled clinical trial of combination therapy versus monotherapy. J Pediatr. 2011;159(4):633-7.
40. El-Hawary M, Hussein S, Abd Elghaffar N, Ibrahim R. Serum Zinc Level In Children With Acute Diarrheal Disease. FYMJ. 2019;4(1):46-60.
41. Fathima M, Pandey S.Serum zinc status of children suffering from diarrhea and acute respiratory infection. Int J Health Sci Res. 2017; 7(1):248-252.
42. Gunes H, Acipayam C, Kirik S, Ipek S. Assessment of Serum Zinc Status of Children. J Pediatr Perinatol Child Health. 2018;2(2):10-5.
43. Habibi R, Hemati M, Rezaei M, Daneshfar A. Comparison of Baseline Serum Zinc Level in 1 - 5-Year-Old Children Hospitalized for Gastroenteritis with Children Hospitalized for Other Diseases at Dr. Mohammad Kermanshahi Hospital in Kermanshah. J Kermanshah Univ Med Sci. 2019;23(3).
44. Patel A, Mamtani M, Dibley MJ, Badhoniya N, Kulkarni H. Therapeutic value of zinc supplementation in acute and persistent diarrhea: a systematic review. PLoS One. 2010;5(4):e10386.
45. Rerksuppaphol S, Na-Songkhla N, RERKSUPPAPHOL L. Serum Zinc Levels in Thai Children with Acute Diarrhoea. J Clin Diagn Res. 2018;12(6).

46. Sezer RG, Ipek IO, Bozaykut A, Bayoglu DS. Zinc Deficiency in Acute Gastroenteritis and Upper Respiratory Tract Infections. ICAN: Infant, Child, & Adolescent Nutrition. 2011;3(1):49-52.
47. Taghavi-Ardakani A, Sharif MR, Kheirkhah D, Moravveji A, Verdi J. Serum zinc level in children with acute gastroenteritis admitted to Kashan Shahid Beheshti hospital. Feyz J Kashan Uni Med Sci 2012;16(2):169-73.
48. IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.
49. Talachian E, Bidari A, Noorbakhsh S, Tabatabaei A, Salari F. Serum levels of vitamins A and D, and zinc in children with acute diarrhea: A cross-sectional study. Med J Islam Repub Iran. 2015;29:207-.

ANNEXURES

INFORMED CONSENT FORM

Date:

I, Mr/Mrs _____, have been explained in my own vernacular language that my child will be included in the study**,CROSS SCETIONAL STUDY OF SERUM ZINC LEVELS IN CHILDREN WITH ACUTE GASTROENTERITIS IN TERITARY CARE HOSPITAL,** hereby give my valid written informed consent without any force or prejudice for recording the observations of haematological and clinical parameters. The nature and risks involved have been explained to me, to my satisfaction. I have been explained in detail about the study being conducted. I have read the patient information sheet, and I have had the opportunity to ask any question. Any question that I have asked have been answered to my satisfaction. I provide consent voluntarily to allow my child as a participant in this research. I hereby give consent to provide history, undergo a physical examination, undergo investigations and provide its results and documents etc. to the doctor/institute etc. For the academic and scientific purpose, the operation/procedure, etc. may be video graphed or photographed. All the data may be published or used for any academic purpose. I will not hold the doctors/institute etc. responsible for any untoward consequences during the procedure/study.

A copy of this Informed Consent Form and Patient Information Sheet has been provided to the participant.

_____ _____

(Signature & Name of Pt. Attendant) (Signature/Thumb impression & Name of Patient/Guardian)

(Relation with patient)

Witness:

_____

(Signature & Name of Research person /doctor)

PATIENT INFORMATION SHEET

Principal Investigator: **DR. AKSHATHA S/ DR. KRISHNAPPA J**

I, Dr Akshatha S, a post-graduate student in Department of Paediatrics at Sri Devaraj UrsMedical College, will be conducting a study titled "**Cross-sectional study of serum zinc levels in children with acute gastroenteritis in a tertiary care hospital**" for my dissertation under the guidance of Dr. Krishnappa J, Professor, Head of the department, Department of Paediatrics. The participants of this study, i.e. children with acute gastroenteritis will be included in the study where children with acute gastroenteritis will be examined in detail and serum zinc levels, will be measured. You will not be paid any financial compensation for the participation of your child in this research project.

All the data will be kept confidential and will be used only for research purpose by this institution.

You are free to provide consent for the participation of your child in the study. You can also with draw your child from the study at any point of time without giving any reasons whatsoever.

Your refusal to participate will not prejudice you to any present or future care at this institution.

Name and Signature of the Principal Investigator

Date:

KEY TO MASTER SHEET:

Key variables	Key code
Age	1=<12 months 2=13 to 36 months 3=37 to 60
Gender	1=Male, 2= Female
Dehydration	0= No dehydration, 1= Some dehydration, 2= Severe dehydration
Malnutrition	1= Moderate malnutrition,2= Severe malnutrition, 3= Normal
CRP	1=Positive, 2= Negative

MASTER CHART

IP number	Age	Gender	Dehydration	Malnutrition	Hemoglobulin	Red blood cell	Packed cell value	White blood cell	Platelets	CRP	Serum zinc	Sodium	Pottasium	Urea	Creatinine
692,392	3	2	0	3	11.8	4.9	29.9	10	389	2	14	134	5	28	0.2
693,800	2	1	0	3	9.9	4.9	32.7	6.4	270	1	12	134	3.7	17	0.3
694,387	3	1	1	3	12	5	47	7.4	325	1	9.7	126	4.1	39	0.4
694,434	3	2	1	3	13	5.9	39	7	346	2	13	132	3.6	12.1	0.1
689,623	3	2	0	3	11.8	4.9	32	10	389	2	14	126	4.1	16	0.5
689,515	3	1	0	3	11.8	4.9	26.1	10	389	1	11	136	3.2	16	0.3
698,306	1	1	1	3	8.1	4.3	25.9	10.2	379	2	10.4	138	4.3	25	0.3
686,356	1	1	1	3	10.3	4.5	30.6	10.3	149	2	11.2	135	3	18	0.2
705,014	3	1	1	3	9.6	4.8	29.9	13.2	257	2	9.3	137	2.7	8	0.2
705,124	1	1	2	3	11.7	4.3	32	18	300	1	5	138	4.5	38	0.8
710,860	3	2	1	3	13.8	5.6	39.4	11.3	409	1	13	131	4.1	45	0.7
763,174	2	1	1	2	11.3	4.7	33	11.5	423	2	9.4	138	6.9	17	0.3
706,489	1	1	2	3	13.8	4.7	29.9	6	303	2	8	159	6.7	165	2.9
709,819	3	1	0	3	12	5	47	7.4	123	2	16	135	3.7	18	0.2
715,255	2	2	2	2	10.7	5.1	33	5	325	2	4.8	140	3.6	26	0.2
715,728	1	2	0	3	13.9	4.4	38.8	16.5	657	1	13	140	5.2	28	0.4
758,607	1	1	0	3	7.8	4.9	27	6.5	425	2	10.4	134	4	38	0.6
724,683	3	1	2	3	12	5	47	7.4	325	2	11.2	143	3.2	46	0.5
732,636	2	2	2	3	7.7	4.2	24.2	11.4	117	2	9	129	3.7	22	0.3
724,722	3	2	1	3	12	5	47	7.5	405	2	12.9	137	4	28	0.5
740,476	3	2	2	2	13.2	5.3	22	10.8	283	2	6.3	135	6.2	60	1
742,168	1	1	1	3	9.6	4	29.2	10.9	297	2	12	134	4.4	22	0.2
689,626	3	2	0	3	11.8	4.9	29.9	10	389	2	11	127	4.5	48	0.4
747,883	2	1	1	3	8.2	4.7	27	11.3	466	2	9	137	5.5	46	0.2
748,706	1	1	2	3	4	2.5	20.8	13.9	496	2	6.8	143	4.7	62	0.2
713,523	1	1	1	3	14.3	5.3	40.3	6.6	133	2	10	139	4.1	18	0.2
763,175	1	1	1	1	11.3	4.7	33.6	11.4	423	2	11.3	133	6.9	17	0.3
751,509	1	1	1	2	7.7	3.4	40.2	19	346	1	12	130	3.8	16	0.2
700,761	2	2	2	2	3.3	2.9	14.3	15.3	289	2	8	131	3.8	10	0.2
765,810	2	1	0	3	9.7	5.1	32.2	12.1	484	2	15	144	4.2	36	0.4
719,499	3	1	2	3	10.1	5.6	39.4	11.3	409	2	12	134	4.6	24	0.4
758,606	2	2	1	3	10.9	4.8	32.1	10.6	384	1	6	135	4.3	28	0.9

MASTER CHART

761,393	3	2	0	3	6.9	4.4	25.4	8.2	351	1	13	136	4.5	32	0.2
767,138	1	1	0	3	11.3	4.8	33.8	11.6	423	1	17	133	6.9	17	0.2
707,409	1	1	0	3	13	5.2	34	9.8	324	2	17	132	4.6	34	1.1
771,412	1	2	0	3	11.1	4.4	32.5	10.7	502	2	18	140	3.4	46	0.4
780,406	3	2	1	3	9.8	4.7	29.6	13	443	1	4.9	139	4.3	28	0.3
744,968	2	1	0	3	12.9	4.4	38.9	8.3	198	2	6	128	4.4	9	0.4
819,112	3	2	0	3	12.1	5.2	36.5	4.7	216	1	13	142	3.4	56	0.2
819,373	3	1	0	3	13.9	4.8	38.2	16.7	466	2	5	138	4.3	32	0.5
823,268	3	2	1	3	9.9	4.9	33.5	7.2	468	2	4	136	4.5	24	0.4
822,263	1	1	2	2	7	5.2	26.4	4.1	592	2	5	137	5	33	0.4
824,574	1	2	0	3	11.6	4.9	34.3	9	413	1	13	135	4.5	21	0.3
827,233	3	1	0	3	12.8	5.4	38.3	12.1	262	2	10	136	4.1	31	0.4
828,629	1	1	1	3	7.8	4.9	26.4	11.6	733	2	9	135	4.4	14	0.2
830,132	3	1	1	3	11.7	4.6	35.3	6.6	199	2	8	134	3.8	38	0.5
830,176	1	2	0	3	10.2	4.7	29.9	12.3	392	1	9	148	3	32	0.4
831,824	1	2	1	3	11.2	3.8	30	10.4	198	2	10	135	3.2	30	0.6
816,446	2	1	0	3	12	4.9	36.3	14.1	364	2	11	130	3.2	30	0.4
836,840	3	1	0	3	10.8	3.9	29.6	5.1	221	2	10	135	4.2	24	0.3
837,948	3	2	1	3	9.5	4	28.4	6.8	303	2	8	136	3.5	26	0.5
839,230	2	2	1	3	5.7	5	22.8	9.3	492	2	14	129	3.3	29	0.3
839,278	3	2	0	3	9.1	4.7	28.6	9.1	387	1	18	137	4.4	14	0.3
710,419	1	2	0	3	11.7	4.9	33.6	14.7	422	2	14	134	4	9	0.2
710,421	1	1	0	3	11.9	4.9	35.5	17.2	448	2	13	135	5.1	20	0.2
839,252	3	2	0	3	9.1	4.6	29.1	7.7	370	2	13	138	4.6	32	0.8
839,670	1	1	0	3	10.1	4.8	29.9	12.8	435	2	14	139	3.6	26	0.4
840,353	1	1	0	3	13.5	5.4	37.9	12.5	302	2	6	137	5.8	23	0.2
733,779	1	2	2	3	10.3	4.3	30.5	12.3	504	2	6	136	3.9	14	0.7
791,378	3	2	1	1	12.6	5.3	37.5	18.8	470	2	7	133	5.2	19	0.3
732,683	2	1	0	3	13.2	5	30.4	10.4	234	2	6	132	4.5	18	0.3
798,284	1	2	0	3	10	4.3	30.2	20.1	482	2	13	133	4.6	14	0.2
807,572	3	1	0	3	11.1	4.5	32.1	10.3	229	2	6	137	4.9	30	0.4
807,574	3	1	0	3	12.1	5.4	36.1	12.7	305	2	13	129	4.6	31	0.6
807,513	3	2	1	1	5.1	4.3	21.3	12.7	446	1	4	134	4	15	0.2
812,678	3	2	0	3	9.2	4.3	24	11.3	284	2	13	129	5.4	25	0.9
812,699	3	2	0	3	15	4.5	43.2	6.7	262	2	6	134	5.2	35	0.8
787,703	1	1	1	1	13	4	32	13.4	234	2	6	130	5.5	30	0.6
828,028	2	1	1	3	12.8	5	38.9	9.1	409	1	7	138	4.5	20	0.2
827,605	2	1	1	3	7.4	4.6	26.3	6.2	312	1	15	135	3.6	28	0.3

MASTER CHART

830,977	2	2	0	3	10.7	5.1	33.4	11.7	399	1	5	141	4.1	30	0.3
809,581	3	1	0	3	10.8	4.5	32.9	7	368	2	13	142	4.3	18	0.6
751,509	1	1	2	3	7.7	3.4	24	14.1	346	2	7.5	130	3.8	16	0.2
757,197	1	2	2	3	11.7	4.2	29.4	24.2	753	1	5	122	3.9	9	0.2
783,092	2	2	0	3	8.1	5.4	29.1	18.9	553	2	13	146	2.8	12	0.4
799,609	3	1	0	3	10.1	4.8	29.5	6.9	290	1	12	131	2.2	17	0.5
803,616	3	2	0	3	13.5	5.1	39.3	2.6	156	1	6	126	4.5	39	0.6
807,513	3	2	1	3	8.5	5.2	29	10.8	378	1	7	134	4	15	0.2
808,218	2	1	1	2	6.8	4.3	24.2	11.2	531	1	7	134	4.7	4	0.2
815,517	1	1	1	3	9.5	3.4	28.2	19.3	497	1	6	136	5.2	33	0.3
820,546	1	1	0	3	8.9	4.7	28.7	7.8	595	1	9	132	4.1	9	0.6
818,694	2	1	0	1	10.5	4.4	31.1	5.7	227	2	10	140	3.2	24	0.3
819,092	1	2	1	3	7.3	4.7	25.3	10.8	555	1	10	140	6.2	46	0.3
822,246	1	1	2	2	10.3	4.1	30.9	7.2	344	2	9	133	4.5	32	0.4
822,743	1	1	0	3	9.4	4.5	29.4	8.9	284	2	4	136	3.4	35	0.5
826,803	2	2	2	3	12.7	4.2	35.6	9.4	282	1	4	140	4.3	22	0.5
827,201	1	1	1	3	9.8	3.3	28.6	13.5	555	2	6	153	4.1	43	0.3
830,133	3	2	1	2	10.9	4	32	4.1	129	2	7	138	3.7	58	0.6
830,132	3	1	1	3	11.7	4.6	35.3	6.6	199	2	8	134	3.8	38	0.5
830,176	1	2	2	3	10.2	4.7	29.9	12.3	392	1	4	145	3.5	57	0.5
831,548	2	2	1	2	12.3	4.8	35	11.9	323	2	5	141	5	54	0.6
834,163	2	2	1	3	6	4.9	23.8	11.1	273	2	6	141	3.6	21	0.7
837,219	3	2	1	3	11.6	4.3	33.5	11.9	451	1	10	131	4.4	22	0.4
839,252	2	2	1	3	9.1	4.6	29.1	7.7	370	1	10	138	4.6	32	0.8
841,064	2	2	1	3	10.2	3.9	12.4	13.4	234	2	4	143	2.9	47	0.9
841,267	2	1	1	3	12	3.6	13.6	12.4	250	2	4	134	3.8	48	0.7
803,088	2	1	0	3	10.2	4.9	30	13.4	234	2	9	138	4.2	42	0.3
818,694	2	2	2	1	9.4	4	32	3.8	129	2	4	136	4	50	0.2
650,890	2	1	0	3	11.7	4.6	35.3	7	230	2	14	134	3.9	40	0.5
824,083	2	1	0	3	10.2	4.7	29.9	12.3	445	1	4	138	3.7	28	0.3
800,440	3	1	0	3	11	4.6	33	9.4	255	2	4.6	144	3.9	23	0.3
800,431	3	2	1	3	9	3.9	32	6.7	190	2	4	142	3.5	23	0.2
807,831	1	1	0	3	8.8	3.6	29	7.7	325	2	14	133	3.3	55	0.4
808,944	2	1	2	2	13	3.5	32	13.5	240	1	4	138	4	38	0.2
809,976	2	2	0	3	9.5	4.3	38	21.3	345	1	13	128	4.2	45	0.5
810,369	2	2	0	3	10.5	3.7	42	6.7	485	2	13	126	4.3	60	0.4
813,056	3	1	0	3	11.3	3.6	37	9.5	220	2	14	134	4.2	36	0.2
814,203	1	2	1	3	8.9	2.6	30	18	420	2	9	139	3.6	42	0.4

MASTER CHART

814,856	3	1	1	3	11	4.2	32	10.6	300	2	12	137	3.4	12	0.5
815,150	3	2	0	3	10.9	3.3	34.2	7.9	229	1	9	136	2.9	16	0.5
817,700	3	1	0	3	11	3.6	33.6	9.2	163	2	14	167	3	29	0.3
819,074	3	2	2	3	11	2.9	29	18.6	365	2	4	143	3.4	17	0.3
819,462	1	2	0	3	13	4.7	40	9.7	266	2	9	146	4.6	33	0.2
819,392	3	2	2	2	11.6	4.9	34.3	9	413	2	11	145	4.4	30	0.6
821,754	1	1	0	3	12.8	5.4	38.3	12.1	262	2	16	145	4.2	29	0.2
770,873	1	2	0	2	7.8	4.9	26.4	11.6	733	2	17	136	3.3	23	0.3
828,909	3	2	0	3	11.7	4.6	35.3	6.6	199	2	15	133	3.2	44	0.6
828,629	1	1	1	3	10.2	4.7	29.9	12.3	392	2	9	134	3.3	23	0.4
830,133	3	2	2	3	13.5	5.4	37.9	12.5	302	2	10	136	3.8	30	0.3
814,662	1	2	1	3	14.4	5.2	38	13	342	2	20	137	4.2	27	0.2