

Cairo University
Faculty of Medicine
Department of Clinical and Chemical pathology

***VIRAL AND TOXIN INDUCED
ACUTE DIARRHEA IN CHILDREN***

Thesis

*Submitted in Partial Fulfillment of
Master Degree in Clinical and Chemical pathology*

By
Yasmin Adel M. El. Mahdy
(M.B.,B.Ch.)

Supervised by

Prof. Dr. Amani El. Kholy

**Professor of Clinical Pathology
Faculty of Medicine
Cairo University**

Dr. Maha Gaafar

**Assistant Professor of Clinical Pathology
Faculty of Medicine
Cairo University**

Dr. Mariam Adel

**Lecturer of Clinical Pathology
Faculty of Medicine
Cairo University**

**Faculty of Medicine
Cairo University
2012**

بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم
سورة البقرة الآية (٣٢)

ACKNOWLEDGMENT

First great thanks to "Allah" who gave me the power to complete this work. Without his care nothing could be achieved.

I am greatly honoured to express my deep respect and gratitude to the eminent Professor of Clinical Pathology Prof. Dr. Amani El Kholi who I had the honor of working under her supervision. A special gratitude and appreciation to Dr. Amani for her guidance, help and most of all her constructive criticism throughout the completion of this work.

I also have to thank Prof. Dr. Maha Gaafar for her very kind supervision and her multiple significant additions to the various sections of this work. Dr. Maha broke the boundary between the professor and her resident by being a good friend who was always there for me when I needed her.

I would also like to thank Dr. Mariam Adel for spending a lot of time and effort in assisting me in every way possible to complete this work.

My gratitude is also directed to all the members of Cairo University Children Hospital (Al Monira Microbiology Laboratory) especially Dr. Mina William, and Mrs. Safaa for their sincere help and support and for the immense time they gave me which contributed significantly to this work.

Yasmin Adel El Mahdy

2012

LIST OF ABBREVIATIONS

AGE	Acute Gastroenteritis
CDAD	Clostridium difficile Associated Disease
CPE	Clostridium Perfringens Enterotoxin
CT	Cholera Toxin
E. AggEC	Enteraggreative Escherichia Coli
E.Coli	Escherichia coli
EHEC	Enterohemorrhagic Escherichia coli
EIEC	Enteroinvasive Escherichia coli
ELISA	Enzyme Linked Immunosorbent Assay
EPEC	Enteropathogenic Escherichia coli
ETE	Emetic Toxin
ETEC	Enterotoxigenic Escherichia coli
G. Lamblia	Giardia lamblia
Gb3	Globotriaosylceramide
GBS	Guillain- Barre ´syndrome
HBL	Haemolytic Enterotoxin
HC	Haemorrhagic Colitis
HLA-B27	Human leucocytic antigen B27
HUCVs	Human Caliciviruses
HUS	Haemolytic Uremic Syndrome
ICT	Immunochromatographic Test

ID	Infectivity Dose
KIA	Kligler Iron Agar
LA	Latex Agglutination
LEE	Locus for Enterocyte Effacement
LFLA	Lacto Ferrin Latex Agglutination
LIA	Lysine Iron Agar
LPS	Lipopolysaccharides
LT	Heat Labile Enterotoxin
Mac	MacConkey Agar
MHC	Major Histocompatibility Complex
MIO	Motility Indole Ornithine Agar
Nhe	Diarrheagenic Enterotoxin
ORT	Oral rehydration therapy
PCR	Polymerase Chain Reaction
REA	Acute Reactive Arthritis
RF	Rheumatoid Factor
RIA	Radioimmunoassay
RPLA	Reverse Passive Latex Agglutination
RVGE	Rotavirus Gastroenteritis
S. Boydii	Shigella boydii
S. Dysenteriae	Shigella dysenteriae
S. Flexneri	Shigella flexneri
S. SONNEI	Shigella sonnei

Sd1	Shigella dysenteriae Type 1
SMac	Sorbitol MacConkey Agar
SRSVs	Small round structured viruses
SS	Salmonella/Shigella Agar
ST	Heat Stable Enterotoxin
ST1/ST2	Shiga toxin 1 / Shiga toxin 2
STEC	Shiga toxin Escherichia coli
Cl.difficile	Clostridium difficile
Stx	Shiga toxin
TIR	Translocated Intamin Receptor
TSI	Triple Sugar Iron Agar
TTP	Thrombotic Thrompocytopenic Purpura
WHO	World Health Organization

LIST OF CONTENTS

		Page
	Introduction	1
Review of littaer		
Chapter (1)	Gastroenteritis	4
Chapter (2)	Toxin –induced diarrhea in children	23
Chapter (3)	Viral -causes of diarrhea in children	48
Chapter (4)	Diagnosis of gastroenteritis	58
Chapter (5)	Material and method	76
Chapter (6)	Result	90
Chapter (7)	Discussion	112
Chapter (8)	Summary , Conclusion and Recommendation	126
	References	130

LIST OF TABLES

Figures		Page
1	Guidelines for assessment of dehydration status	18
2	The frequency distribution of different bacteria from stool culture	90
3	The frequency distribution of pathogens among all enrolled cases	91
4	The frequency distribution of pathogens among cases with identified etiology	91
5	Distribution of viruses using ELISA and Rapid test	92
6	Distribution of organisms in relation to age	93
7	The residence of patients compared to type of pathogen	94
8	The distribution of water sources compared to organisms	95
9	The water source versus the residence of patients	96
10	Animal contact versus isolated organisms	96
11	Animal contact versus residence	97
12	Frequency distribution of pathogens among Inpatient and outpatient	98
13	The clinical presentation of patients compared to the type of organism	99
14	The distribution of clinical presentations in relation to age group	100

15	The dehydration score in dehydrated patient in relation to isolated organisms	100
16	Antibiotic intake versus isolated organisms	101
17	Antibiotic intake versus the type of isolated bacteria on culture	101
18	Frequency distribution of clinical presentations according to history of antibiotic intake	102
19	ELISA versus rapid test for rotavirus	103
20	ELISA versus rapid test for adenovirus	103
21	ELISA versus rapid test for norovirus	103
22	The Sensitivity and Specificity of rapid test compared to ELISA	104
23	Frequency distribution of rotavirus according to age groups	105
24	Frequency distribution of rotavirus according to residence	105
25	Frequency distribution of rotavirus according to water source	106
26	Frequency distribution of clinical presentation of rotavirus infected patients	106
27	Frequency distribution of rotavirus infected patients according to antibiotic intake	107
28	The clinical and demographic characteristics of all patients studied	108
29	Microbiological results of all patients	110

LIST OF FIGURES

Figures		Page
1	Morphological effect of CPE on human ileal mucosa	29
2	Pathogenesis of <i>Cl. difficile</i> associated disease	43
3	Specific sets of biochemical reaction	63
4	Direct and indirect ELISA	67
5	Colonies on Salmonella Shigella agar and Hecktone agar	79
6	Test cassette and interpretation of result of immunochromatographic test for Norovirus	84
7	Illustrate test cassette and interpretation of result ImmunoCard STAT EHEC kit	87
8	Distribution of organisms in different age groups	94

Abstract

Every year more than one billion diarrhea episodes occur among children younger than 5 years of age. Most of cases occur in socioeconomically developing countries causing 2 to 2.5 million deaths. Over one year from November 2009 to September 2010 the prevalence of enteric viruses and toxin inducing agent were studied in 80 successive cases of children less than 9 years of age. It was found that viral etiology was identified in 48.8%, bacterial etiology in 2.5% and mixed in 7.5%. Viral etiology was more predominant than bacterial and toxin-mediated diarrhea in our study. Rotavirus was the most prevalent cause of viral gastroenteritis, contributing to 55.3% of total isolated viruses, and most frequently identified pathogen in urban and rural areas, followed by norovirus, and adenovirus. The peak age group for all viruses including rotavirus was less than 2 years.

Key words: Diarrhea, Viral gastroenteritis, Rotavirus, Norovirus, Adenovirus.

Introduction

Every year more than one billion diarrhea episodes occur among children younger than 5 years of age. Most of cases occur in socioeconomically developing countries causing 2 to 2.5 million deaths. More than twenty viral, bacterial, and parasitic enteropathogens are currently associated with acute diarrhea (*Laura et al., 2004*)

Despite much progress in the understanding of pathogenesis and management, diarrheal illnesses remain one of the most important causes of global childhood mortality and morbidity (*Thapar and Sanderson, 2004*).

Enteric viruses are major etiologic agents of acute gastroenteritis among infants and young children worldwide (*Hart, 2009*).

Rotavirus is the most common single cause of severe diarrhea leading to dehydration and death (*Parashar et al., 2005*).

Other recognized viral causes of pediatric gastroenteritis include norovirus, astrovirus, and enteric adenovirus (*Hart, 2009*).

Nearly every child in the United States is infected with rotavirus by age 5 years, and the majority will have symptomatic gastroenteritis and it is an important cause of infant mortality in the developing world (*Payne et al., 2009*).

After rotavirus, the most important cause of acute infantile gastroenteritis probably is norovirus infection. Seroepidemiologic studies have shown that antibodies to Noroviruses are present in 50-90% of children younger than 2 years in Kuwait, Italy, Kenya, China, London, and South Africa (*Ramani et al., 2009*).

Researchers have recognized for a long time that certain enteric adenoviruses are an important cause of infantile gastroenteritis. Recent studies confirmed that they cause 2-6% of cases (**Ramani et al., 2009**).

Escherichia coli, a common cause of gastroenteritis, accounts for 30% of the total number of diarrheal pathogens in some regions. Strains of E. coli, which cause diarrhea in humans, can be divided into at least seven different categories based on distinct epidemiology, clinical syndromes, and virulence properties (**Larke et al., 2001**).

Shiga toxin-producing E. coli (STEC) has been shown to be the most important group in developed countries because of numerous outbreaks and life threatening complications, such as haemolytic-uraemic syndrome (HUS) and hemorrhagic colitis (HC). However, self-limiting diarrhea caused by STEC has frequently been encountered (**Larke et al., 2001**).

Clostridium difficile-associated disease (CDAD) represents a considerable public health hazard. In the United States, it is responsible for more deaths than all other intestinal infections combined (**Redelings et al., 2007**).

CDAD can range from uncomplicated diarrhea to sepsis and even death. CDAD rates and severity are increasing, possibly due to a new strain. Transmission of Cl.difficile occurs primarily in health care facilities via the fecal-oral route following transient contamination of the hands of health care workers and patients; contamination of the patient care environment also plays an important role (**Sunenshine and Mcdonald , 2006**).

Several studies have examined the role of specific enteropathogens in childhood diarrheas in Egypt; however, no comprehensive studies

describing the prevalence of viral, bacterial and parasitic enteropathogens, especially newly recognized ones in Egyptian children have been reported in recent years.

The present study was the first recent survey to assess the prevalence of viral and toxin induced diarrhea.

Aim of the study:

Our aim was to study the prevalence of rotavirus, adenovirus, norovirus, shiga toxin producing *E. coli* (STEC) and *Clostridium difficile* associated disease in children less than or 5 years of age who presented with acute diarrheal diseases to Cairo University Children Hospital at AlMonira (Abo El Reesh pediatric hospital).

Gastroenteritis

Gastroenteritis is defined as inflammation of the mucosa of the stomach and intestine. Major symptoms include nausea and vomiting, diarrhea, and abdominal cramps. Intestinal infection is the most common cause of diarrhea worldwide and is responsible for the deaths of 3–4 million individuals each year, the majority of whom are pre-school children (*WHO, 1996*).

In some countries in the developing world children may suffer many attacks of acute diarrhea every year, each of which contributes to the infection-malnutrition cycle and consequent impaired growth and development. Most of the deaths from acute infectious diarrhea result from excessive fluid and electrolyte losses, which result in dehydration and acidosis (*Farthing, 2000*).

Thus, the majority of these deaths are avoidable providing fluid and electrolyte losses are replaced promptly. The major burden of infectious diarrhea falls upon individuals, particularly infants and young children, who live in the developing world. However, despite industrialization, wealth, and public health interventions to ensure water quality and sewage disposal, acute intestinal infections are increasing in the Western world. This is particularly due to food borne infections such as *Salmonella* species, *Campylobacter jejuni*, and enterohaemorrhagic *Escherichia coli* O157: H7 (*Farthing, 2000*).