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Human Norovirus Detection among Children with Gastroenteritis in Diyala Governorate

A Thesis

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بِسْمِ اللَّــهِ الرَّحْمَـٰنِ الرَّحِيمِ

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

صدقَ اللَّهُ العظيم

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Dedication

- To the absent present, my eldery brother "Maad" may Allah have mercy upon his soul.
- To the most potent moral force in my life "my parents " who see the best of me and unconditionally teach, inspire as well as encourage me. No words can ever express my gratitude to you.
- To the sunshine of my life, these whom always there for me when I need them, pick me up when I fall, stick up for me when no one else will my sister "Maysam" and brothers "Mokhallad" and "Marwan", thank you for believing in me and encouraging me.
- To the stars of my sky my nephews especially "Bassam".

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Marwa

Summary

Acute gastroenteritis remains a global public health problem. It causes significant morbidity and mortality among children worldwide. Human noroviruses are a major cause of gastroenteritis and severe diarrheal disease around the world.

The present study is designed to determine the rate of human norovirus infection among children with gastroenteritis in Diyala governorate using enzyme linked immunosorbent assay and immuneochromatography, also, to evaluate genogroup 1(GGI) and genogroup 2(GGII) by nested polymerase chain reaction among study population and study the association between the rate of infection and different parameters such as age, body mass index, gender, the education level of the mothers, water source, type of feeding and clinical aspects.

A cross sectional study was carried out for patients with acute gastroenteritis who attended to the Emergency Department of Pediatrics in Al-Batool Teaching Hospital for Maternity and Pediatric in Baqubah city, during the period from 6th of September 2018 to 4th of March 2019. A total of 182 children under the age of five years old (115 males and 67 females) are admitted during the study period. The stool samples were collected from each participant and stored as frozen at -70 °C until used to an enzyme immune assay and immunochromatographic test for the qualitative identification of human norovirus genogroups I and II in human stool samples, as well as used nested polymerase chain reaction after RNA extraction among positive samples from study population.

The results of this study shows that, the rate of human norovirus infection was 6.04% (11 out of 182) samples by enzyme linked immunosorbent assay and immuneochromatography techniques; infection

among females was (54.55%) higher than males (45.45%). The positive results 8 (72.73%) were in age group (1-12) months and 3 (27.27%) in age group (13-24) months, while no positive cases among other ages. All the positive patients for human norovirus were from Baqubah city. The education level of the mothers of the positive patients were highest rate with primary education 6(54.55 %) followed by 2 (18.18%) for each secondary and higher education.

The distribution of positive human norovirus infection regarding the type of feeding showed that 9 cases (81.82%) were used artificial milk and 2 cases (18.18%) were mixed feeding that drinking artificial and breast feeding, while there were no positive results recorded among children with breast feeding. Concerning the sources of water use, the highest infection rate was noticed among patients were used filtered and boiled water 6 cases (54.54%) followed by filtered water about 3 cases (27.28 %) and boiled tap water 2 cases (18.18 %).

The signs and symptoms of infection were fever 5(45.45%), nausea 7(63.63%), vomiting 10 (90.09%), weight loss 4(36.36%) and dehydration 6(54.54%). Plus, all patients had abdominal pain 11(100%).

Only two cases, (18.18%) that acquired the infection while they were hospitalized infections. However, three cases (27.27%) were have other cases with the same signs and symptoms in the same family, six cases (54.55%) were having non-sporadic infection.

The result of nested polymerase chain reaction demonstrated that only one case was positive for human norovirus genogroup 2(GGII).

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List of abbreviations

Abbreviation	Meaning
AGE	Acute gastroenteritis
BMI	The body mass index
CD	Cluster of differentiation
CDC	Centers for Disease Control and Prevention
ID	Infecting dose
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EM	Electron microsope
GG	Genogroup
HBGAs	Histo-blood group antigens
HLADR	Human leukocyte antigen complex on chromosome 6 region 6p21.31
ICTV	International Committee on Taxonomy of Viruses
IFNs	Interferons
IL	Interlukin
MENA	Middle East and North Africa
MHC	Major histocompatibility complex
NK cells	Natural killer cells
NSP	Non-structural proteins
ºC	centigrade
ºF	Fahrenheit
ORF	Open reading frames
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase–polymerase chain reaction
RT-qPCR	Quantitative real time polymerase chain reaction
TNF-α	Tumor necrosis factor alpha
VLP	Viral like particle
VPg	Viral protein genome
WHO	World health organization

Chapter One
Introduction

1.1 Introduction.

Acute gastroenteritis (AGE) remains a global public health concern, causes significant morbidity and mortality among children worldwide (Arowolo *et al*., 2019; Plants-Paris *et al.*, 2019).

The causes of acute gastroenteritis in children vary depending on multiple factors such as location, season, and the population studied (Dennehy, 2005). There is a wide range of infections that can cause acute gastroenteritis. These are viruses (rotavirus, norovirus, astrovirus, sapovirus, adenovirus), bacteria (*Shigella*, *Escherichia coli*, *Campylobacter*, *Salmonella*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Aeromonas*), and protozoa such as *Cryptosporidium*, *Entamoeba histolytica*, *Giardia intestinalis*. In addition, *Clostridium difficile* may induce diarrhoea when the antibiotic treatment alters the intestinal microbial balance, and bacterial toxins may cause gastroenteritis without enteric infection such as *Staphylococcus aureus* (Kabayiza, 2014).

Viruses are the most frequently implicated pathogens causing pediatric acute gastroenteritis and viral diarrhea in pediatric patients in both outpatient, emergency department, and inpatient settings (Trang *et al.*, 2012). Enteric viruses, particularly rotaviruses and human noroviruses, are a leading cause of gastroenteritis worldwide. Rotaviruses primarily affect young children, human noroviruses affect people of all ages, and are a leading cause of foodborne disease and outbreaks of gastroenteritis worldwide (Krisztián *et al*., 2018). Human noroviruses remain a major cause of gastroenteritis and severe diarrheal disease around the world (Karst and Tibbetts, 2016).

Human noroviruses which are small, non-enveloped, positive-stranded RNA viruses belong to Caliciviridae family is now comprised of five genera, including Norovirus, Sapovirus, Lagovirus, Nebovirus, and Vesivirusl (Green, 2013). The human norovirus genus can be subdivided in seven genogroups, of which genogroups GI, GII and GIV have been detected in humans, and can be further subdivided into more than 40 genotypes (Vinje, 2015).

Human norovirus causes ~20% of all acute gastroenteritis and ~200,000 deaths per year, primarily in young children. Most epidemic and all pandemic waves of disease over the past 30 years have been caused by type GII.4 human norovirus strains (Lias *et al.*, 2019).

The transmission of human norovirus occurs primarily via the fecal oral route, including direct person to person contact, consumption of contaminated food or water, or contact with contaminated environmental surfaces (CDC, 2011). Common symptoms of illnesses include increase in bowel movement frequency with or without vomiting, fever, abdominal cramping, headache, dehydration and myalgia (Sattar and Shashank, 2018). The illness is generally mild and short duration (1-2 days) (Bok and Green, 2012).

It is not well known whether human norovirus infections induce any lasting protective immunity (Simmons *et al.*, 2013). Extent immunity protects against exposure to different strains. This is important because noroviruses are highly genetically and antigenetically diverse this complexity that is a big challenge for the development of an efficient human norovirus vaccine (Rackoff *et al.*, 2013). Infections are notoriously difficult to prevent and control, owing to their low infectious

dose, high shedding titer, and environmental stability (Barclay *et al.*, 2014).

Globally, human norovirus resulted in a total of $4.2 billion in direct health system costs and $60.3 billion in societal costs per year. Disease amongst children <5 years cost society $39.8 billion, compared to $20.4 billion for all other age groups combined. Costs per norovirus illness varied by both region and age and was higher among adult ≥55 years, low- and middle-income countries and high-income countries had a similar disease incidence (Bartsch *et al.*, 2016).

In Iraq, several studies have been conducted in various provinces to determine the rate of human norovirus infections by using different techniques; such as study done by Al-Mashhadani *et al*., (2008) in Kurdistan region, Thwiny and Hassan (2015) in Basrah, Al-Marsome *et al*., (2016) detected the virus by enzyme linked immunoassay (ELISA) and polymerase chain reaction (PCR) in Basrah city and Mohamed *et al.*, (2016) in Mosul city detected the virus using (RT-qPCR) for NVGI and NVGII got the same rate, Al-Khoweledy (2016) diagnosed the virus in Al-Najaf Provence using reverse-transcriptase-polymerase-chain-reaction (RT-PCR) technique and Al-Moussawi *et al.*, (2018) in Thi-Qar province with infection rates 30%, 8% , 28% , 37.9% and 17.5 % for each one respectively. To the best of our knowledge, there is no previous study done in Diyala governorate about human norovirus genotyping.

1.2 Aims of the study.

The study aims at.

1. To detection the rate of human norovirus infections among children with acute gastroenteritis in Diyala governorate by enzyme linked immunosorbent assay and immuneochromatography.

2. To determine genogroup 1(GGI) and genogroup 2(GGII) by nested polymerase chain reaction among study population.

3. To study the association between the rate of infections and different parameters such as age, gender, the education level of the mothers, water source, type of feeding and clinical aspects.