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Detection and Comparison of Rotavirus, Norovirus, Sapovirus among Children under 5 years with Acute Diarrhea

A Thesis

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Chapter One
Introduction

1. Introduction

Diarrhea is the second greatest cause of mortality in children under 5 years of age worldwide (Anderson *et al.*, 2010), with 10% deaths each year. Among the etiologic agents of diarrhea, viruses lead the way with 80% of cases. Among these viruses, rotaviruses are the most represented (Agbla *et al.*, 2018). Rotaviruses are non-enveloped, icosahedral, double-stranded RNA viruses (dsRNA) comprising a genus within the family Reoviridae. Seven groups of rotavirus (A–G) have been described, with group A rotaviruses being the leading cause of severe dehydrating gastroenteritis in children <5 years of age worldwide (Al-Shuwaikh, 2016). In 2009, the World Health Organization (WHO) recommended that all countries should introduce rotavirus vaccines into their national immunization programs (World Health Organization, 2009).

Enteric caliciviruses in the genera norovirus and sapovirus are significant causes of gastroenteritis in humans and animals, with noroviruses alone causing approximately 200,000 deaths per annum in children <5 years of age (Patel *et al.*, 2009; Ayukekbong, 2015; Alfajaro *et al.*, 2017). However, in countries where universal rotavirus vaccination has been implemented, noroviruses have become the most prevalent in children admitted to hospital with acute gastroenteritis (Patel *et al.*, 2009; Ayukekbong *et al.*, 2015). Numerous studies have shown that asymptomatic norovirus infections are common within different populations. This renders clinical judgement on its contribution to diarrhoeal disease difficult (Ayukekbong *et al.*, 2015).

Human sapovirus, formerly known as sapporo-like viruses, is a significant leading cause of gastroenteritis. It is a single-stranded positive sense RNA viruses. The virions are composed of a single structural capsid protein, with icosahedric symmetry (Romani *et al.*, 2012). Sapovirus peaks in winter and is relatively common among young children. Sapovirus has been identified more frequently in infants and toddlers, with almost all children having antibody against sapovirus by the age of 5 years, while norovirus tends to be more common in older children (Anderson, 2010). Using real-time RT-PCR, studies from low-, middle-, and high-income countries have shown that the prevalence of sapovirus in children <5 years of age ranges from 3.3 to 17%. Although earlier reports described sapovirus infection as one with less severe clinical symptoms than norovirus and rotavirus, more recent studies have shown that infections with sapovirus may result in hospitalizations and severe dehydration (Diez-Valcarce *et al.*, 2018). There is no previous study about prevalence of sapovirus infection in Iraq.

1.2 The present study aims at:

1. Serological and molecular detection of the sapovirus, rotaviruses, norovirus and study the prevalence of theses viruses in children with acute gastroenteritis.
2. Estimating the demographic and risk factors associated with infection such as age, sex, source of water and level of mother education.

Summary

Acute gastroenteritis remains a global public health problem. Rotavirus, Norovirus and Sapovirus are recognized as important causes of acute gastroenteritis among children worldwide. The present study aimed to determine the rates of infections, co-infections, clinical features, risk factors of sapovirus, rotavirus and norovirus infections among children with gastroenteritis in Diyala governorate using two methods, immunological method by using enzyme-linked immunosorbent assay (ELISA) to evaluate the presence of human rotavirus and human norovirus antigens and molecular method by using real-time PCR (RT-PCR) to determine the presence of human sapovirus.

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 Children in Baqubah city, during the period from 31 October till 22 December, 2019. A total of 93 children under the age of five years old (57 males and 36 females) were admitted during the study period.

The positive samples of human sapovirus that detected by RT-PCR method were (6.5%) and the positive samples of rotavirus and norovirus by ELISA method were (79.6%) and (1.1%) respectively. Statistically, rotavirus showed the higher positivity rate among viruses with significant difference ($P<0.05$). The present study, showed that there were 7 cases of rotavirus co-infection, most common co-infection is between 6 sapovirus cases and rotavirus (6.45%), while only one (1.1%) case of co-infection between norovirus with rotavirus.

There was significant difference (P<0.05) noticed between males and females as their rates of infection were (10.5%) and (0%), respectively in sapovirus cases and (80.7%), (77.8%), respectively in rotavirus cases but this difference was not statistically significant (P>0.05).While the only norovirus positive case was male (1.8%) and none of female were positive.

Highest infection rate was recorded among children in age group (6-12) months in sapovirus and rotavirus (8.2%), (90.0%), respectively. While , norovirus positivity was detected only in one child (6.3%) in age group (>12) months. However, there was statistically significant association only between rotavirus positivity and the different age group (P<0.05).

The distribution of positive sapovirus and norovirus infection regarding the sources of water use, the highest infection rate was noticed among patients who used boiled filtered water (10.6%), (2.2%), respectively. While the highest infection rate among patients with rotavirus infection was noticed in those who used boiled bottled water (82.9%) followed by boiled filtered water (78.8%). However, there was no statistically significant association between viruses positivity and the different sources of water used (P>0.05)

The clinical features from children with sapovirus, rotavirus and norovirus gastroenteritis are similar and non-specific but highly suggestive and should raise the indexes of suspicion of infection with these viruses. Regarding the signs and symptoms of human sapovirus infection, 6 (100%) had diarrhea, 5(83.3%) had dehydration, 5(83.3%) had vomiting, 5(83.3%) had abdominal pain and 5(83.3%) had fever. While the most frequent clinical signs and symptoms among patients with

human rotavirus infection were diarrhea 74 (100%), dehydration 52 (70.3%), vomiting 60 (81.1%), abdominal pain 58 (78.4 %) and fever 61 (82.4%). Generally only one child had been affected by norovirus, the signs and symptoms in this patient included diarrhea, dehydration, vomiting, abdominal pain and fever 1 (100%).

In addition, the present study showed that there was no significant association between sapovirus, rotavirus and norovirus infection and the level mother education ($P>0.05$). Also there was no significant association between the source of water used for milk preparation and the level of mother education ($P>0.05$). Interestingly, all the parents enrolled in the present study denied their knowledge if their child vaccinated or not to rotavirus, therefore this information was excluded in the present study.

According to the results of the current study, it can be concluded that human rotavirus can be considered one of the most important causes of diarrhea in children and more research are required with a larger sample size to investigate sapovirus and norovirus prevalence, genotyping and confirm their roles as a primary etiology of pediatric diarrhea.