# Distribution of Norovirus GII.P4R among Children with Acute Gastroenteritis in Alnajaf Alashraf City/Iraq

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## Abstract

Diarrhea is the second commonest infectious cause of death in children less than five years old where Norovirus represented one of the four major viral groups that cause diarrhea. This research aimed to investigate the distribution of Norovirus GII.P4R among children under 5 year's old suffering from acute gastroenteritis.

A total of 100 stool specimens were collected from patients suffering from gastroenteritis (150 -200 ml of stool specimens) in a sterile wide mouthed universal container during the period from Feb, 2020 to Feb, 2021. Direct RNA extraction from all specimens have been carried out. RT qPCR technique was use for direct detection Norovirus GII.P4R (Nor. GII.P4R).

The results showed that 57(57%) specimens gave positive results for amplification of Norovirus GII.P4. A high percentage of infection reported among male with age group1day — 6 months (70.27%) in comparison with female at the same age group (32.43%).

Keywords: Acute gastroenteritis, Norovirus GII.P4R, RT qPCR technique.

## 1. Introduction

Norovirus is one of the most diverse human viruses described, and can be divided into five different genogroups, where GGI, GGII, and GGIV cause disease in humans while GGIII contains bovine strains, whereas GV contains murine strains [1]. GGII.4 is the most frequently occurring and clinically severe genotype which is often further divided into specific variants to account for the recurrent seasonal pandemics [2].

Norovirus is a well-described cause of epidemic gastroenteritis in both adult and pediatric populations across a wide range of geographic regions [3]. A systematic review of all reports of norovirus detected by reverse transcriptase PCR (RT-PCR) attributed 5 - 31% of cases of gastroenteritis in hospitalized patients and an additional 5 - 36% of cases in all patients seeking outpatient evaluation to norovirus [4]. The general population is broadly vulnerable to disease across all age groups, but the majority of morbidity and mortality occurs at the extremes of age. The fecal-oral route is the main mode of transmission, although several other modalities have been described,These modalities include transmission via aerosolized viral particles in vomitus [5] and through food, water, and environmental contamination [6].

This research aimed to investigate the distribution of Norovirus GII.P4R among children under 5 year's old suffering from acute gastroenteritis.

## 2. Methods

Study inhabitance: This study was accomplish on 100 cases with age group 1 day to 5 years whom admitted to AL-Zahraa Teaching Hospital , during the period extended from Feb,2020 up to Feb, 2021in ALnajaf ALashraf City/ Iraq. Questionnaires were used to obtain information from the parents or guardians accompanying the child to hospital. Information included clinical history of patients, signs and symptoms of illness.

Inclusion Criteria: Babies under 5 years with acute gastroenteritis. Children below 5 years with diarrhea alone.

Specimens Collection: A total 150 -200 ml of stool specimens were collected in sterile wide mouthed universal container during acute stage of gastroenteritis (<3 days of onset of symptoms). All specimens were kept in ice box to maintain ideal temperature (2-8 $C^{O}$) and transported immediately to the microbiological laboratory for direct extraction of viral RNA.

Extraction of viral RNA: Viral Gene-spin TM Viral DNA/ RNA Extraction kit (iNtRON, Korean) has been used for direct extraction of viral RNA from feces specimen by followed up it manufactural recommendation. All extracted RNA undergoes RT—qPCR technique.

Synthesized oligonucleotide: the sequences of primers that used for detection of Norovirus GII.P4R have been designed during this research which involved Nor GII.P4F — TGAGCTAGCACCACTCTCCT — and Nor GII.P4R — GCCCATATGCACCAAGGTCT — all primer were prepare with a final concentration 100 Pmol/µl as a stock solution.

RT—qPCR technique: the mixture was prepared with a final volume 20 µl by mixing all content of amplification materials as mentioned in Table 1. All mixture were centrifuged for short spin at 10000 rpm/ min then, transferred to thermo cycler. The real time – qPCR thermos cycler was adjusted on SYBR Green quantitative PCR.

**Table1: The contents of Real time – qPCR for amplification of Norovirus GII.P4R**

| Items | Volume |
|---|---|
| ONE-STEP RT-PCR PreMix Kit | 8.0 µl |
| F. primer (10 Pmol/µl) | 2.0 µl |
| R. primer (10 Pmol/µl) | 2.0 µl |
| Nuclease – Free Water | 4.0 µl |

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| | |
|---|---|
| RNA template | 4.0 µl |
| Total volume | 20.0 µl |

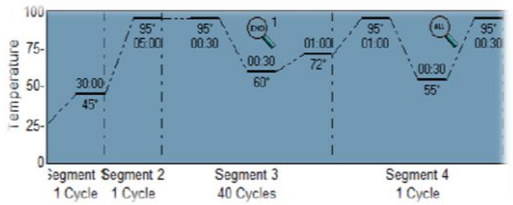


Figure 1: The condition of amplification with ONE STEP RT PCR PreMix for amplification of Norovirus GII.P4R

The conditions of amplification with ONE-STEP RT-PCR PreMix (iNtRON, Korean) are shown in figure 1

## 3. Results and Discussion

The results of detection of Norovirus GII.P4 according to the primer which design in this study depending on the sequences of ORF 1, 2, and 3 showed that 57( 57%) specimens gave positive results for amplification of Norovirus GII.P4 in which the Ct value were range between 16-35 (Figure 2A and B). According to the Ct values, the results showed that 41(71.9 % ) specimens gave a strong positive reaction with abundant target nucleic acid where Ct values ≤ 29 , while 16 (28.1%) specimens showed a positive reactions with moderate amounts of target nucleic acid where Ct value between 30-37.

The result of percent study showed a wide distribution of viral infection among both sex with different age group Where a high percentage of infection reported in male which was 64.91% in comparison with female which was 35.08% (Table 2). Also the results showed that a high percentage of infection with Norovirus GII.P4 occurred in male with age groups 1daye –6 months which was 70.27, while low percentage of infection with NorovirusGII.P4 observed in male with age groups 13-18 months which was 05.40%. Among female a high percentage of infection occurred among age groups 1daye –6 months which was 32.43%, while a low percentage of infection observed with age groups 19-24 months which was 05.00%.

Norovirus (NoV) is one of the leading causes of acute gastroenteritis(AGE) and represented the main cases of diarrhea after Rotavirus [7, 8]. Globally it causing both community-acquired and healthcare-associated outbreaks [9]. Noroviruses were estimated to be the causes of 18% of AGE cases worldwide affecting people of all ages. Norovirus GII.P4 outbreaks are frequently associated with health- or childcare settings predominantly in the winter months from November to April [10].

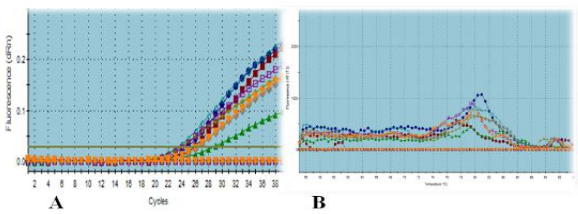


Figure 2: Real time qPCR threshold curves for amplification and dissociation of Norovirus GII.P4. A: Amplification curve B: Dissociation curve

**Table 2: The percentage of Norovirus GII.P4infection among male and female with different age groups.**

| Sex | NO.(%) of viral infection among age groups | | | | |
|---|---|---|---|---|---|
| | 1daye –6 months | 7-12 months | 13-18 months | 19-24 months | Total (%) |
| Male | 26 (70.27) | 9 (24.32) | 2 (05.40) | 10 (27.02) | 37 (64.91) |
| Female | 12 (32.43) | 4 (20.00) | 3 (15.00) | 1 (05.00) | 20 (35.08 ) |
| Total (%) | 38(66.66) | 13(22.80) | 5(8.77) | 11(19.29) | 57(57) |

The primer which design in present study targeted ORF (ORF 1,2 and 3) for detection of norovirus GII.P4. A new variants and new recombinant strains emerge in the Norovirus GII.P4 population was resulted from mutation and recombination [2, 11] founded that Norivirus GII.P4 was associated with 65.3% of diarrhea infection while Parra et al. [12] reported that Norovirus GII.P4 was the dominate virus associated with diarrhea infection in Australia and New Zealand where the percentage of infection was 57% and 43.2% respectively. Also Ji et al. [13] showed that the percentage of Norovirus GII.P4 was 83%.

## 4. Conclusion

Norovirus GII.P4 represented one of enteric viral causes of diarrhea among children under 5 years old and its widely associated with age group1daye –6 months.

## 5. Acknowledgments

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