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Molecular detection of norovirus and TNF-α polymorphism in children with acute gastroenteritis infection in Samawa, Iraq

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***Abstract*---**Human Norovirus is the leading cause of non-bacterial gastroenteritis in children worldwide, and research into it is critical for outbreak prevention and infection control. The multiplex real time PCR technique was evaluated in this study using 100 specimens (stool and blood) collected from children in Al-Muthanna province. RT-Qpcr for NVGI and NVGII was used to test all samples for Norovirus. The overall incidence rate of human Norovirus infection was 26%, with the first genogroup G1 accounting for only one case (2%) and the second genogroup accounting for 12 cases (24%). The findings revealed a high prevalence of previous virus infection in the age group (1-10) months. Infections occurred equally among males and females, with no significant difference in infection rates; females were infected at a rate of 42%, while males were infected at a rate of 58%. Males were infected at a higher rate (53.8%) than females (46.2%). The results of Tetra ARMS -PCR after DNA extraction for patients and amplification using the previous method show that there are three genotypes for G/G among patients compared to two results for A/A in the control groups. As a result, association and both homozygous as risk factors for HNV infection development.

***Keywords*---**acute gastroenteritis, human noroviruses GI, human noroviruses GII, real time–PCR, TNF-α, SNPs.

Introduction

Acute gastroenteritis (AGE) is a common syndrome caused by an infectious viral disease that causes nausea, diarrhea, vomiting, and abdominal pain. It is a leading cause of morbidity and mortality worldwide (Abdulaziz *et al.*, 2020). Each

year, noroviruses are responsible for an estimated 1.1 million hospitalizations and up to 218,000 deaths in this age group. Noroviruses are an evolutionary diverse group of envelopeless, single-stranded, positive-sense RNA viruses that belong to the Caliciviridae family and cause a significant proportion of acute viral gastroenteritis in humans. (Miura *et al.*,2018). Symptomatic Norovirus infections are common in various populations, making clinical judgment on the virus's contribution to diarrheal disease difficult (Ayukekbong *et al.*, 2015).

Norovirus

Noroviruses are small (27–35 nm in diameter), round-shaped, non-capsulated particles that spread from person to person via the fecal-oral route, though droplet transmission after vomiting episodes is also possible. Norovirus strains are highly contagious due to their small infective dose (10 viral particles), long-term shedding, multiple modes of transmission, wide genetic diversity, and resistance to environmental factors. Human noroviruses are highly diverse viruses that can be genetically classified into ten genogroups (GI–GX), but only genogroups GI, GII, GIV, GVIII, and GIX can infect humans, with GII being the most common (Vinjé,2015; Chhabra et al.,2019). Most gastroenteritis outbreaks are currently caused by the GII.4 genotype, though cases caused by other GII genotypes, such as GII.2 and GII.17, are on the rise. Human Norovirus has three open reading frames (ORFs), with ORFs 2 and 3 encoding the major (VP1) and minor (VP2) capsid proteins (Zhang et al., 2021). The expressed VP1 can self-assemble into virus-like particles (VLPs) that are nearly identical to native virus particles. The shell (S) and protruding (P) domains of the VP1 capsid monomers can be structurally separated. The structural core is formed by the S domain, while two P domains wrap around each other to form the base unit dimer) Zhang, Fu, and Hu, 2021(. Isolated P dimers retain some functional features of virus particles, such as ligand-binding and antigenic sites (Tan et al.,2004; Tan et al,2008) Several Human Norovirus subunit vaccine candidates based on VLPs and P particles have been identified (Ramesh et al., 2020). In light of the foregoing introduction and the scarcity of studies on the subject, we designed this study to detect Human Norovirus by RT-PCR in patients suffering from acute gastroenteritis disease.

The human TNF alpha (TNFα)

It became cloned in 1984 and corresponds to chromosome 6p21.3, subsequent to the LTα and LTβ genes, withinside the important histocompatibility complex (Hajeer et al., 2000). TNF (tumor necrosis factor) is a pleiotropic cytokine that has a function in modulating inflammatory and immunological responses in addition to mobileular survival (Young et al.,2008)) TNF- is a kind of inflammatory cytokine this is produced withinside the body.TNF-α is synthesized as a transmembrane protein and it's far secreted after cleavage via way of means of the metalloprotease ,TNF-α changing enzyme (TACE). Binding of both TNFR-1 or 2 to their ligand induces the receptor`s trimerisation. Both receptors bind TNFα, with TNFR 1 being constitutively expressed in all cells, whilst TNFR-2 is inducibly regulated specifically in haematopoietic and endothelial cells (Idress et al.,2020).TNF-α interacts with Chapter Two Literature of Review 23 awesome receptors, TNF-R1 and TNF-R2 that exist as transmembrane and soluble forms. TNF-R1 is

discovered on maximum cells withinside the body, and TNF-R2 is typically expressed on hematopoietic cells. TNF-R1 is activated via way of means of the soluble ligand, whilst TNF-R2 specifically via way of means of the membrane incorporated form. The activation of those receptors ends in the recruitment of intracellular adaptor proteins that spark off more than one sign transduction pathways, which can be related to irritation and mobileular survival (Tian et al.,2014) .

Materials and Methods

Study Population and Sample Collection

A total of 100 blood and stool specimens were collected between November 2020 to April 2021 from children less than 5 years of age who were admitted with acute gastroenteritis (defined as three or more passages of liquid or semi-liquid stool within the preceding 24 hrs.) to the Women and children Hospital, Iraqi Samawa. Demographic and clinical data were collected through administration of a questionnaire. Stool samples were stored at VTM while blood in EDTA tubes until use and extraction then transfer at 80c in the freeze. All specimens were transported to the laboratory for processing and investigations.

Human NOROVIRUS RNA detection by Real Time –PCR POWER CHEK

Real-time PCR (qPCR) is based on two major processes: Firstly, isolation of viral genome (RNA) from specimens, and Secondly, Real Time amplification for each sample. In real-time PCR (qPCR), the accumulating amplified product can be detected at each cycle with fluorescent dyes. This increasing signal allows achieving sensitive detection and quantification of pathogens.

Extraction of Viral Nucleic Acid from Clinical Specimens

By using specific viral DNA/RNA extraction kit (Intron/Korea); the viral genomic was extracted and purifying from the stool and blood of infants and children patients with acute gastroenteritis infection as a first step to amplify the target Norovirus-RNA. The viral genomic was and migrated using agarose gel. The concentration of agarose used was 2%; gels are prepared as percentage weight/volume solutions. Thus, a 0.5% gel agarose in 100 ml buffer were loaded on to 2% agarose gel containing ethidium bromide (0.2µg/ml).After extraction Norovirus group I and II were detected by multiplex RT-PCR technique (Fig.1).

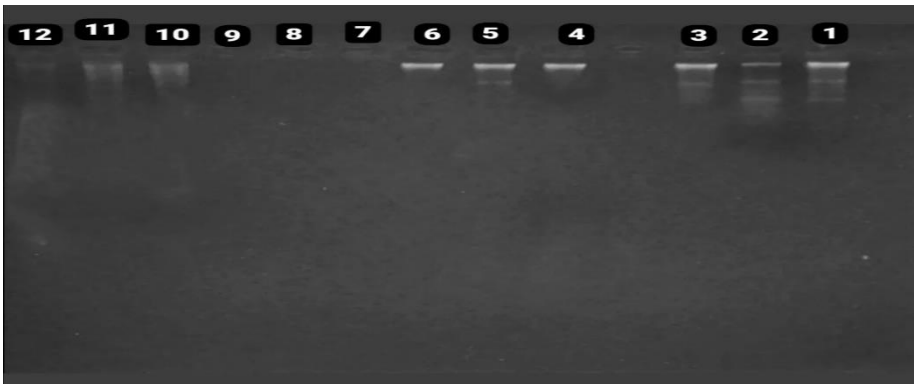

Figure 2. 1: Extraction of Viral Genome from Patients with AGEI ,0.5 % Agarose Gel Electrophoresis , TBE 1X ,at Voltage 100 Volt for 20 min, Lanes (1-11) were Positive

Detection of Norovirus infection by Real Time –PCR det-Qpcr

Norovirus GI and GII Detection by Real-time RT-PCR assay is a qualitative Triplex Real-time RT-PCRtest, which includes cDNA synthesis by reverse transcriptase and PCR amplification using Taq. DNA polymerase, specific primer and probe labeled with the fluorescent dye in a single tube. The target sequences of the specific genes for Norovirus GI, GII and the Internal Control (IC) are detected through the FAM, VIC (JOE or HEX) and Cy5 channels respectively Table 1.

Table 1: Specific Detection on Fluorescence Channels

Analyze	Fluorophore
Norovirus GI	FAM
Norovirus GII	VIC (JOE or HEX)
IC	Cy5

Results and Discussion

Norovirus infection

Hundred stool and blood specimens involved in this study 50 were AGEI patients and 50 were healthy control. This study used R.T PCR to evaluate the presence of human norovirus in stool and blood samples of children with acute gastroenteritis who have attended to children and women Hospital in Samawa city. The rate of human norovirus infection obtained was 26% (13 out of 50) as showed in Table 2 and Figure (1).

Total Viral genome	No.	%	P value
Positive	13	%26	P=0.00 (P<0.05)
Negative	37	%74	
Total	50	100%	

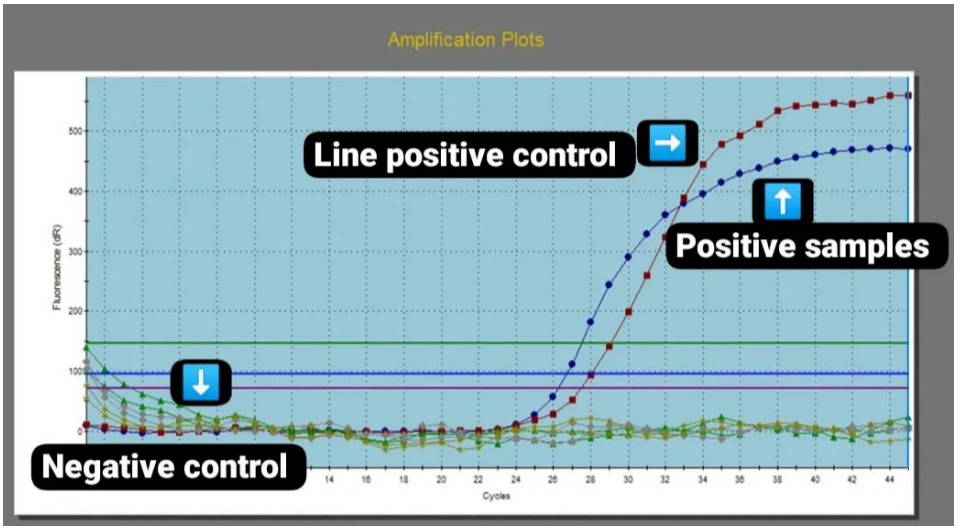

Figure (3.1) shows line positive control and line negative control and also positive samples and negative samples, according to detection kit (qRT-PCR) of HRV which used in this study the curves that represent positive samples appear between the line positive control and line negative control

This study used R.T PCR to evaluate the presence of human Norovirus in stool samples of children with acute gastroenteritis who have attended to children and women Hospital in Samawa city. In this study the rate of human norovirus infection obtained was 26% (13 out of 50) as showed in Figure (3-1) Norovirus by rapid test, 29/100 (29%) were positive, and when examined by RT-PCR technique, the total number of positive samples was 11 (37.9%) (Al-khoweledy, 2016), by Enzyme linked assay and conventional PCR in Mousel. The study found that 28 (28%) of 100 samples tested positive for G1 Norovirus with a PCR product size of approximately (380) bp, while 72 (72%) tested negative in Basra ((Sameh Arif et al., 2016).

Infection rates in MENA countries ranged between 0.82 percent and 36.84 percent, and the majority of studies were clinical observational studies assessing human Norovirus rates, primarily among children.(Kreidieh et al., 2017), Using (RT-qPCR) for NVGI and NVGII. Enteric viruses were detected in 42 out of the 100 (42%)(Mohamed, Hameed and Al-Rubai, 2016). while Norovirus was detected in 8% of cases using RT.PCR in Basrah city(Thwiny, 2015).

On the other wise were the rate of infection in this study was higher than in Diyala city was (1.1%)(Abdulazeez, Al-shuwaikh and Latif, 2020) also other study in Diyala city obtained was 6.04%(Diraa, Hussein and Alezzi, 2020). This study in Diyala backs up the findings of many other studies on the subject, such as (6.23 percent) in a study conducted by Chen et al., (2017) in China, and Al-Ali (2011) in Lebanon with a rate of (6.32 percent). Noroviruses were found in 47 (12.53 percent) of all stool samples tested with ELIZA in the south of Iran (Najafi, 2014). In Brazzaville, the rates of norovirus infection were higher (27, 14%), according to reports made on the same virus. (Mikounou et al., 2019).

The variety in rates can be credited to the distinctions between populaces, the varieties on schedule of study or to the research center tests utilized (Lorrot et al., 2011). The low degree of cleanliness and sterilization there, just as the low admittance to consumable water worked with the spread of human norovirus strains (Huynen et al., 2019). The high recurrence of gastroenteritis brought about by human norovirus in a few nations can be because of different components unique in relation to the center east. For model, the utilization of crude marine items.Temperature, moistness, and precipitation are the most significant ecological factors overseeing the human norovirus pestilence cycle. It was tracked down that low temperature between - 6.6 and 20 °C, relative dampness somewhere in the range of 10 and 66 %, and precipitation from 1 day to 90 days before an episode are compelling scopes of the ecological components, which favor the recurrence of human norovirus. Some other natural components may have a relationship with the pattern of human norovirus pestilences(Diraa, Hussein and Alezzi, 2020).

In AGEI, the most commonly affected age stratum infected with RNA -HNV was (1-10 months) which constituted 14% (7 out of 50 cases), while the age stratum (11-18 months) was constituted 8% (4 out of 50 cases), followed by 4 %(2 out of 50) in age stratum (21 – 48 months). Statistical comparison of these age stratum revealed significant differences ($p< 0.05$) Table (3). The results recorded the higher rate in the male but in the little difference as the table 4

Table 3.1: Frequency of HNV RT-PCR Signal among the Patients with AGEI According to the Age Stratum

Age Stratum	Months	HNV			P value
		No.	Positive	Negative	
	1-10	33	7	26	
		%66	14%	52%	
	11-20	10	4	6	Anova test P=0.00 (P<0.05)
		%20	8%	12%	
	21-48	7	2	5	
		%14	4%	10%	
Total		50	13	37	
		100%	26%	74%	

According to the findings, there has been an increase in the number of Norovirus infections among children aged (48 months). As shown in Table 1, the highest rate of infection was found among infants aged 1 to 10 months (33%) and approximately (10%) in the age group 11 to 20 months (4-1). This finding appears to be consistent with other studies that found a higher prevalence of human norovirus infection among children. Viruses cause approximately 70% of episodes of infectious diarrhea in the pediatric age group, and children may have been infected with different genotypes of norovirus in 6 to 12 months. Asymptomatic infection is common in children under the age of five (Weeb and Staar, 2005; Castilho et al., 2006). A review of recent studies that used molecular methods to detect enteric viruses revealed that noroviruses accounted for approximately 12% (range 4.4–30.7%) of severe gastroenteritis cases in children. The Baghdad study

found that children of all ages were infected with Norovirus, with the highest infection occurring in children aged (2-5) years (32.6 %), followed by children aged (5 years) (23.8 %). This finding was consistent with the findings of another study by Li-Juan et al, (2010), who found no significant difference in the distribution of age in Norovirus infection. In this day and age, there are numerous causes of infection. We have two ideas to discuss. The first is that, theoretically, children (both females and males) will have greater immunity (low risk) while on breast feeding or moderate risk on oral feeds (bosom/bottle), and will be at high risk in children who are completely free on phony feeding strategy. Furthermore, maternal invulnerability will be reduced after the age of about a year due to the opportunity of presenting semi-strong food, which may put the children in danger with poor hygiene. According to this and other studies, the following idea is fundamental .

Table 3. 2. Frequency of HNV RT-PCR Signal Among The Patients With AGEI According to the Age Stratum

Age Stratum	Months	Patients in Each Group		HNV		P value
				Positive	Negative	
	1-10	No	33	7	26	
		Percent	66%	14%	52%	
	11-18	No	10	4	6	Anova test
		Percent	20%	8%	12%	P=0.00 H.S**
	21-48	No	7	2	5	(P<0.05)
		Percent	14%	4%	10%	
Total		No	50	13	37	
		Percent	100%	26%	74%	

**Highly significant at 1% level

Table 3.3: Percentage of HNV in AGEI Patients According to Their Gender

AGEI Patients	HNV- Infection	
	+	%
Male	7	%53.8
Female	6	%46.2
Statistical analysis	(P >0.05) = 0.00	

The current study obtained, more infections were noticed among males 7(53.8%) than females 6(46.2%) Table (3-3). This results are in agreement with the finding done by Dirraa (2019),Thiwany and Hassan (2015) in Iraq and that of(Qi et al., 2017)in China. In any case, in correlation with the review done by Hussein et al., in (2018), concerning the sex, a review uncovered countless guys patients than females patients in Diyala additionally with other review done by Gentile et al., in (2018) in Argentina who recorded (59%) among guys. So a few examinations revealed that there is no huge contrasts related with sexual orientation and human norovirus disease like the review done in Baghdad by Al-Moussawi et al.,(2018) and Mahmood et al., (2015) they decipher the outcome by young men and young ladies presented to chance of disease because of every one of them

were living under similar conditions and environments of illness (Mahmood et al., 2016). With everything taken into account the above assessments, have reflected that the speed of human norovirus sickness has no effect in sexual direction and both sex are correspondingly influenced.

The Results of TNF-α *rs*361525
Extraction Total Genome DNA from the Stool and Blood Specimens

By using specific Total genome DNA extraction kit (G-Spin total DNA Extraction kit, Intron / Korea) the whole (total) genomic (DNA) was extracted , and migrated using agarose gel from the stool and blood specimens of children patients with gastroentrities infection as well as healthy control groups as a first step to amplify the target to detect the single nucleotide polymorphisms (SNPs) TNF-α gene polymorphism .After using the gel electrophoresis technique the result was almost the appearance of the DNA in 50 patients with AGEI and 50 as HC samples as shown in Figure (3.3). The results observed there are 45% of positive sample for SNPs in rs 361525-TNF alpha (Table 4.7). The age group of 1-10 months was the highest (8%) followed by the age group of 11-18 months (2%).However, in the age group 24-48 there was no appearance of SNPs in rs-361525 of TNF alpha. The statistical analysis showed highly significant differences between them (Table 3.8.).

Figure 3.2: Extraction Human Total Genome DNA from AGEI & HC, 0.5% Agarose Gel Electrophoresis, TBE 1X, at Voltage 100 volt for 15 min.

Table 3.4 The distribution of SNPs in rs-361525 TNF alpha

TNF alpha	No.	%	P value
Positive	5	10%	P=0.00
Negative	45	%90	(P<0.05)
Total	50	100%	

Table 3.5. The distribution of SNPs in rs-361525 TNF alpha according to age stratum

Age Stratum	Months	TNF alpha			P value
		No.	Positive	Negative	Anova test

	1-10	33	4	29	P=0.00**
		66%	8%	58%	(P<0.01)
	11-20	10	1	9	
		20%	2%	18%	
	21-48	7	0	7	
		14%	0	14%	
Total		50	5	45	
		100%	10%	90%	

**Highly significant at 1% level

However, we were not really prepared to use data on different age groups and variants affecting the rs361525 SNPs for TNF-alpha in our model with previous studies because of lack of acceptance and lack of reliable information. The SNPs in rs-361525 TNF alpha were in male higher than female. The ratio was 60% and 40% respectively. The differences between male and female genders were highly significant (p=0.00) Table (3.5).

Table.3.6: The distribution of SNPs in rs-361525 TNF alpha according to gender

TNF alpha	SNPs -rs361525	
	Positive	Percent
Male	3	60%
Female	2	40%
Statistical analysis	(P <0.01) = 0.00**	

**Highly significant at 1% level

The consequences of this examine are typically agreed with distinctive studies. Bank et al., 2014 performed a examine in University of Liouville in America and found out that because of the polymorphism on this gene the consequences have been near among ladies and adult males, with ratios of 59% and 41%. In addition, Wei et al., 2011 discovered a near consequences that the proportion of male and ladies changed into 64.5% and 35.5%. In comparison to the existing examine, Ferguson et al., 2008 found out that, withinside the town of New Zealand, the contamination price in ladies changed into 62.2% at the same time as in adult males the price of contamination changed into 35.8% in addition to in ladies. From the higher consequences that display for us the proportions are comparable and might have an effect on each sexes with out the other.

Genotyping of TNF-α Gene Polymorphisms

The amplified of TNF-α (*rs*361525) goal sequences of studied corporations had been through ARMS approach are summarized in table (4-10) and figures (3-4 A&B). The end result of amplified became seemed the presence of bands (G Allele= 176 bp and A Allele= 124 bp (because of the presence of the G>A mutation. Whereas the wild kind became recognized through a unmarried three hundred bp fragment. It may be visible that the frequency of GG and AA genotypes in sufferers with AGEI and HC corporations which reached 6%, 4% respectively. wherein AA genotype improved as fee OR=5.2062 as compared with GG genotypes in studied corporations. On the opposite hand, the frequency of A

and G genotype in sufferers with AGEI and HC corporations became (15.3%, 38.4 %) respectively in table (3-6), that reduced in sufferers as compared with manipulate group . From the table(3-6) now no longer confirmed the statically evaluation observed a excessive huge in TNF-α polymorphism of that Iraqi patient.

Table 3.7 Allele Frequency Distribution and Odd Ratio of TNF-α Gene Polymorphism between AGEI patients infected with HNV and HC

Allele frequency		OR (CL 95%)	P-Value
Infected (13)	control (50)		
G: 38.4 (%) A:15.3 (%)	G:1 (2 %) A:0 (0%)	30.6250 (3.15 to 297.45) 21.956 (0.9859 to 488.9609)	P = 0.0032** P = 0.0510N.S

Tumor Necrosis Factor-α is a primary cytokine withinside the inflammatory reaction to infection. TNF-α generally capabilities to set off cell immunity and to offer safety in opposition to microbes, however immoderate ranges of this cytokine will bring about intense tissue damage, septic surprise or even death(Abbas AK et al.,2015). The TNF-α rs361525 polymorphism ought to alternate TNFα gene transcription and alter TNF-α generation (Kaluza et al., 2000) .in step with observe in china e observed TNF-α rs361525 polymorphism multiplied the threat of GC amongst Asians, at the same time as this SNP regarded to defend Caucasians from GC with gastric cancer.

Patients with CD who exhibit more severe oral soft tissue alterations were significantly more often A allele carriers of rs361525 than G allele carriers (14.2% versus 2.2%; P <0.001). Furthermore, A allele carriers had a higher mean periodontal probing depth (P <0.05).TNF-α -238 G/A in aNew Zealand the control of AA 0 ,AG 12.1%,GG 87.9% while reached for CD and UC (bowel disease) 0.5% AA,AG 10.9%,GG 89.9%.(Ferguson *et al.*, 2008) In studies on the same gene and SNP, contradictory results were shown in Iran in study about association of Tumor Necrosis Factor Alpha Gene Polymorphisms with bowel disease , the -238 mutant allele frequencies were higher in CD patients comparing to control group, but this difference was not statistically significant.

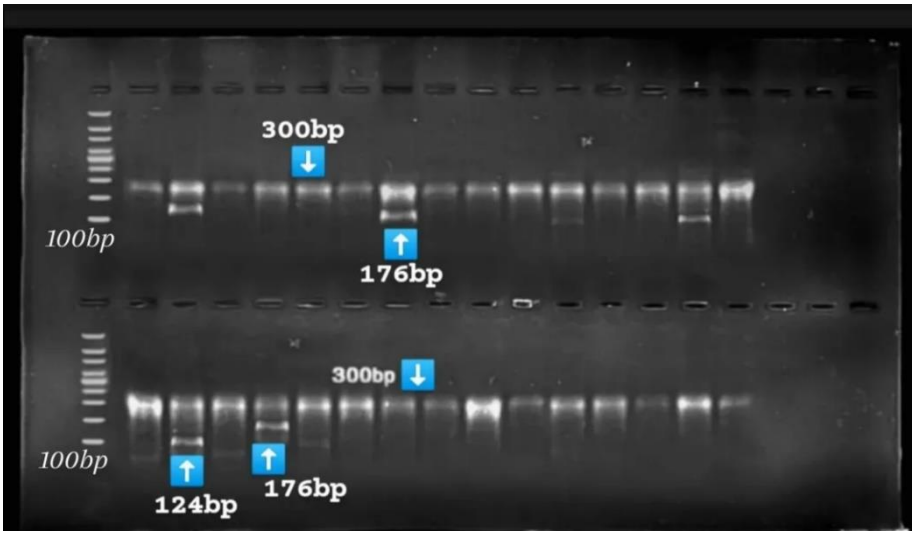

Figure (3.3):Allelo typing patterns of TNF- α gene using PCR-ARMS; Showed a monozygote allele (GG) and (AA) had a one band (176 bp) and(124bp) molecular size respectively in AGEI .M:DNA ladder 100-1500bp .The amplified products using PCR-ARMS migrated into 2 agarose ,100v,for 20 min;20µl in each well;stained with ethidium bromide

Conclusions

The following can be concluded from the present study.

1. The highest rate (18%) was recorded among females and male within age group less 1 year.
2. whole positive results were from different places in Samawa.Human, Norovirus may cause sporadic infection in closely groups of people.
3. There is risky association between TNF-α gene polymorphism in rs361525 and HNV infection.

References

Abdulazeez, M. S., Al-Shuwaikh, A. M., & Latif, I. I. (2020). Detection and comparison of Sapovirus, rotavirus and norovirus among infants and young children with acute gastroenteritis. *Annals of Tropical Medicine and Public Health*, *23*, 231-439.

Abdulazeez, M. S., Al-Shuwaikh, A. M., & Latif, I. I. (2020). Detection and comparison of Sapovirus, rotavirus and norovirus among infants and young children with acute gastroenteritis. Annals of Tropical Medicine and Public Health, 23, 231-439.

Al-Khoweledy, A.J.S. (2015). Investigation of norovirus among diarrheal children in Al-Najaf province. IASJ., 8(3): 1-6.

Al-Marsome HT, Jabbar SH, Hala SA, Abdulrhman (2016) MHH. Diagnosis of noroviruses in Iraqi children suffering from gastroenteritis by enzyme linked assay and conventional PCR. IASJ;34(1):42-9.

Al-Moussawi, D.K., Rahman, L.C. and Basim, A.J. (2018). Genotyping and

phylogenic of norovirus as main cause of children gastritis in Thi Qar province, University of Thi - Qar Journal of Science, 6(4) :35-41.

Ayukekbong, J. A., Mesumbe, H. N., Oyero, O. G., Lindh, M., & Bergström, T. (2015). Role of noroviruses as aetiological agents of diarrhoea in developing countries. Journal of General Virology, 96(8), 1983-1999.

Blazevic, V.; Lappalainen, S.; Nurminen, K.; Huhti, L.; Vesikari, T. (2011).Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. Vaccine, 29, 8126–8133 .

Castilho, J. G., Munford, V., Resque, H. R., Fagundes-Neto, U., Vinjé, J., & Rácz, M. L. (2006). Genetic diversity of norovirus among children with gastroenteritis in Sao Paulo State, Brazil. Journal of clinical microbiology, 44(11), 3947-3953

Dennehy, P. H. (2005). Acute diarrheal disease in children: epidemiology, prevention, and treatment. *Infectious Disease Clinics*, *19*(3), 585-602.

DİRAA, M. G., AL-EZZİ, J. İ., & HUSSEİN, A. A.(2020). Detection of Human Norovirus among Children with Gastroenteritis in Diyala Governorate. Pediatric Practice and Research, 8(1), 1-6 .

Djunaid, R., Sahar, J., Widyatuti, W., & Priyo, S. (2022). Predictive factors contributing to violent behavior in adolescents . *International Journal of Health Sciences*, *6*(2), 1148–1159. https://doi.org/10.53730/ijhs.v6n2.11483

Ferguson, L. R. et al. (2008) 'Single nucleotide polymorphism in the tumor necrosis factor-alpha gene affects inflammatory bowel diseases risk', 14(29), pp. 4652–4661.

Gentile, A., Juan, A.S., Juan, Ignacio, D., Maria, D.V., Maria, F.L., Marisa, T., Liliana, S., Solana, R. and Maria, S.A. (2018).Active Norovirus Surveillance in Children Under 5 Years with Diarrhea Following Rotavirus Vaccine Introduction in Argentina, Open Forum Infect. Dis., 2018; 5(Suppl 1): S753–S754.

Hajeer, A. H., & Hutchinson, I. V. (2000). TNF α gene polymorphism: Clinical and biological - implications. Microscopy research and technique, 50(3), 216-228.

Hussein, A.A., Rawaa, A.H. and Mohammed, J.S. (2018). Enteric Viruses co-infection with giardiasis among diarrheal children in Diyala province – Iraq, J. Pure Appl. Microbiol., 12(2): 793-799.

Huynen, P., Mauroy, A., Melin, P., & Thiry, E. (2019). Noroviruses, these unknown worldwide epidemic enteropathogens. Revue medicale de Liege, 74(1), 41-41.

Huynen, P., Mauroy, A., Melin, P., & Thiry, E. (2019). Noroviruses, these unknown worldwide epidemic enteropathogens. Revue medicale de Liege, 74(1), 41-41.

Idress, M., Milne, B. F., Thompson, G. S., Trembleau, L., Jaspars, M., & Houssen, W. E. (2020). Structure-based design, synthesis and bioactivity of a new anti-TNFα cyclopeptide. Molecules, 25(4), 922.

Jalilian S, Samarbaf-Zadeh AR, Mozhgani SHR, Makvandi M, Parsa-nahad M (2012) Relative frequency of norovirus infection in children suffering from gastroenteritis referred to Aboozar hos pital, Ahvaz, Iran. Jundishapur J Microbiol 5:355–358

Kocher, J., & Yuan, L. (2015). Norovirus vaccines and potential antinorovirus drugs: recent advances and future perspectives. Future virology, 10(7), 899-913.

Kreidieh, K., Charide, R., Dbaibo, G. and Melhem, N.M.(2017). The enidemiology of norovirus in the middle east and north africa (MENA) : asystemic review, Virology Journal, 14(1), 220.

Li-Juan Liu, Wei Liu, Yun-Xi Liu, et al.(2010)Identification of Norovirus as the Top Enteric Viruses Detected in adult Cases with Acute Gastroenteritis Am. J. Trop. Med. Hyg.; 82(4). pp. 717–722

Lorrot M, Bon F, El Hajje MJ, Aho S, Wolfer M, Giraudon H et al.(2011). Epidemiology and clinical features of gastroenteritis in hospitalised children: prospective survey during a 2-year period in a Parisian hospital, France. Eur J Clin Microbiol Infect Dis, 30 (3):361–368.

Mikounou, V. et al. (2019) 'International Journal of Infectious Diseases Molecular characterization of norovirus infection responsible for acute diarrhea in Congolese hospitalized children under fi ve years old in Brazzaville , Republic of Congo', 88, pp. 41–48.

Miura, F., Matsuyama, R., & Nishiura, H. (2018). Estimating the asymptomatic ratio of norovirus infection during foodborne outbreaks with laboratory testing in Japan. Journal of epidemiology, JE20170040.

Mohamed, N. S., Hameed, M. F., & Al-Rubai, H. K. (2016). Detection of norovirus and rotavirus in children under five years during winter 2012-2013 in Mosul Province. Al-Nahrain Journal of Science, 19(3), 101-107.

Mohamed, N.S., Mohammed, F.H. and Haider, K.A. (2016). Detection of norovirus and rotavirus in children under five years during winter 2012-2013 in Mosul province, I.A.S.J., 19(3): 101- 107.

Mohamed,. N. S., Hameed, M. F., & Al-Rubai, H. K. (2016). Detection of norovirus and rotavirus in children under five years during winter 2012-2013 in Mosul Province. Al-Nahrain Journal of Science, 19(3), 101-107.

Najafi, A. (2014) 'Two year monitoring of norovirus in children with acute gastroenteritis in the south of Iran', (October 2012). doi: 10.5812/jjm.7866.

Najafi, A. (2014) 'Two year monitoring of norovirus in children with acute gastroenteritis in the south of Iran', (October 2012).

Okoye, C. N. (2016). Knowledge, Attitude and Adoption of Appropriate Feeding Practices by Mothers and Anthropometric Indices of Their Children in Aguata Local Government Area Of Anambra State, Nigeria (Doctoral dissertation).

P. Chhabra, M. De Graaf, G. I. Parra et al., (2019)"Updated classification of norovirus genogroups and genotypes," Journal of General Virology, vol. 100, no. 10, pp. 1393–1406.

Qi, L.M., Xinzhi, X., Ying, X., Hua, L., Huangcheng, S., Wenwen, D., Wenge, T., Tao, S., and Qin, L. (2017). Outbreak of acute gastroenteritis caused by norovirus genogroup II attributed to contaminated cold dishes on a cruise ship in Chongqing, China. Int. J. Environ. Res. Public Health., 15: 2823.

Ramesh, A. K. (2020). Study of enteric virus infection and parenteral vaccines in the gnotobiotic pig model (Doctoral dissertation, Virginia Tech).

Sameh Arif, H. et al. (2016) 'Diagnosis of Noroviruses in Iraqi children suffering from Gastroenteritis by Enzyme linked assay and conventional PCR', The Medical Journal of Basrah University, 34(1), pp. 42–49.

Sameh Arif, H., M Hassan Hadi, A., Thaher Al-Marsome, H., & Salman Hassan, J. (2016). Diagnosis of Noroviruses in Iraqi children suffering from Gastroenteritis by Enzyme linked assay and conventional PCR. The Medical Journal of Basrah University, 34(1), 42-49.

Scheuer, K. A., Oka, T., Hoet, A. E., Gebreyes, W. A., Molla, B. Z., Saif, L. J., & Wang, Q. (2013). Prevalence of porcine noroviruses, molecular characterization of emerging porcine sapoviruses from finisher swine in the United States, and unified classification scheme for sapoviruses. *Journal of clinical microbiology*, *51*(7), 2344-2353.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, *5*(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

Suryatika, I. B. M., Anggarani, N. K. N., Poniman, S., & Sutapa, G. N. (2020). Potential risk of cancer in body organs as result of torak CT-scan exposure. *International Journal of Physical Sciences and Engineering*, *4*(3), 1–6. https://doi.org/10.29332/ijpse.v4n3.465

Tan, M.; Fang, P.; Chachiyo, T.; Xia, M.; Huang, P.; Fang, Z.; Jiang, W.; Jiang, X. (2008) Noroviral P particle: Structure, function and applications in virus-host interaction. Virology, 382, 115–123.

Tan, M.; Hegde, R.S.; Jiang, X.(2004)The P domain of norovirus capsid protein forms dimer and binds to histo-blood group antigen receptors. J. Virol., 78, 6233–6242.

Thwiny, H.T., Hassan, J.H. (2015). Detection of norovirus in stool samples by RT-PCR from under five years age children hospitalized for acute gastroenteritis in Basrah, Iraq, Journal of Thi-Qar Science, 5(2): 47-53.

Tian, T., Wang, M., & Ma, D. (2014). TNF-α, a good or bad factor in hematological diseases?. Stem cell investigation, 1.TNF-A Receptor Subtypes is Essential for Optimal Skin Tumour Development. Oncogene 2004, 23, 1902.

Vinjé, J. (2015). Advances in laboratory methods for detection and typing of norovirus. Journal of clinical microbiology, 53(2), 373-381..

Webb A, Starr M. (2005). Acute gastroenteritis in children. Australian family physician, 34(4), 227-31.

Young, C. N., Koepke, J. I., Terlecky, L. J., Borkin, M. S., Boyd, S. L., & Terlecky, S. R. (2008). Reactive oxygen species in tumor necrosis factor-α-activated primary human keratinocytes: implications for psoriasis and inflammatory skin disease. Journal of Investigative Dermatology, 128(11), 2606-2614.

Zhang, M., Fu, M., & Hu, Q. (2021). Advances in Human Norovirus Vaccine Research. Vaccines, 9(7), 732.