جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية العلوم

قسم علوم الحياة

دراسة جزيئية للنوروفيروس والفيروس النجمي وعلاقته بتعدد الاشكال الجيني لمستقبل الشبيه بالتول نمط ٧ والانترفيرون كاما في الاطفال المصابين بالتهاب المعدة والامعاء

أطروحة مقدمة

الى مجلس كلية العلوم في جامعة بابل، وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في العلوم / علوم الحياة

من قبل

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(ماجستير علوم حياة – احياء مجهرية ٢٠١٩ – جامعة بابل)

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College of Sciences

Department of Biology

Molecular Study of Norovirus and Astrovirus in Relation to TLR-7 and IFN-γ Gene Polymorphism among Children with Gastroenteritis

A Thesis

Submitted to The Council of The College of Science and The Committee of Postgraduate Studies of Babylon University in Partial Fulfillment of The Requirements for The Degree of PhD in Biology

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Ph.D Molecular Virology

2022 A. D **1444 A.H**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ
أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ
خَبِيرٌ

صـــدَقَ الله العَظِـيم.

سورة المجادلة , الآيه 11

Dedication

To .. my supporter dear "Father" Who taught me tender without waiting .. To whom I carry his name with all pride ..

To .. Big heart , great lady and a successful source …

To the smile of life and the secret of existence..

My mother

To the eyes and heartbeat … to…

My brother & sisters

ISRAA

الخلاصـــــه

يعد فايروس النورو وفايروس الأسترو (الفيروسات النجميه) من العوامل المسببة لالتهاب المعدة والأمعاء الفيروسي الذي يصيب جميع الفئات العمرية، ولكن في أغلب الأحيان يكون اكثر حدوثا عند الشباب وكبار السن والأشخاص في المجتمعات شبه المغلقة مثل المستشفيات ودور رعاية المسنين والقواعد العسكرية والسفن السياحية.

إن التشخيص الحساس والسريع للعوامل المسببة لالتهاب المعدة والأمعاء الفيروسي هو مفتاح التنفيذ الفعال لأنظمة مكافحة العدوى. يتميز إنترفيرون كاما (IFN-) بقدرته على تنظيم الاستجابات المناعية للمضيف ؛ ومع ذلك ، فإن نشاطه المباشر المضاد للفيروسات لم يتم دراسته جيدًا.

المستقبل الشبيه بالرسم 7 (TLR7) هو مستقبل للتعرف على الأنماط يتعرف على الحمض النووي الريبي الفيروسي بعد الالتقام الخلوي للفيروس ويبدأ استجابة مناعية قوية تتميز بإنتاج IFN وإنتاج السايتوكين المحفزة للالتهابات.

تهدف الدراسة الحالية إلى تحديد الأنماط الجينية لفيروس نوروفيروس و الاسترو فايروس (الفايروسات النجميه) وكذلك تأثير الانترفيرون كاما والمستقبل شبيه بالرسم 7 عند الرضع والأطفال الذين يعانون من التهاب المعدة والأمعاء .

كانت هذه الدراسة عبارة عن دراسة حالة- ضابطة شملت 200 طفل تم تقسيمها للمجاميع التالية: 150 مريضًا مصابًا بألتهاب الامعاء, 50 مريضًا يبدو أنهم يتمتعون بصحة جيدة (116 ذكور و 84 أنثى) تم جمعها من مستشفيات الأمومة والأطفال التعليمية في محافظات الفرات الاوسط في العراق. كانت المجموعات المدروسة لها أعمار مختلفة تتراوح بين 6-122 شهرًا. أجريت الدراسة في قسم الأحياء المجهريه ، كلية العلوم، جامعة بابل خلال الفترة ما بين فبراير 2021 ويونيو 2022. تم جمع مسحات من البراز وتم اخذ 3-5 مل من عينة الدم ثم تم جمع الجينوم الفيروسي والحمض النووي الكلي. تم الحصول عليها وتخزينها عند -20 درجة مئوية / -80 درجة مئوية حتى يتم استخدامها. باستخدام تفاعل البلمرة المتسلسل تم اكتشاف الأنماط الجينية للفايروسات النوروفايروس والفايروسات النجميه ، وأخيراً عزل جينات النوروفايروس والفايروسات النجميه التي تم إرسالها للتسلسل. أجريت فحوصات روتينية أخرى للانترفيرون كاما ,المستقبل الشبيه بالرسم- 7 عند الرضع والأطفال الذين يعانون من التهاب المعدة والأمعاء.

•الانماط الجينية في هذه الدراسة لنوروفيروس (النمط الجيني الاول ,الثاني ,الثالث) موجبًا في 29 (37.6٪) من مجموع 77 وسلبيًا في 48 (62.4٪) من مجموع 77 مريضًا. وكان هناك فرق معنوي (P- Value= 0.03) بين مجموعة المرضى , والمجموعة الضابطة.

أشارت النتائج الحالية النوروفايروس النمط الاول ,الثاني ,الثالث إلى وجود أربعة متغيرات من النيوكليوتيدات في العينات التي تم فحصها ، وهي G> A56 و A> T147 و C> T 231 و T> C 288. تم الحصول على تسلسل جميع النوكليوتيدات في هذه الدراسة.

من أجل ايجاد التسلسل الجزئي للجين VP-1 تمت محاذاته بشكل أكبر وثمانية فقط (سته من النمط الجيني الثاني واثنان من النمط الجيني الثالث) تم إيداعها في قاعدة بيانات البنك الجيني للمركز الوطني لمعلومات التكنولوجيا الحيوية مع تعريف أرقام الانضمام من (SUB11565544 Seq1 ON678619 ؛ ON678620 ؛ ON678621 ؛ ON678622 ؛ ON678623 ؛ ON678624) و SUB11565548 Seq1 ON678617 و ON678618 ؛ على التوالى.

• تم إنشاء الشجرة التطورية للتسلسل الجزئي للجين للنوروفايروس باستخدام 8 عزلات من هذه الدراسة مع تسلسلات من النوكليوتيدات المشابه من البنك الجيني ذو رقم الانضمامEU310927.1 .

• النتيجة الإيجابية الإجمالية لـ الفايروسات النجميه وفقًا لتفاعل البلمرة التسلسل تظهر أن 18.1٪ (14 من 77 حالة) كانت إيجابية أقل من 81.9٪ (63 من 77 حالة) سلبية. ذات فارق معنوي (0.04 =) بين مجموعة المرضى. أشارت النتائج الحالية لـ AsV إلى وجود ثلاثة متغيرات للحمض النووي في العينات التي تم فحصها ، وهي C> T86 و G> A131 و G> A239 المكتشفة في العديد من العينات السريرية التي تم فحصها. تم اكتشاف جميع تسلسلات النوكليوتيدات التي تم تسجيلها في هذه الدراسة من أجل التسلسل الجزئي للجين ORF خصيصًا للنمط الجيني للفايروسات النجميه وقد ظهرت ستة تسلسلات مسجلة حديثًا فقط تم تسجيلها في قاعدة بيانات البنك الجيني في مركز الوطني لمعلومات التكنولوجيا الحيوية مع تعريف أرقام التسلسل (Astrovirus BankIt2589912 Seq1 ON669284 ؛ ON669285 ؛ ON669286 ؛ ON669287 ؛ ON669288 ؛ و ON669289.

• شجرة النشوء والتطور للتسلسل الجزئي عملت باستخدام 6 عزلات من هذه الدراسة مع تسلسلات مماثلة للحمض النووي الفايروسي في البنك الجيني ذو رقم التسلسل (MH933759.1).

• في هذه الدراسة ، تم تحديد ستة عزلات من تسلسلات مسجلة حديثًا (أرقام: LC715217 ؛ LC715218 ؛ LC715219 ؛ LC715220 ؛ LC715221 ؛ LC715222) من طفرة جين TLR-7 (نطاق واحد 408 نقطة أساس) من 45 عزلة (30٪). والتي كشفت ما يقع في المواقع 22 ؛ 341 ؛ 354 طفرة إحلال A → C ؛ الموقع 8 ؛ 361 تعويض T → A ؛ موضع 18 استبدال A → T ؛ وآخر واحد في الموضع 113 استبدال A → C وفقًا لمحاذاة التسلسل المرجعي لجين TLR-7 البشري.

في النتائج الحالية ، تم تحديد أربع عزلات من متواليات مسجلة حديثًا (أرقام: LC715223 ؛ LC715224 ؛ LC715225 ؛ LC715226) من طفرة IFN - γ (نطاق واحد 441 bp) من 32 عزلة (21.3٪). والتي كشفت ما يقع في المواقع 20 ؛ 21 ؛ 74 طفرة إحلال A → G ؛ الموقع 48 استبدال T → C ؛ موضع 19 استبدال T → A ؛ وآخر واحد في الموضع 405 ، استبدال G → A وفقًا لمحاذاة التسلسل المرجعي لجين IFN - البشري.

• كان متوسط تركيز TLR-7 و IFN في المصل لـ AHC والمرضى الذين يعانون من مجموعات GE 1.78 ± 19.66 .1.0pg./ml ± 20.60 جزء من الغرام / مل و 67.28 ± 2.0 جالون / مل ؛ 52.9 ± 1.9 على التوالي.

تم العثور على علاقة إيجابية قوية (مع ارتباط كبير للغاية) بين نوفمبر؛ أست ؛ SNP TLR-7 وكذلك IFN γ - في GE. تم العثور على علاقة إيجابية قوية (مع ارتباط كبير للغاية) بين نو في. أسف. SNPs لـ TLR-7 و γ - IFN وفقًا لأعمار المرضى المصابين بعدوى GE. ومع ذلك ، لا توجد ارتباطات ذات دلالة إحصائية بين النوروفيروس ؛ AsV و SNPs لـ TLR-7 و IFN - وفقًا لجنس مجتمع الدراسة.

• استنتاجات من هذه الدراسة:

•تعتبر فيروسات نوروفيروس والفيروسات النجمية أهم سبب لالتهاب المعدة والأمعاء الفيروسي عند الرضع والأطفال. تُظهر هذه الفيروسات مرونة على جينومها يمكن أن تؤدي إلى متغيرات متعددة تدور في نفس الوقت بين البشر. يبدو أن جميع الملاحظات تشير إلى حدث متعدد العوامل يتضمن تفاعلات فيروس مضيف وتغييرات ، على الأقل ، في منطقتين مختلفتين من الجينوم (VP-1 و ORF1).

• يمكن أن يشير تعدد الأشكال لـ TLR-7 و IFN - γ مع عدوى نو في و AsV إلى دور مهم للغاية لهذه العوامل الجزيئية في مرضى الطفولة العراقيين المصابين بجنرال إلكتريك. بالإضافة إلى ذلك ، يمكن أن

يشير التركيز العالي لمستوى TLR-7 و IFN في المصل في المرضى الذين يعانون من التهاب الامعاء إلى أنه يعمل كعامل خطر في التسبب فيه.

Summary:

Norovirus and Astrovirus are causative agents of viral gastroenteritis affecting all age groups, but most frequently the young, the elderly and persons in semi-closed communities such as hospitals, nursing homes, military bases and cruise ships. The sensitive and rapid detection of causative agents of viral gastroenteritis is key to the effective implementation of infection control systems. Interferon gamma (IFN-γ) is best known for its ability to regulate host immune responses; however, its direct antiviral activity is less well studied. Toll-like receptor 7 (TLR7) is a pattern recognition receptor that recognizes viral RNA following endocytosis of the virus and initiates a powerful immune response characterized by IFN production and pro-inflammatory cytokine production.

The current study was aimed to determine the genotypes of norovirus (NoV) ; astrovirus (AsV) as well as effect of IFN-γ; TLR-7 genes polymorphism in infants and children suffering from gastroenteritis (GE).

A case control study including 200 childhoods (150 patients with GE and 50 apparently healthy control); (116 males and 84 females) who were recruited from different Maternity and Children Teaching Hospitals in Mid-Euphrates Governorates of Iraq. Studied groups were had different ages that range from 6-122 months. The study was carried out at the department of Microbiology, College of Science, University of Babylon during the period between February 2021 and June 2022. Stool swabs as well as three to five ml of blood sample were collected and then viral genome and total DNA were obtained and stored at -20°C/-80°C till used. NoV and AsV genotypes detection using Real time- PCR, finally isolates of NoV-VP1- and AsV-ORF-1 genes that were sent for sequencing. Other routine

investigations were done for IFN-γ; TLR-7 genes polymorphism; serological tests for IFN-γ; TLR-7 markers in infants and children suffering from gastroenteritis.

•In this study, Norovirus (GI ; GII&GIII) was positive in 29 (37.6%) out of 77 and negative in 48 (62.4%) out of 77 patients. Statistically significant differences (p = 0.03) among patients group.

•The present results of Norovirus (GI ; GII&GIII) indicated the presence of four Nucleotide variants in the investigated samples, namely 56G>A, 147A>T, 231C>T, and 288T>C. All nucleotide sequences reported in this study have been discovered for VP-1 gene partial sequencing specifically for NoV genotype were further aligned and only eight (six for NoV GII and Two for NoV –GIII) newly registered sequences that were deposited in the National Center for Biotechnology Information GenBank database with definition of accession numbers of (SUB11565544 Seq1 ON678619; ON678620; ON678621; ON678622; ON678623; ON678624) and SUB11565548 Seq1 ON678617 and ON678618; respectively.

•Phylogenetic tree of NoV-VP-1 gene partial sequence constructed using 8 isolates from this study along with similar referring reference nucleic acid sequences (GenBank acc. no. EU310927.1).

•The total positive result of AsV according to qRT-PCR shows 18.1% (14 out of 77 cases) as positive less than 81.9% (63 out of 77 cases) as negative. Statistically significant differences (p = 0.04) among patients group. The present results of AsV indicated the presence of three nucleotide variants in the investigated samples, namely 86C>T, 131G>A, and 239G>A detected in several investigated clinical samples. All nucleotide sequences reported in this study have been discovered for ORF gene partial sequencing specifically for AsV genotype were further aligned and only six newly registered sequences that were deposited in the National Center for

Biotechnology Information GenBank database with definition of accession numbers of (Astrovirus BankIt2589912 Seq1 ON669284,ON669285,ON669286; ON669287, ON669288 and ON669289. Respectively).

•Phylogenetic tree of AsV-ORF gene partial sequence constructed using 6 isolates from this study along with similar referring reference nucleotide sequences (GenBank acc. no. MH933759.1).

•In this study, six isolates from a newly registered sequences (NUMBERS : LC715217 , LC715218 , LC715219 , LC715220 , LC715221, LC715222) of **TLR-7** gene (single band 408 bp) mutation were identified out of 45 isolates (30%). Which revealed that which located at positions 22; 341; 354 a substitution mutation A→C; positions 8; 361 substitutions A→ T; position 18 substitution T→ A; and last one at position 113 substitution C→ A according to the reference sequence alignment of the human **TLR-7** gene.

•In current results, four isolates from a newly registered sequences (NUMBERS : LC715223 , LC715224 , LC715225 , LC715226) of **IFN-γ** gene (single band 441 bp) mutation were identified out of 32 isolates (21.3%). Which revealed that which located at positions 20; 21;74 a substitution mutation G→A; positions 48 substitution C→ T ; position 19 substitution A→ T; and last one at position 405 substitution A→ G according to the reference sequence alignment of the human **IFN-γ** gene.

•The mean of serum **TLR-7** and **IFN-γ** concentration for apparently healthy control and patients with GE groups were **20.60 ± 1.0**pg./ml; 19.66± 1.78 pg./ml and **67.28 ± 2.0**pg./ml; 52.9 ±1.9, respectively.

A strong positive relationship (with highly significant correlation) was found between Nov, Ast , SNP TLR-7 as well as **IFN-γ** in GE. A strong positive relationship (with highly significant correlation) was found between NoV, AsV , SNPs of TLR-7 and **IFN-γ** according to ages patients who have GE infection.

However, there are no significant correlations among NoV; AsV and SNPs of TLR-7 and **IFN-γ** according to the sex of study population**.**

•**Conclusions from this study**:

• Norovirus and Astrovirus have become the most important cause of viral gastroenteritis in infants and children. These viruses exhibit a plasticity on their genome that could result in multiple variants co-circulating at the same time in the human population. All observations seem to point to a multifactorial event that involves host-virus interactions and changes on, at least, two different regions of the genome (VP-1 and ORF1).

•Polymorphism of TLR-7 and **IFN-γ** with NoV and AsV infection could indicate highly important role of these molecular factor in Iraqi childhood patients with GE. In addition, highly serum concentration of TLR-7 and **IFN-γ** level in patients with GE could point that act as a risk factor in the pathogenesis of idiopathic GE.

Appendix

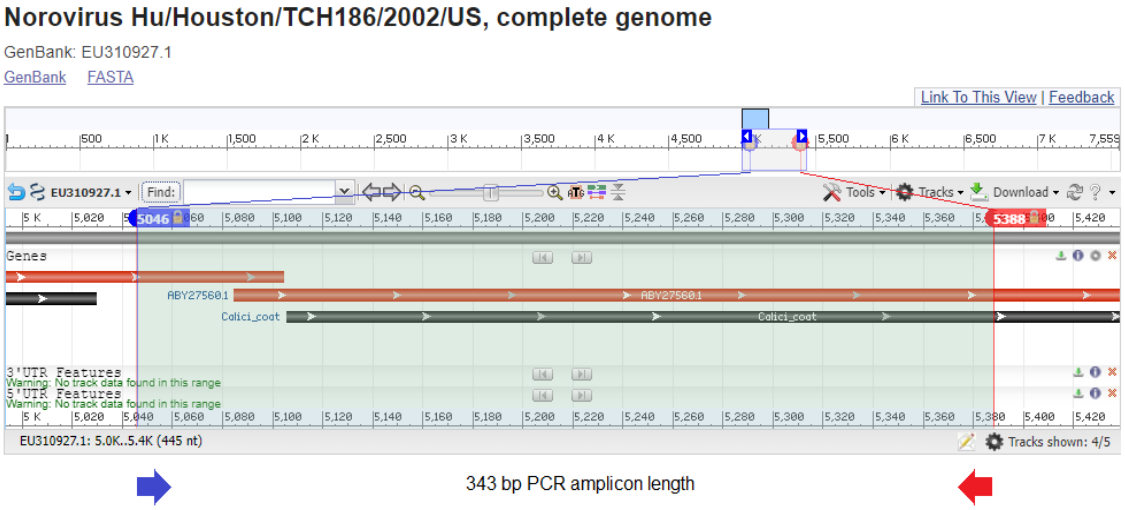

Figure 1. The exact position of the retrieved 343 bp amplicon partially covered the coding portions of the VP1 gene within Human norovirus genomic sequences (GenBank acc. no. EU310927.1). The blue arrow refers to the starting point of this amplicon while the red arrow refers to its endpoint.

Sample sequence were Submitted in NCBI and the Accession Number of nucleotide sequences of Norovirus GII

SUB11565544 Seq1	ON678619
SUB11565544 Seq2	ON678620
SUB11565544 Seq3	ON678621
SUB11565544 Seq4	ON678622
SUB11565544 Seq5	ON678623
SUB11565544 Seq6	ON678624

Appendix

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LOCUS       ON678619                343 bp    RNA     linear   VRL 10-JUN-2022
DEFINITION  Norovirus GII isolate ISRAA-S1 nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678619
VERSION     ON678619.1
KEYWORDS    .
SOURCE      Norovirus GII
  ORGANISM  Norovirus GII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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      121 gctttggagc ccgttgttgg tgccgcaatt gcggcacctg tagcgggcca acaaaatgta
      181 attgacccct ggattagaaa taattttgta caagcccctg gtggagagtt tacagtatcc
      241 cctagaaacg ctccaggtga aatactatgg agcgcgccct tgggccccga tctgaatccc
      301 tacctttctc atttggccag aatgtacaat ggttatgcag gtg
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Appendix

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LOCUS       ON678620                  343 bp    RNA     linear   VRL 18-JUN-2022
DEFINITION  Norovirus GII isolate ISRAA-S2 nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678620
VERSION     ON678620.1
KEYWORDS    .
SOURCE      Norovirus GII
  ORGANISM  Norovirus GII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /collection_date="Apr-2021"
                     /collected_by="Israa Habeeb Naser"
                     /note="genotype: GII"
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     CDS             <1..59
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     gene            40..>343
                     /gene="ORF2"
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                     /note="major capsid protein"
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      121 gcttttggagc ccgttgttgg tgccgcaatt gcggcacctg tagcgggcca acaaaatgta
      181 attgacccct ggattagaaa taattttgta caagccccctg gtgggagagtt cacagtatcc
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Appendix

```
LOCUS       ON678621                 343 bp    RNA     linear   VRL 10-JUN-2022
DEFINITION  Norovirus GII isolate ISRAA-S5 nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678621
VERSION     ON678621.1
KEYWORDS    .
SOURCE      Norovirus GII
  ORGANISM  Norovirus GII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /mol_type="genomic RNA"
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                     /host="Homo sapiens"
                     /db_xref="taxon:122929"
                     /country="Iraq"
                     /collection_date="Apr-2021"
                     /collected_by="Israa Habeeb Naser"
                     /note="genotype: GII"
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                     /protein_id="UBQ32888.1"
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     mat_peptide     <1..56
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     gene            40..>343
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     CDS             40..>343
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                     /note="major capsid protein"
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                     /product="VP1"
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       61 gccagcccat ctgatgggtc cacagccaac ctcgtcccag aggtcaacaa tgaggttatg
      121 gcttttggagc ccgttgttgg tgccgcaatt gcggcacctg tagcgggcca acaaaatgta
      181 attgacccct ggattagaaa taattttgta caagcccctg gtggagagtt cacagtatcc
      241 cctagaaacg ctccaggtga aatactatgg agcgcgccct tgggccctga tctgaatccc
      301 taccttttctc atttggccag aatgtacaat ggttatgcag gtg
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Appendix

```
LOCUS       ON678622                  343 bp    RNA     linear   VRL 10-JUN-2022
DEFINITION  Norovirus GII isolate ISRAA-S6 nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678622
VERSION     ON678622.1
KEYWORDS    .
SOURCE      Norovirus GII
  ORGANISM  Norovirus GII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /country="Iraq"
                     /collection_date="Apr-2021"
                     /collected_by="Israa Habeeb Naser"
                     /note="genotype: GII"
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     mat_peptide     <1..56
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                     /product="RdRp"
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     CDS             40..>343
                     /gene="ORF2"
                     /note="major capsid protein"
                     /codon_start=1
                     /product="VP1"
                     /protein_id="URQ32891.1"
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      121 gctttggagc ccgttgttgg tgccgcaatt gcggcacctg tagcgggcca acaaaatgta
      181 attgacccct ggattagaaa taatttttgta caagcccctg gtggagagtt tacagtatcc
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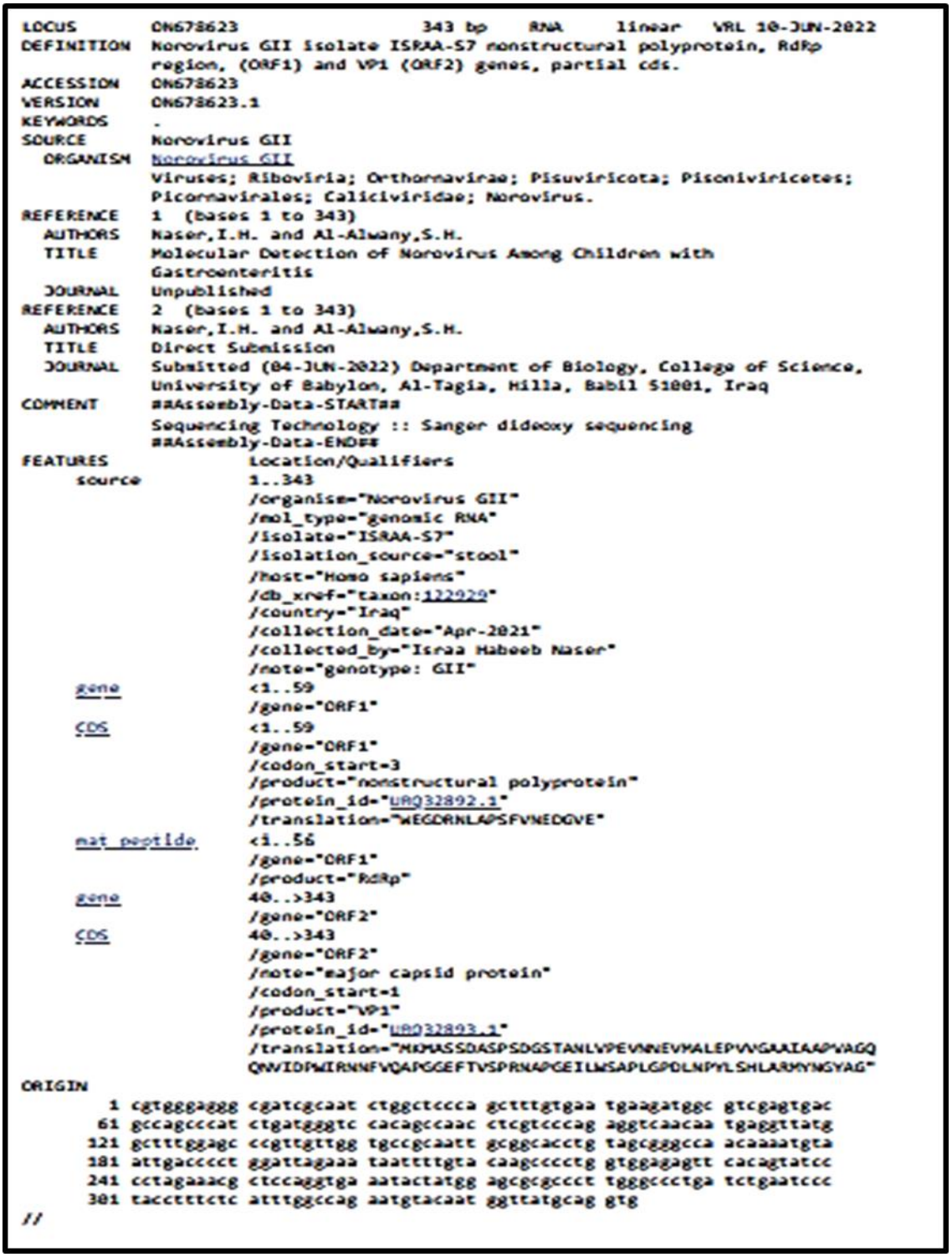
Appendix



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LOCUS       ON678623                343 bp    RNA     linear   VRL 10-JUN-2022
DEFINITION  Norovirus GII isolate ISRAA-S7 nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678623
VERSION     ON678623.1
KEYWORDS    .
SOURCE      Norovirus GII
  ORGANISM  Norovirus GII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
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                     /collected_by="Israa Habeeb Naser"
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                     /translation="WEGDRNLAPSFVNEDGVE"
     mat_peptide     <1..56
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                     /product="RdRp"
     gene            40..>343
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     CDS             40..>343
                     /gene="ORF2"
                     /note="major capsid protein"
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                     /protein_id="UAQ32893.1"
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      181 attgacccct ggattagaaa taatttttgta caagcccctg gtggagagtt cacagtatcc
      241 cctagaaacg ctccaggtga aatactatgg agcgcgccct tgggccctga tctgaatccc
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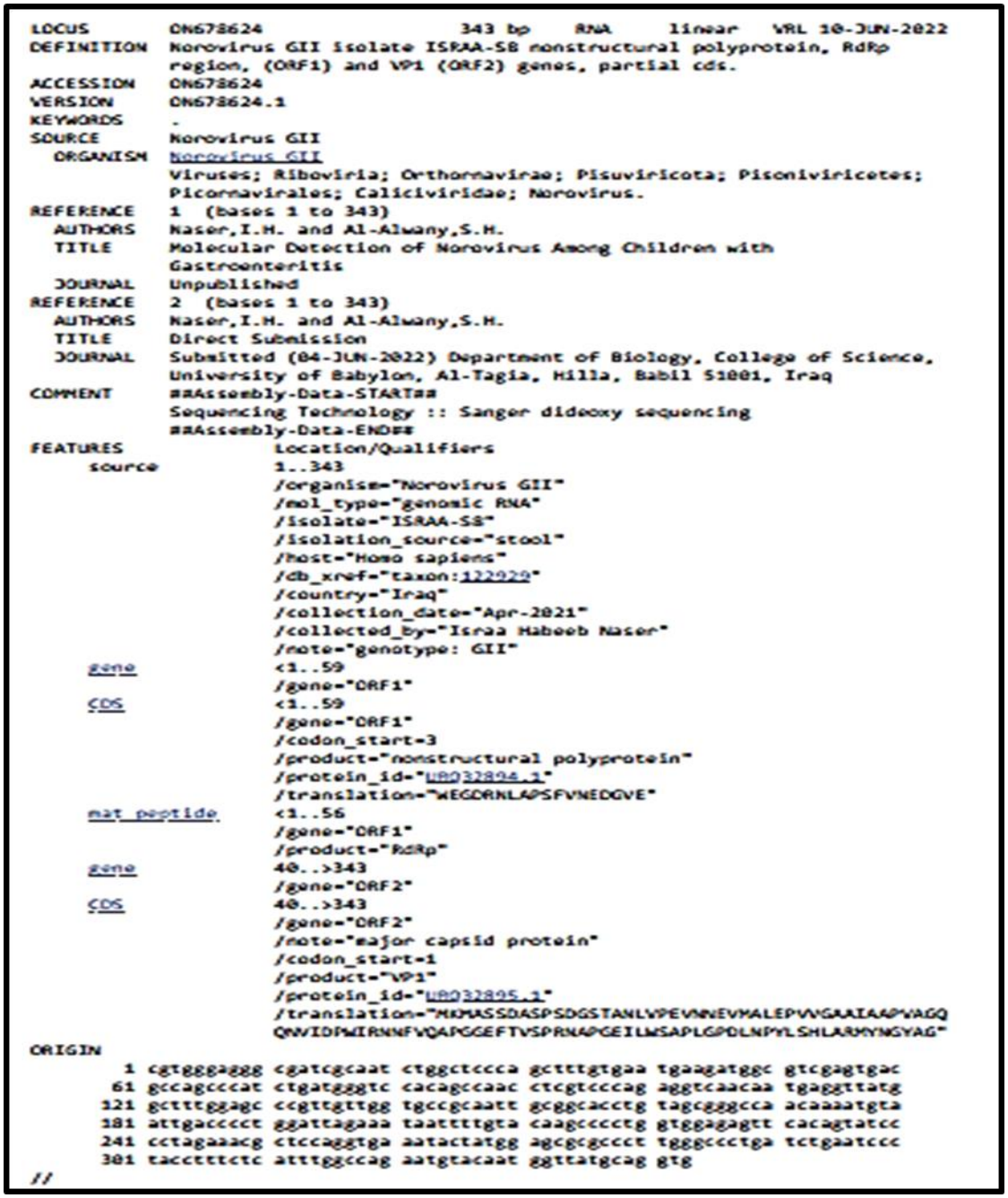
Appendix



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DEFINITION  Norovirus GII isolate ISRAA-SB nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678624
VERSION     ON678624.1
KEYWORDS    .
SOURCE      Norovirus GII
  ORGANISM  Norovirus GII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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      301 taccttttctc atttggccag aatgtacaat ggttatgcag gtg
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Appendix

Sample was submitted in NCBI and the accession number of nucleotide sequences of Norovirus GIII

SUB11565548 Seq1 ON678617
SUB11565548 Seq2 ON678618

```
LOCUS       ON678617                343 bp    RNA     linear   VRL 10-JUN-2022
DEFINITION  Norovirus GIII isolate ISRAA-S3 nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678617
VERSION     ON678617.1
KEYWORDS    .
SOURCE      Norovirus GIII
  ORGANISM  Norovirus GIII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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     CDS             <1..59
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                     /product="RdRp"
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     CDS             40..>343
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                     /codon_start=1
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                     /protein_id="UBQ32881.1"
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                     QNVIDPWIRNNFVQAPGGEFTVSPRNAPGEILWSAPLGPDLNPYLSHLARMYNGYAG"
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      181 attgacccct ggattagaaa taattttgta caagcccctg gtggagagtt cacagtatcc
      241 cctagaaacg ctccaggtga aatactatgg agcgcgccct tgggccctga tctgaatccc
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Appendix

```
LOCUS       ON678618                  343 bp    RNA     linear   VRL 10-JUN-2022
DEFINITION  Norovirus GIII isolate ISRAA-S4 nonstructural polyprotein, RdRp
            region, (ORF1) and VP1 (ORF2) genes, partial cds.
ACCESSION   ON678618
VERSION     ON678618.1
KEYWORDS    .
SOURCE      Norovirus GIII
  ORGANISM  Norovirus GIII
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Picornavirales; Caliciviridae; Norovirus.
REFERENCE   1  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Molecular Detection of Norovirus Among Children with
            Gastroenteritis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 343)
  AUTHORS   Naser,I.H. and Al-Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2022) Department of Biology, College of Science,
            University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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                     /collection_date="Apr-2021"
                     /collected_by="Israa Habeeb Naser"
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     CDS             <1..59
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                     /translation="WEGDRNLAPSFVNEDGVE"
     mat_peptide     <1..56
                     /gene="ORF1"
                     /product="RdRp"
     gene            40..>343
                     /gene="ORF2"
     CDS             40..>343
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                     /product="VP1"
                     /protein_id="UAQ32883.1"
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      181 attgaccccct ggattagaaa taattttgta caagcccctg gtggagagtt cacagtatcc
      241 cctagaaacg ctccaggtga aatactatgg agcgcgccct tgggccctga tctgaatccc
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Appendix

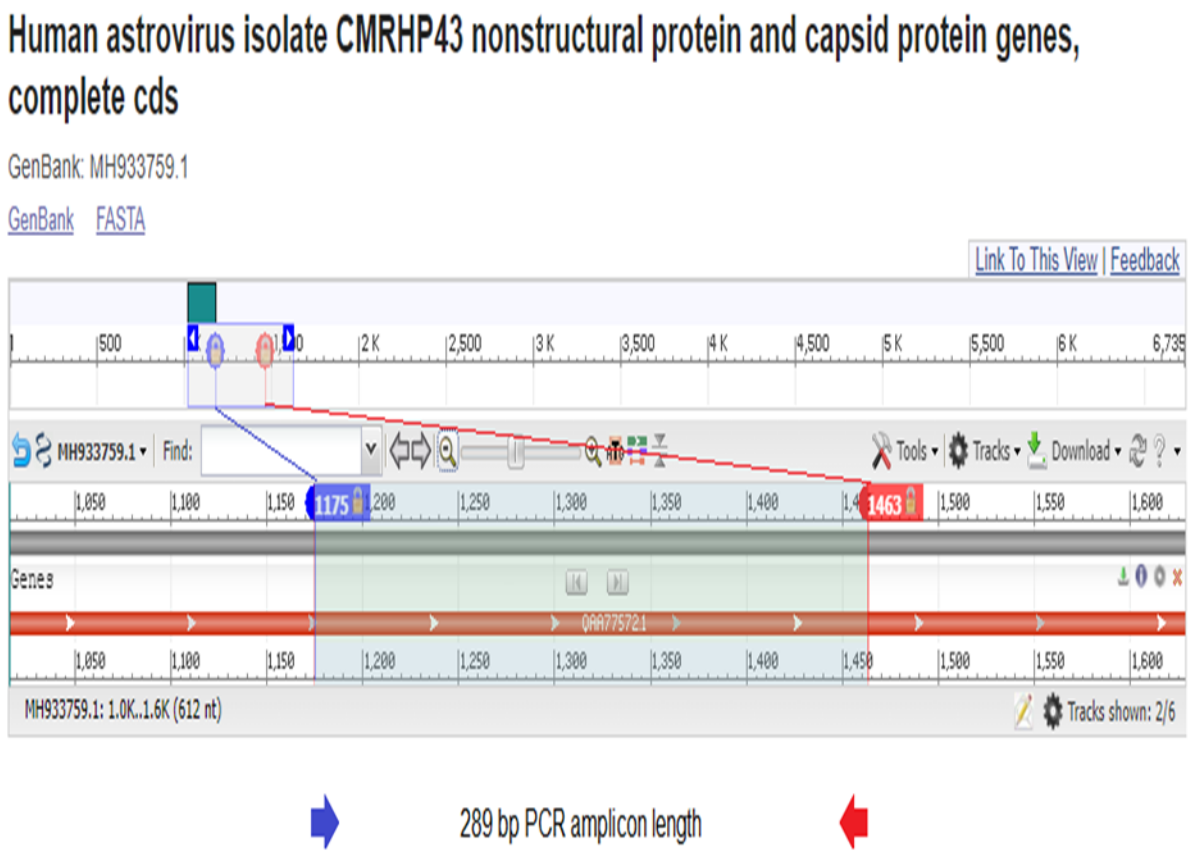

Figure 2 : The exact position of the retrieved 289 bp amplicon partially covered the coding portions of the ORF1a gene within Human astrovirus genomic sequences (GenBank acc. no. MH933759.1). The blue arrow refers to the starting point of this amplicon while the red arrow refers to its endpoint.

The recording six new recording in gene bank NCBI & American bank Unde

ACCESSION NUMBERS :

LC715217 ; LC715218 ; LC715219 ; LC715220 ; LC715221; LC715222

Appendix

```
LOCUS       LC715217                408 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS24 TLR7 gene for toll like receptor 7, partial cds.
ACCESSION   LC715217
VERSION     LC715217.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     TLR7 Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 408)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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Appendix

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LOCUS       LC715217                 488 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS24 TLR7 gene for toll like receptor 7, partial cds.
ACCESSION   LC715217
VERSION     LC715217.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     TLR7 Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 488)
  AUTHORS   Janeel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Janeel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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      121 tcttggtatg ttttagaaca atgatttgtt ctttcttata ctttcaggtg tttccaatgt
      181 ggacactgaa gagacaaatt cttatccttt ttaacataat cctaatttcc aaactccttg
      241 gggctagatg gtttcctaaa actctgccct gtgatgtcac tctggatgtt ccaaagaacc
      301 atgtgatcgt ggactgcaca gacaagcatt tgacagaaat tcctggaggt attaccacga
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Appendix

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LOCUS       LC715218                408 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS25 TLR7 gene for toll like receptor 7, partial cds.
ACCESSION   LC715218
VERSION     LC715218.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     TLR7 Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 408)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 48 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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                     /map="Xp22.2"
                     /cell_type="leukocyte"
                     /tissue_type="blood"
                     /country="Iraq"
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                     /protein_id="BDM75814.1"
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                     HVIVDCTDKHLTEITGGIPTNTTNLTLTINHIPDIS"
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      121 tcttggtatg ttttagaaca atgatttgtt ctttcttata ctttcaggtg tttccaatgt
      181 ggacactgaa gagacaaatt cttatccttt ttaacataat cctaatttcc aaactccttg
      241 gggctagatg gtttcctaaa actctgccct gtgatgtcac tctggatgtt ccaaagaacc
      301 atgtgatcgt ggactgcaca gacaagcatt tgacagaaat tactggaggt attcccacga
      361 acaccacgaa cctcacccтc accattaacc acataccaga catctccc
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Appendix

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LOCUS       LC715219                408 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS29 TLR7 gene for toll like receptor 7, partial cds.
ACCESSION   LC715219
VERSION     LC715219.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     TLR7 Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 408)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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                     /protein_id="BDM75815.1"
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      241 gggctagatg gtttcctaaa actctgccct gtgatgtcac tctggatgtt ccaaagaacc
      301 atgtgatcgt ggactgcaca gacaagcatt tgacagaaat tcctggaggt attcccacga
      361 tcaccacgaa cctcaccctc accattaacc acataccaga catctccc
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Appendix

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LOCUS       LC715228                408 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS48 TLR7 gene for toll like receptor 7, partial cds.
ACCESSION   LC715228
VERSION     LC715228.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     TLR7 Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 408)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 48 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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                     /cell_type="leukocyte"
                     /tissue_type="blood"
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                     /protein_id="BDM75016.1"
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                     HVIVDCTDKHLTDIPGVIPTNTTNLTLTINHIPDIS"
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                     /number=3
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      181 ggacactgaa gagacaaatt cttatccttt ttaacataat cctaatttcc aaactccttg
      241 gggctagatg gtttcctaaa actctgccct gtgatgtcac tctggatgtt ccaaagaacc
      301 atgtgatcgt ggactgcaca gacaagcatt tgacagacat tcctggagtt attcccacga
      361 acaccacgaa cctcaccctc accattaacc acataccaga catctccc
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Appendix

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LOCUS       LC715221                408 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS53 TLR7 gene for toll like receptor 7, partial cds.
ACCESSION   LC715221
VERSION     LC715221.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     TLR7 Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 408)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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                     /protein_id="BDM75817.1"
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                     /number=3
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      241 gggctagatg gtttcctaaa actctgccct gtgatgtcac tctggatgtt ccaaagaacc
      301 atgtgatcgt ggactgcaca gacaagcatt tgacagaaat tactggaggt attcccacga
      361 acaccacgaa cctcaccctc accattaacc acataccaga catctccc
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Appendix

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LOCUS       LC715222                408 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS5S TLR7 gene for toll like receptor 7, partial cds.
ACCESSION   LC715222
VERSION     LC715222.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     TLR7 Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 408)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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      181 ggacactgaa gagacaaatt cttatccttt ttaacataat cctaatttcc aaactccttg
      241 gggctagatg gtttcctaaa actctgccct gtgatgtcac tctggatgtt ccaaagaacc
      301 atgtgatcgt ggactgcaca gacaagcatt tgacagaaat tactggaggt attcccacga
      361 acaccacgaa cctcacccctc accattaacc acataccaga catctccc
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Appendix

The recording four new recording in gene bank NCBI & American bank Unde ACCESSION NUMBERS :

LC715223 ; LC715224 ; LC715225 ; LC715226

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LOCUS       LC715223                441 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS21 IFNL4 pseudogene, partial sequence.
ACCESSION   LC715223
VERSION     LC715223.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     INF Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 441)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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      181 cgagggggctt tgctgggggga gcgcggagtg caattcaacc ctggttcgcg ccttcgggga
      241 gctccctggt tcagtacacg acaggcacga ccgtgcgctg ccagtaccca tccacgtcca
      301 ggaatcccag actgtgcaga ggttaggggc cctggcgagg gggcctagcc gtatgcgata
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Appendix

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LOCUS       LC715224                441 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS22 IFNL4 pseudogene, partial sequence.
ACCESSION   LC715224
VERSION     LC715224.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     INF Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 441)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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      241 gctccctggt tcagtacacg acaggcacga ccgtgcgctg ccagtaccca tccacgtcca
      301 ggaatcccag actgtgcaga ggttaggggc cctggcgagg gggcctagcc gtatgcgata
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      421 ccgcctcagg agggatcctc c
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Appendix

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LOCUS       LC715225                441 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS23 IFNL4 pseudogene, partial sequence.
ACCESSION   LC715225
VERSION     LC715225.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     INF Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 441)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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      181 cgaggggctt tgctgggggg gcgcggagtg caattcaacc ctggttcgcg ccttcggggg
      241 gctccctggt tcagtacacg acaggcacga ccgtgcgctg ccagtaccca tccacgtcca
      301 ggaatcccag actgtgcaga ggttaggggc cctggcgagg gggcctagcc gtatgcgata
      361 agcgccgctt gtcccgcagg aggaagaggc gctgagctgg gggcagcgca actgctcctt
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Appendix

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LOCUS       LC715226                 441 bp    DNA     linear   PRI 22-JUN-2022
DEFINITION  Homo sapiens IS24 IFNL4 pseudogene, partial sequence.
ACCESSION   LC715226
VERSION     LC715226.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Naser,I.H. and Mohammed Al Alwany,S.H.
  TITLE     INF Gene Polymorphism
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 441)
  AUTHORS   Jameel,Z.I. and Mohammed Al Alwany,S.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-JUN-2022) Contact:Zahraa Isam Jameel Babylon
            University, Biotechnology; 40 Street, Babylon, Hilla 51001, Iraq
FEATURES             Location/Qualifiers
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       61 atgtcagcgc ccacaattcc caccacgaga ccccccgcagt ccccgtcgtc agcgcgaacg
      121 caggctcagg gtcaatcaca gaagggagcc ctgccgggag gactcggctc caggtcgggg
      181 cgaggggctt tgctgggggа gcgcggagtg caattcaacc ctggttcgcg ccttcggggа
      241 gctcccctggt tcagtacacg acaggcacga ccgtgcgctg ccagtaccca tccacgtcca
      301 ggaatcccag actgtgcaga ggttaggggc cctggcgagg gggcctagcc gtatgcgata
      361 agcgccgctt gtcccgcagg aggaagaggc gctgagctgg gggcagcgca actgctcctt
      421 ccgccctagg agggatcctc c
//
```

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1.1. Introduction

Gastroenteritis constitutes the main cause of morbidity and mortality among children younger than 5 years and are also a major cause of malnutrition and diminished growth. Diarrheal diseases cause 1.6 million deaths, and constitute about 27% of all deaths among children aged less than 5 years annually. Infantile diarrheas are caused by viruses, parasites, bacteria, and some toxins produced by fungi. Human arboviruses (HAstVs) are a major cause of viral gastroenteritis after rotaviruses and noroviruses in children, adults and elderly (Omosigho *et al*., 2022).

Noroviruses are an important cause of gastroenteritis in humans and animals. Their genome is 7.5 kb in length and organized in three open reading frames (ORF1-3). ORF1 encodes a polyprotein that is enzymatically cleaved by the viral protease into six proteins, including RNA-dependent RNA polymerase (RdRp). ORF2 and ORF3 encode for the major and minor capsid protein (VP1 and VP2), which make up the virus capsid (Chhabra *et al*.,2019).

VP1 is composed of the conserved shell-domain and the protruding (p)-domain, which contains the receptor binding sites that recognize histo-blood group antigens (HBGAs), and the antigenic sites. Based on phylogenetic analysis of VP1 sequences, 10 Geno groups have been identified (GI-GX), which are further divided into 49 genotypes, of which some include several variants. Viruses within Geno groups I, II, IV, VIII, and IX infect humans, with GI and GII being the most commonly detected genotypes. Viruses from the other Geno groups have been found in a broad range of animals including cattle and sheep (GIII), cats and dogs (GIV, GVI, and GVII), rodents (GV), bats (GX), and harbor porpoises (GNA1). Despite this large number of genotypes, viruses within GII.4 are most commonly detected in humans and are responsible for the majority of outbreaks. Norovirus

diversity is additionally increased by recombination events between ORF1 and ORF2, resulting in new strains. New variants, genotypes, and recombinants frequently emerge in the human population, yet their origin is unknown (Villabruna *et al*.,2020).

Astrovirus are leading causes of infectious diarrhea in children, the elderly, immunocompromised individuals, and a wide range of animals. The major clinical symptom is watery diarrhea. The diseases may be fatal in infants and juvenile animals. HAstV-1 was first detected in humans in 1975. Since then, the reported incidence of astrovirus infection in humans and animals has increased. Astrovirus infections are ubiquitous and ~90% of the human population aged >9 years presents with anti-HAstV-1 antibodies (Zhang *et al*., 2022).

Astrovirus (AstVs) is divided into two genera, Mamastrovirus and Avastrovirus, whose members infect mammals and birds, respectively. The genome contains of three open reading frames (ORFs): ORF1a, ORF1b, and ORF2 (Yin *et al*., 2021). High-throughput next-generation sequencing (NGS) methods have recently disclosed that astroviruses may be associated with aseptic encephalitis, meningitis, and meningoencephalomyelitis in humans and animals (Roach and Langlois, 2021).

Innate immunity encompasses an elaborate system of physical and chemical barriers, secreted and membrane proteins, as well as a myriad of effector cells that provide rapid nonspecific protection from an invading pathogen. The interferon (IFN) response pathway is a central component of this system, and begins with detection of pathogen-associated molecular patterns (PAMPs) by a diverse network of host receptors, leading to production of IFNs and generation of an antiviral state in affected cells (Lazear *et al*.,2019). Although IFN-γ was originally discovered

due to its ability to "interfere" with virus infection, most studies of IFN-γ have focused on its immunomodulatory effects in both innate and adaptive immunity, such as enhancement of NK- and T-cell-mediated cytotoxicity, B-cell differentiation, surface antigen expression, and macrophage activation. IFN-γ plays a crucial role in curtailing enteric viral infection (Shan *et al*.,2019).

TLRs are pattern recognition receptors (PRRs) of the innate immune system that have been shown to exhibit tissue or mucosa-specific expression patterns. Each TLR has its own agonist (or set of agonists), known as pathogen associated molecular patterns (PAMP). These TLR agonists, or PAMP, include bacterial ligands, virus-specific ribonucleotide motifs (i.e., dsRNA), and imidazoquinoline compounds and all are currently studied as adjuvants. Toll-like receptor-7(TLR7) is a forms from family of intracellular nucleic acid sensors maintained under strong Purifying selection, which attests of their essential role in host survival to viral infection (Wicherska *et al*.,2021).

TLR7 localizes to the endosomal compartment, where it binds microbial or self-derived single-stranded RNA ligands. In plasmacytoid dendritic cells (pDCs), TLR7engagement elicits Strong Type-I interferon(IFN) production and is critical to the induction no f antiviral immune responses. TLR7is also an essential component of antibody-mediated immunosurveillance against there-activation of endogenous viruses (Patinote *et al*.,2020).

1.2. Aim of the Study

Considering all these points, this research study was designed for detection of norovirus (NoV), astrovirus (AsV) as well as IFN-γ and TLR-7 genes polymorphism in infants and children suffering from gastroenteritis (GE) through achieving the following Objectives:

1- To determine the percentage of Nov. and AsV in stool swabs in case and control.

2- VP1 gene sequencing of Norovirus.

3- ORF1 gene sequencing of Astrovirus.

4- Phylogenetic study of Norovirus and Astrovirus.

5- Estimation of the genetic polymorphism for IFN-γ and TLR-7 genes in case and control by Sequencing.

6- Compared levels serum concentration of IFN-γ and TLR-7 with healthy childhood to patients with GE by ELISA.

7- Detect the association between these of IFN-γ and TLR-7 genes polymorphism in relation to Nov. and AsV genotypes among study population.

2.1. Gastroenteritis in the pediatric

2.1.1. Introduction

Gastroenteritis in the pediatric population is a very common disease that could be lethal. It accounts for around 10% of pediatric deaths, estimating 70 million deaths per year around the globe making it the second cause of death worldwide. The most common cause in infants younger than 24 months old is rotavirus, after 24 months of age, *Shigella* displaces it to second most common (Rivera *et al*.,2022).

2.1.2. Etiology

Gastroenteritis occurs when there is a fecal-oral contact, ingestion of contaminated water or food, and person to person. This is the most common way of acquiring this infection and making it the main cause for norovirus and *Shigella* outbreaks. This disease is associated with bad hygiene and poverty. In the United States, rotavirus and noroviruses (accountable for almost 58% of all cases) are the most common viral agent that causes diarrhea, followed by enteric Adenoviruses, Sapovirus, and Astroviruses (Jeffs *et al*.,2019). The main risk factors for gastroenteritis are environmental, seasonal, and demographics, being you children more susceptible. Other diseases like measles and immunodeficiency's put the patient at a higher risk for a gastrointestinal (GI) infection. Malnutrition is another significant risk factor, like vitamin-A deficiency or zinc deficiency. Most of the episodes are acute diarrheas, lasting less than one week. When diarrhea lasts more than 14 days, it is considered persistent diarrhea and accounts for 3% to 19% of episodes. Around 50% of death cases due to diarrhea (Chen *et al*.,2020).

2.1.3. Epidemiology

Children under 5 years of age are the most affected population, and diarrheal episodes happen more frequently in Asia and Africa, accounting for 80% of annual incidence (Hartman *et al*.,2019; Rivera *et al*.,2022).

2.1.4. Pathophysiology

The pathophysiology depends on the organism causing the disease. Enterotoxin production agents cause noninflammatory diarrhea; the virus frequently destroys the villus surface, and parasites adhere to the mucosa. In the case of inflammatory diarrhea, there are fluid, proteins, and leukocytes entering the interstitial lumen. Viruses like adenovirus can directly invade the microvillus or through calcium-dependent endocytosis, causing the loss in the ability to absorb (Grigsby *et al*.,2019; Rivera *et al*.,2022). On a molecular level, agents affect the interstitial lumen by activating enterocyte intracellular signal transduction, affecting the cytoskeleton of the host cells. This will alter the water and electrolyte fluxes across the enterocytes. For toxic diarrhea, there is an increase of cAMP and inhibition of NaCl abortion. When infiltration occurs, histologic damage will reduce glucose, stimulated Na, and electroneutral NaCl absorption (Hartman *et al*.,2019).

2.1.5. Viral infection and Gastroenteritis

Rotaviruses, noroviruses, adenoviruses, and astroviruses are known to cause viral gastroenteritis. Rotavirus is the most common cause of gastroenteritis in children, and produces similar rates in both the developed and developing world. Viruses cause about 70% of episodes of infectious diarrhea in the pediatric age group. Rotavirus is a less common cause in adults due to acquired immunity. Norovirus is the cause in about 18% of all cases. Generally speaking, viral gastroenteritis accounts for 21–40% of the cases of infectious diarrhea in

developed countries (Barlow *et al*.,2020; Rivera *et al*.,2022). Norovirus is the leading cause of gastroenteritis among adults in America accounting for about 90% of viral gastroenteritis outbreaks. These localized epidemics typically occur when groups of people spend time proximate to each other, such as on cruise ships, in hospitals, or in restaurants. People may remain infectious even after their diarrhea has ended. Norovirus is the cause of about 10% of cases in children (Barlow *et al*.,2020; van Dongen *et al*.,2021).

2.2. Norovirus (NoV)

2.2.1. Historical Review of NoV

The NoV was originally named the "Norwalk agent" after Norwalk, Ohio, in the United States, where an outbreak of acute gastroenteritis occurred among children at Bronson Elementary School in November 1968 (although an outbreak had already been discovered in 1936 in Roskilde, Denmark, where it is commonly known as "Roskilde syge" or "Roskilde illness"). In 1972, electron microscopy on stored human stool samples identified a virus, which was given the name "Norwalk virus"(Tam *et al*.,2012). The cloning and sequencing of the Norwalk virus genome show that these viruses have a genomic organization consistent with viruses belonging to the family Caliciviridae. The name "norovirus" (*Norovirus* for the genus) was approved by the International Committee on Taxonomy of Viruses (ICTV) in 2002. In 2011, however, a press release and a newsletter were published by ICTV, which strongly encouraged the media, national health authorities and the scientific community to use the virus name Norwalk virus, rather than the genus name Norovirus, when referring to outbreaks of the disease. This was also a public response by ICTV to the request from an individual in Japan to rename the Norovirus genus because of the possibility of negative associations for people in Japan and elsewhere who have the family name "Noro". Before this

position of ICTV was made public, ICTV consulted widely with members of the Caliciviridae Study Group and carefully discussed the case (Bucardo *et al*.,2014). In addition, to "Norwalk agent" and "Norwalk virus", the virus has also been called "Norwalk-like virus", "small, round-structured viruses" (SRSVs), Spencer flu and "Snow Mountain virus". Common names of the illness caused by noroviruses still in use include "Roskilde illness", "winter vomiting disease", "winter vomiting bug", "viral gastroenteritis", and "acute nonbacterial gastroenteritis" (Tam *et al*.,2012; Bucardo *et al*.,2014).

2.2.2. Taxonomy and Classification of NoV

Norovirus, previously known as Norwalk-like viruses, belongs to the family, Caliciviridae (Rani *et al*.,2021). Currently, NoV genetic classification is based on complete VP1 amino acid (aa) sequences and partial RNA-dependent RNA polymerase (RdRp) regions. Based on this classification system, NoVs can be categorized into 10 genogroups (GI–GX) (Chhabra *et al*., 2019) . Viruses of GI, GII and GIV infect humans however GII also includes three genotypes (GII.11, GII.18 and GII.19) detected in fecal specimens from swine and GIV viruses include a genotype (GIV.2) that has only been detected in carnivore species (cats and dogs). Since the nomenclature criteria could not be met for distinguishing GII.4 variants, it was decided that the subtyping of GII.4 strains into variants will be based on phylogenetic clustering and that new GII.4 variants will only be recognized after they become epidemic in at least two geographically diverse locations (Chhabra *et al*.,2019; Rani *et al*.,2021).

(unranked): Virus ; ***Realm*:** *Riboviria* ; **Kingdom:** *Orthornavirae* ; **Phylum:** *Pisuviricota* ; **Class:** *Pisoniviricetes* ; **Order:** *Pisoniviricetes* ; **Family:** *Caliciviridae* ; **Genus:** *Norovirus ;* **Species:** ***Norwalk virus***

2.2.3. Morphology and Structure of Norovirus

2.2.3.1 Particles

Viruses in Norovirus are non-enveloped, with icosahedral geometries with icosahedral geometries. Capsid diameters vary widely, from 23 to 40 nm in diameter. The larger capsids (38–40 nm) exhibit T=3 symmetry and are composed of 180 VP1 proteins. Small capsids (23 nm) show T=1 symmetry, and are composed of 60 VP1 proteins. The virus particles demonstrate an amorphous surface structure when visualized using electron microscopy (Snowden *et al.*,2020).

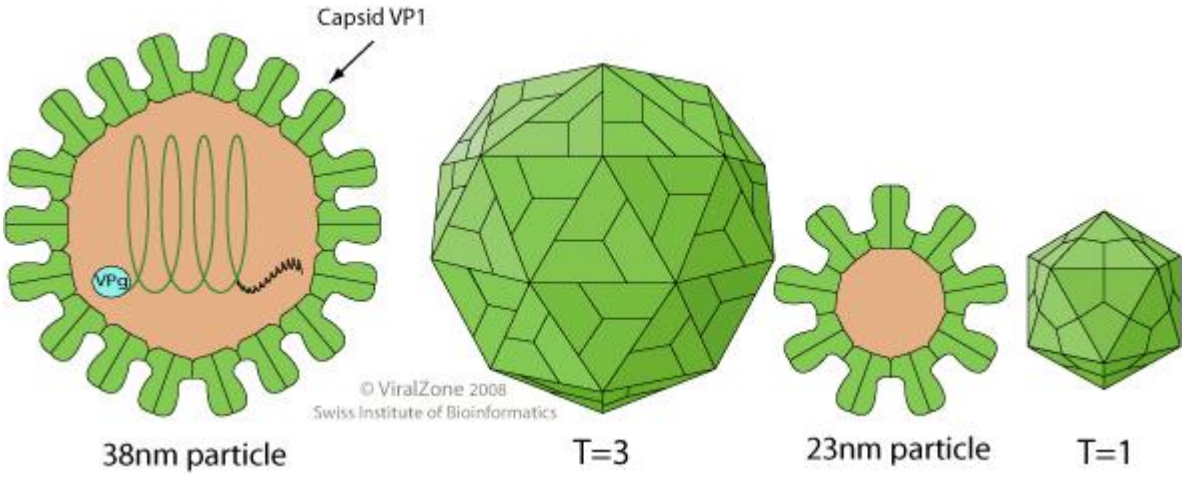

Figure 2.1: Norovirus particle. (Snowden *et al.*,2020).

2.2.3.2. Norovirus Genome Organization

Noroviruses contain a linear, non-segmented, positive-sense RNA genome of approximately 7.5 kilobases, encoding a large polyprotein which is cleaved into six smaller non-structural proteins (NS1/2 to NS7) by the viral 3C-like protease (NS6), a major structural protein (VP1) of about 58~60 kDa and a minor capsid protein (VP2) (Tohma *et al.*, 2021).The most variable region of the viral capsid is the P2 domain, which contains antigen-presenting sites and carbohydrate-receptor binding regions. NoV genomes consist of three separate open reading

frames (ORFs). ORF1 encodes a large polyprotein that is cleaved by the virus-encoded 3C-like proteinase to release six mature nonstructural proteins that are involved in genome replication (Lee *et al.*, 2019). ORF2 encodes VP1, the main capsid protein, which is divided into two domains; shell (S) and protruding (P). The P domain is subdivided into the P1 and P2 sub-domains. The P2 subdomain is highly variable; it possesses major neutralization epitopes and interacts with attachment factors or receptors. NoVs infect humans and animals via the recognition of host-specific receptors. Histo-blood group antigens (HBGAs) are complex fucose-containing glycans that are abundantly distributed on the mucosal epithelia of the intestinal tract, where they most likely serve as host attachment ligands to specific human NoVs (HuNoVs) (Yang *et al.*, 2019). NoVs from dogs and bats appear to attach to HBGAs, similar to HuNoVs . ORF3 encodes the minor capsid protein VP2, which is possibly involved in capsid stabilization and viral entry (Wang *et al.*,2022) Figure (2-2).

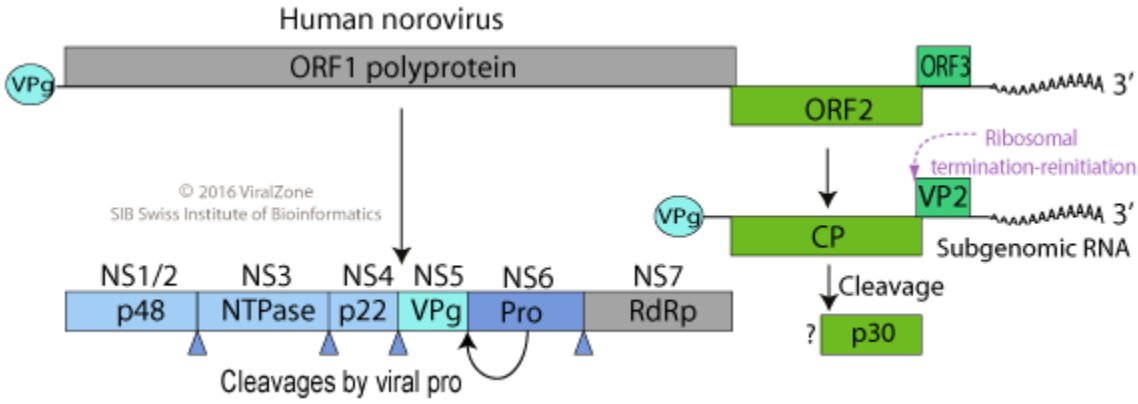

Figure 2.2: Schematic of norovirus genome map (Tohma *et al.*,2021).

2.2.4. Norovirus Tropisms and Life Cycle

I. NoV Tropisms and Entry

Human NoV is an enteric pathogen, the vast majority of reported attempts (and likely more unreported) at cultivating HuNoV have been focusing on human gastrointestinal tract epithelial cells, together with some common human and animal cell lines. Notably, in some cases, the classification of host molecules involved in viral entry can be dependent on viral strain, host cell type, and culture conditions. Interestingly for noroviruses, both cell-associated and non-cell-associated host molecules that augment binding, albeit by diverse mechanisms, have been described. These molecules include the attachment factors histo-blood group antigens (HBGAs), bile acids, sialic acid, and divalent cations (Graziano *et al*.,2019). A three-dimensional culture system was first developed using rotating wall vessels (RWV) to maintain and differentiate INT-407 or Caco-2 cells, and the initial attempts showed increase of HuNoV viral RNA in infected cells. Primary intestinal organoids (or enteroids) culture system for HuNoV, which demonstrated an enterocytic tropism of HuNoV and virus strain-dependent requirement of bile for infection (Ettayebi *et al*., 2016).

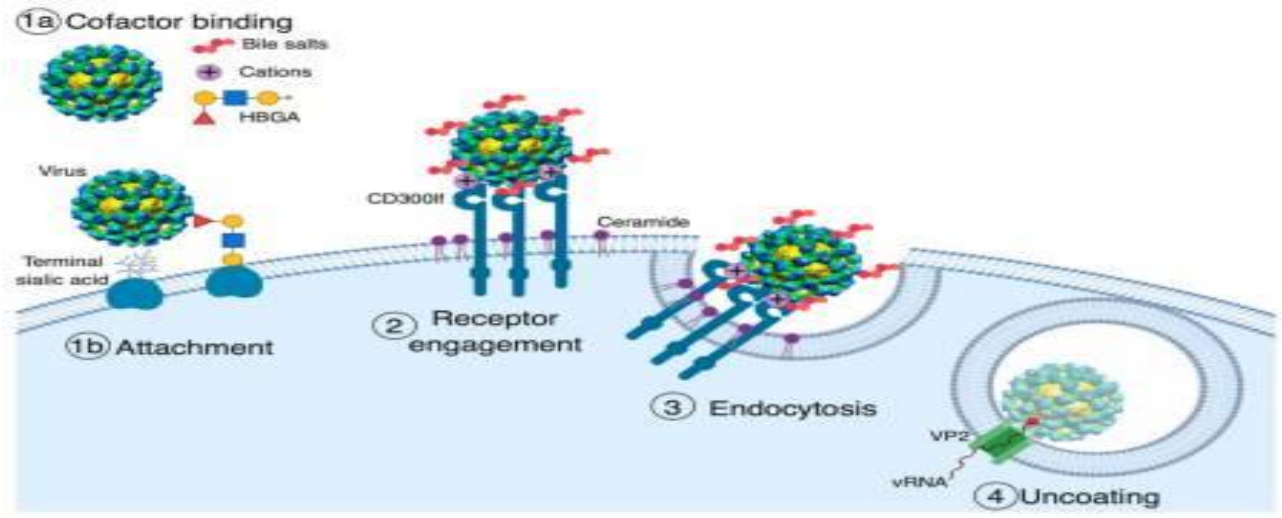

Figure 2.3. Model of norovirus entry. The first and often rate-limiting step of viral entry is viral attachment to the cell surface. Cell-associated host glycans including terminal sialic acid and histo-blood group antigens (HBGAs) can facilitate the entry of mouse (MNoV) and human norovirus (HNoV), respectively (Graziano et al.,2019).

Notably, viral entry is a critical determinant of cell tropism, host range, and pathogenesis. The first and often rate-limiting step of viral entry is virus binding to host cells, which is mediated by both host attachment factors and receptors. Attachment factors are host molecules that concentrate the virus on the cell surface but do not actively induce viral entry. In contrast, viral receptors are essential host molecules that specifically bind the virus particle, induce a conformational change in the virus, and actively promote viral entry. However, it was shown that transfection of VPg-linked Norwalk virus RNA into intestinal epithelial Caco-2 cells resulted in a single round of virus replication. This observation suggests that the restriction of HuNoV infection in epithelial cells is possibly viral entry. Infection of primary macrophages and dendritic cells isolated from peripheral blood showed no evidence of HuNoV replication, nor did the inhibition of innate immune responses with IFN cocktails promoted replication (Jones *et al.*, 2015; Graziano et al.,2019) Figure (2-3).

II. Gene expression of NoV

HuNoV binds to the cell by the interaction of the P2 region present in the *P* domain of the VP1 protein with a still unknown receptor and some host co-receptors such as HBGA .After the interaction of VP1 with the cell receptor(s), internalization of the virus and subsequent disassembly of the viral capsid occur, releasing the RNA into the cell cytoplasm. The exact mechanism by which these two processes take place. Once the viral genome is released, the VPg protein, which is covalently linked at the 5′ end of the viral genome, interacts with the cellular translation initiation factors, such as eIF3, generating a translation complex. This activity has been described in several caliciviruses, suggesting that this function is highly conserved. Subsequently, the major and the minor ribosomal subunits are recruited, resulting in the translation of the non-structural polyprotein which contains the non-structural proteins of the virus (Hosmillo *et al*.,2019).

Once the non-structural polyprotein of HuNoV is generated, the protease is auto-cleaved. Subsequently, the protease co- and post-translationally cleaves the rest of the polyprotein, generating three protein precursors: p48/NTPase, p22/VPg, and Pro/Pol .The enzymatic functions of the p48/NTPase and p22/VPg complexes have not been described. However, the ProPol precursor has two enzymatic functions: protease and polymerase activities. It should be noted that the ProPol complex has a higher enzymatic performance compared to the activity of both proteins separately (Viskovska *et al*.,2019). Subsequently, the three precursors are cleaved by the action of the ProPol complex, which in turn self-cleaves and generates the six individual non-structural proteins. These individual proteins have specific functions in the replicative and the infective cycles. The replication process is carried out by the action of Pol, VPg, and NTPase proteins. The latter has three activities: helicase, NTP hydrolase, and chaperone. Subsequently, the p48 protein will be integrated into this process, which will enhance the Pol activity. Then,

synthesis of the viral genome and the sub-genomes with the VPg protein, attached at their 5′ ends, which recruits the cellular translation initiation factors will occur. Finally, both the genome and the sub-genomes will be mobilized by the NTPase protein chaperone activity (Li *et al*.,2018; Campillay-Véliz *et al*.,2020). A viral polyprotein will be produced from the translation of the viral genome, and VP1 and VP2 proteins will be generated from the sub-genomes. Once the three proteins have been generated, the assembly and the release of the visions take place through a mechanism that remains to be elucidated. While this process occurs, the p48 protein migrates to the endoplasmic reticulum and to the trans-Golgi network where its disassembly is induced, causing interference of the signaling pathways of NFκB, MAPK, and PI3K-Akt, which are important for the host's immune response (Lateef *et al*.,2017). p48 binds to protein A that is associated with VAP-A, which participates in SNARE-mediated vesicular transport and causes a blockage in cell protein transport (Roth and Karst,2016; Lateef *et al*.,2017). Besides that, the effect caused by p48 is reinforced by the action of p22, which synergistically contributes to the disassembly of the trans-Golgi network . P22 also blocks the traffic of COPII-coated vesicles since it has a motif that closely resembles an export signal from the endoplasmic reticulum (ER). p22 and NTPase favor the pro-apoptotic activity of the cell, facilitating the release of HuNoV virions from the host cell (Thorne and Goodfellow ,2014; Campillay-Véliz *et al*.,2020).

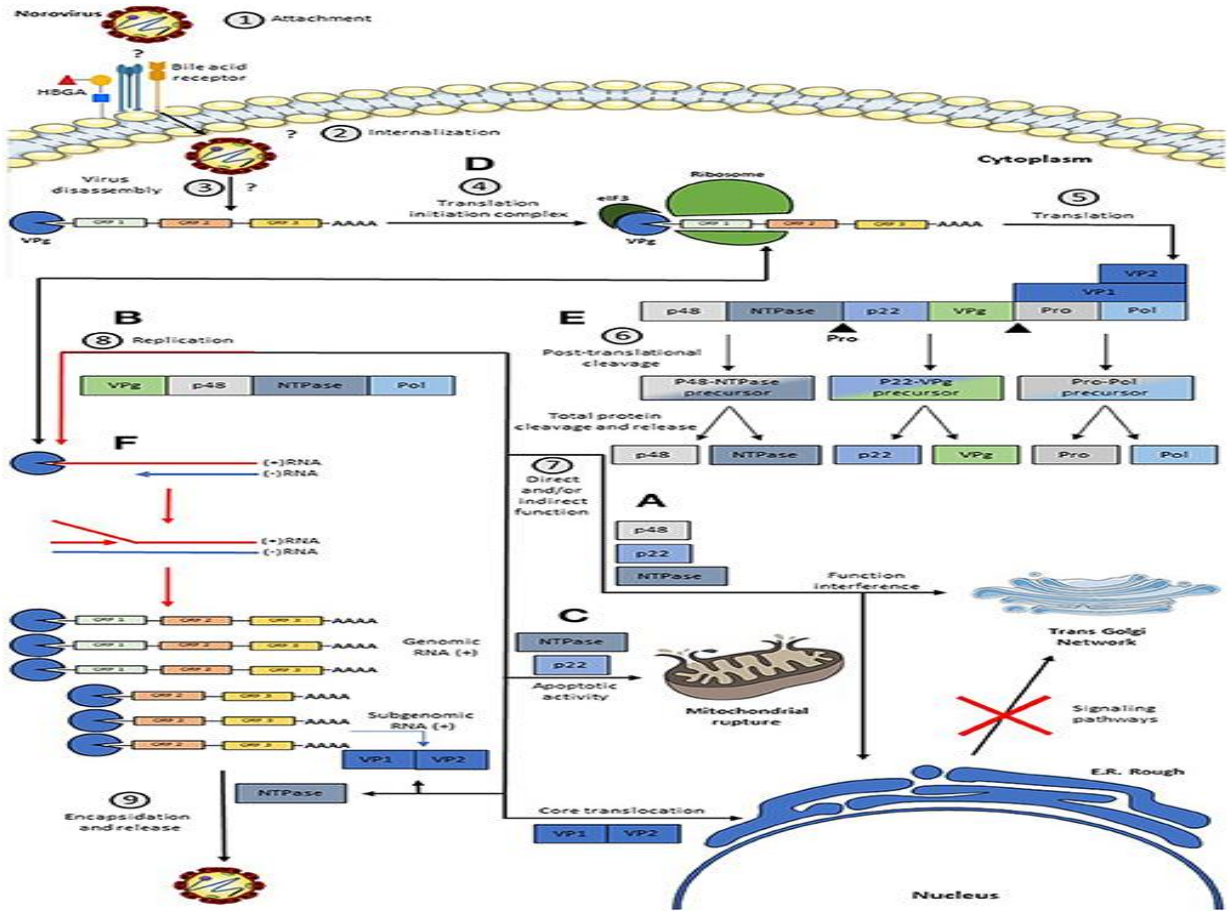

Figure 2.4. **Model of a Replicative HuNoV Cycle and Function of its Proteins (Campillay-Véliz *et al*.,2020).**

2.2.5. Pathogenesis of NoV

Noroviruses are highly transmissible since only 10 visions (ID50 = 10 visions) per individual is required to infect half proportion of those individuals. The virus is highly resistant to extremely low and/or high temperatures, high sugar concentrations, acidic environments, exposure to chlorine, antiseptic solutions and alcohol. The virus has an incubation period of about 1-2 days with symptoms lasting for about 1-3 days. It could remain contagious for up to 3 weeks. Histo-blood group antigens (HBGAs) are polymorphic receptors or cofactors of norovirus. Different Geno clusters bind various HBGAs: Geno group I viruses bind A and O antigens; Geno group II viruses mostly bind A and B antigens. Great

diversity of norovirus strains and the lack of cross-strain or long-term immunity are the causes of recurrent infections. The virus primarily damages the microvilli of the cells of the small intestine. It affects the motility of stomach leading to delayed gastric emptying and eventually nausea and vomiting. The virus is responsible for the enzymatic disorder, leak flux, anion secretion and fat malabsorption at the brush border leading to diarrhea in infected individuals. Colon is intact in this infection so hematochezia is rare (Teunis *et al*.,2020).

2.2.6. NoV Phylogenetic

Phylogenetically, NoV can be segregated into 10 genogroups and further divided into genotypes based on amino acid sequence diversity in the VP1 gene. GII is the largest of the known genogroups, consisting of 26 genotypes, including 23 human NoV genotypes that are responsible for most epidemics, and three porcine NoV genotypes (GII.11/18/19) (Chhabra *et al*.,2020). As the diversity of NoV increased through recombination, dual typing was proposed for NoV classification. Partial nucleotide sequences of the RNA-dependent RNA polymerase (RdRp) region of ORF1 are used for NoV P-type classification independently from genotype. A total of 37 P-types have now been identified for in GII viruses (Zhang *et al*.,2022). Entire genome sequencing followed by phylogenetic analysis of the GII.3 genotype strains indicated that they are new recombinant viruses, because the genome encoding ORF1 is derived from a GII.12 genotype strain, whereas that encoding ORF2-3 is from a GII.3 genotype strain. The putative recombination breakpoints with the highest statistical significance were located around the border of $3D^{pol}$ and ORF2. The change in the prevalent strain of NoV seems to be linked to the emergence of new forms of recombinant viruses. These findings suggested that the swapping of the structural and non-structural proteins of NoV is a common

mechanism by which new epidemic variants are generated in nature (Boonchan *et al.*,2018).

2.2.7. Modes of Transmission of NoV

Norovirus can easily contaminate food and water because it only takes a very small amount of virus particles to make you sick. Food and water can get contaminated with norovirus in many ways, including when (Fraenkel *et al.*,2021):

- An infected person touches food with their bare hands that have feces (poop) or vomit particles on them
- Food is placed on a counter or surface that has feces or vomit particles on it
- Tiny drops of vomit from an infected person spray through the air and land on the food
- The food is grown or harvested with contaminated water, such as oysters harvested from contaminated water, or fruit and vegetables irrigated with contaminated water in the field

2.2.8. Pathophysiology

When a person becomes infected with norovirus, the virus replicates within the small intestine. The principal symptom is acute gastroenteritis, characterized by nausea, forceful vomiting, watery diarrhea, and abdominal pain, that develops between 12 and 48 hours after exposure, and lasts for 24–72 hours. Sometimes there is loss of taste, general lethargy, weakness, muscle aches, headache, cough, and/or low-grade fever. The disease is usually self-limiting. Severe illness is rare; although people are frequently treated at the emergency ward, they are rarely admitted to the hospital. The number of deaths from norovirus in the United States is estimated to be around 570–800 each year, with most of these occurring in the very young, the elderly, and persons with weakened immune systems. Symptoms

may become life-threatening in these groups if dehydration or electrolyte imbalance is ignored or not treated (Hall *et al*., 2013).

2.2.9. Immune responses against NoV

The immunological response to HuNoV in humans is complex, considering the large variability of circulating genotypes and the pre-existing exposures to these viruses. Short-term immunity has been evidenced against HuNoV. Nevertheless, the exact molecular mechanism behind these responses remains to be elucidated (Campillay-Véliz *et al*.,2020).

2.2.9.1. Innate Immune Response

Two main cell pathways of the innate immune system are raised against RNA viruses such as HuNoV, the type I and III interferon (IFN) systems controlling viral replication.Particularly, RNA viruses are mainly detected by the pattern recognition receptors (PRRs), which include the TLR and the RIG-I like receptor (RLR) family members (Lee and Baldridge ,2017). Specifically, IFN-α has been reported to play a role in controlling HuNoV replication in Huh-7 cells and BHK21 cells, suggesting that IFN production may be one of the host antiviral mechanisms in controlling HuNoV infection. Furthermore, the following evidences suggest that the type I and III IFN systems can play an important role in controlling HuNoV infections: (a) high IFN-α cytokine production has been detected in the intestinal contents and the serum of pigs inoculated with a GII.4 strain ; (b) the replication of MNV-1 is sensitive to the type I and III IFN systems both in vivo and in vitro ; (c) NV replication in Huh-7 cells, after transfection with genomic NV RNA, is inhibited when cells are pre-treated with the supernatant from cells that have been transfected with the IFN inductor, poly inosinic/polycytidylic acid (poly I:C), to induce IFNα/β production ; and (d) bile acids allow the replication of GI.1, GII.3, and GII.17 HuNoV strains and enhance it in GII.4 variants in human intestinal

asteroids (Ettayebi *et al*., 2016). Interestingly, bile acids have previously been reported to down-regulate type I and III IFN pathways and enhance the growth of the porcine enteric calicivirus (Lee and Baldridge ,2017; Campillay-Véliz *et al*.,2020).

2.2.9.2. Humoral Immune Response

Rapidly evolving RNA viruses, such as GII.4 HuNoV, elicit complex humoral responses associated with previous exposure. In general, it is not clearly defined whether serum antibody levels against HuNoV are correlated with protection against future infections by these viruses. Several studies in the literature indicate that high levels of serum antibodies against NV in adults correlate with protection against future infections by this virus (GI.1 genotype) (Campillay-Véliz *et al*.,2020).Likewise, the results of other studies, conducted in adult volunteers, revealed that the presence of high serum or fecal titers of specific antibodies for NV before infection decreased the probability of becoming infected by this virus, compared to the case of volunteers with low titers of preexisting antibodies. Additionally, in children, high serum antibody levels appear to be correlated with protection, possibly due to a short-term immunity and recent exposures to these viruses. HuNoV may also induce IgA antibody production, which is known to play a role in short-term immune protection against viruses (Lee and Baldridge ,2017). Indeed, some volunteers who became ill after a NV challenge showed partial immunity against the disease in a re-exposure after 6–14 weeks. However, this partial immune protection was completely lost after 2–3 years. Nevertheless, long-term protective immune response can be evidenced from numerous epidemiological reports from different parts of the world that show periods of "high norovirus activity," which correlate with the apparition of new GII.4 strains (Ettayebi *et al*., 2016). This phenomenon is followed by a period of years with

reduced numbers of HuNoV outbreaks, which suggests a generation of herd immunity in the population that protects against GII.4 HuNoV infections. However, it is important to keep in mind that individuals can become susceptible again to the disease if a new strain of GII.4 appears. Specifically, it was observed that only 20–30% of people who had pre-existing anti-NV antibodies had preexisting blocking antibody titers. Moreover, between 90 and 100% of those volunteers, after the challenge with NV, generated blocking antibody titers (Dai *et al*.,2017).

2.2.9.3. Cell-mediated Immunity

The role that T cells play in controlling NoV infection is complex and not well characterized. Human NoV infection or vaccination elicits a primarily CD4+ Th1 response, leading to increased secretion of IFN-gamma and IL-2 (Ettayebi *et al*., 2016). One study using human-derived PBMCs found that T cell responses were more cross-reactive between GII strains with higher antigenic relatedness, while another study found that T cell responses toward alternate GI strains were more robust than the immunizing GI strain in some individuals. Additional studies using a wider array of genotypes are needed to further characterize T cell responses and their relationships in controlling human infection (Chhabra *et al*.,2019).

2.2.9.4. Norovirus with TLR-7

Pathogen recognition receptors (PRRs) are divided into three groups, which include the toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and nucleotide-binding domain, leucine-rich repeat-containing receptors (NLRs). Members of each receptor family are involved in nucleic acid detection and are expressed either within the plasma membrane or within the endosome membranes. Norovirus infections are a significant health burden globally, accounting for hundreds of millions of cases of acute gastroenteritis every

year (Enosi *et al*.,2018). Several important examples of these factors include nuclear factor-kappa B (NF-B), activator protein-1 (AP-1) and interferon (IFN)-regulatory factors (IRFs) IRF3 and IRF7, which can alter the expression of genes involved in antiviral defense (Kawasaki and Kawai,2014). The benefit of using TLR7 agonists is that they pose a low risk for the development of antiviral resistance (Pawlotsky, 2011). These agonists bind to TLR7, causing homo- or hetero dimerization and subsequent recruitment of the adaptor protein myeloid differentiation primary response protein 88 (MyD88) to Toll and interleukin-1 receptor (TIR) domains of TIR domain-containing proteins (Lester *et al*., 2014).

2.9.2.5. Norovirus and Interferon Gamma (IFN-γ)

Numerous cellular factors and pathways have been identified as playing critical roles in the intracellular life of noroviruses (Walker and Baldridge, 2019). In addition, the key role of the innate immune response in controlling norovirus infection is IFN that activated by HuNoV and degree components of the pathway control norovirus infections (Alwin and Karst,2021).The IFN response pathway is a central component of this system, and begins with detection of pathogen-associated molecular patterns (PAMPs) by a diverse network of host receptors, leading to production of IFNs and generation of an antiviral state in affected cells (Lazear *et al*.,2019). IFN gamma induce expression of IFN-stimulated genes (ISGs) that facilitate the resistance of host cells to viruses, activate immune cells recruited to the sites of infection and upregulate factors required for activation of adaptive immunity, all of which makes them critical in the control of viral infections (Schoggins,2018), For this reason, and on account of the co-evolution of hosts and pathogens, any virus that is able to infect a host is also likely to have evolved mechanisms of counteracting IFN responses (Fensterl *et al*.,2015).

2.9.10. Laboratory Diagnostic of Norovirus

Diagnostic methods for norovirus focus on detecting viral RNA (genetic material) or viral antigen. Diagnostic tests are available at all public health laboratories and many clinical laboratories, and most use reverse transcription- real-time polymerase chain reaction (RT-qPCR) assays to detect norovirus (Cannon *et al*.,2017; Barclay *et al*.,2019).

I.RT-qPCR Assays

TaqMan-based RT-qPCR assays detect the RNA of the virus. They can be used to test stool, vomitus, food, water, and environmental specimens for norovirus. RT-qPCR assays are the preferred method to detect norovirus because they are very sensitive and specific. They can detect as few as 10 to 100 norovirus copies. They use different oligonucleotide primer sets to detect genogroup I, genogroup II, genogroup VIII and GIX noroviruses. RT-qPCR assays can also provide estimates of viral load.

II.Multiplex Gastrointestinal Platforms

Recently, several commercial platforms for detection of multiple gastrointestinal pathogens have become available. They include genogroup I and genogroup II norovirus. The sensitivity of these assays for norovirus is in the same range as RT-qPCR.

III. Enzyme Immunoassays

Rapid commercial enzyme immunoassays (EIAs) that detect norovirus antigen in stool samples are also available. However, these kits have poor sensitivity (50 to 75%), and are, in general, not recommended for testing single samples from

sporadic cases of gastroenteritis. These assays can be used for preliminary identification of norovirus when testing multiple specimens during outbreaks. However, samples that test negative should be confirmed by a second technique, such as RT-qPCR. Thus, EIA kits should not replace RT-qPCR during outbreak investigations.

IV. Genotyping

Genetic characterization of noroviruses detected in stool and environmental samples can be very useful in epidemiologic investigations by linking cases, suggesting a common source, or identifying new emerging virus strains. Norovirus can be genotyped by sequence analysis of a RT-PCR product amplified from a partial region of both the polymerase gene (region B) and capsid gene (region C) in a single reaction for either genogroup I or genogroup II viruses.All laboratories participating in CaliciNet, a national laboratory surveillance network for norovirus outbreaks coordinated by CDC, use dual typing for norovirus. The sequences obtained are compared to CaliciNet reference sequences for typing. An example of dual typing nomenclature is the GII.4 Sydney[P16] norovirus strain that has caused the majority of all norovirus outbreaks in the United States in recent years.

2.3. Astrovirus

2.3.1. Historical Preview:

Astrovirus are a type of virus that was first discovered in 1975 using electron microscopes following an outbreak of diarrhea in humans. Astrovirus infecting other species, particularly mammalian and avian, were identified and classified into the genera Mamastrovirus and Avastrovirus. Mamastrovirus (MAstV) representing genotype species affecting mammalian species and Avastrovirus containing those viruses found in avian species. Through next-generation

sequencing, many new astroviruses infecting different species, including humans, have been described, and the Astroviridae family shows a high diversity and zoonotic potential. Three divergent groups of HAstVs are recognized: the classic (MAstV 1), HAstV-MLB (MAstV 6), and HAstV-VA/HMO (MAstV 8 and MAstV 9) groups (Kauer *et al.*,2019). In animals, Astroviruses also cause infection of the gastrointestinal tract but may also result in encephalitis (humans and cattle), hepatitis (avian) and nephritis (avian). Classic HAstVs contain 8 serotypes and account for 2 to 9% of all acute nonbacterial gastroenteritis in children worldwide. Infections are usually self-limiting but can also spread systemically and cause severe infections in immunocompromised patients. The other groups have also been identified in children with gastroenteritis, but extraintestinal pathologies have been suggested for them as well. Classic HAstVs may be grown in cells, allowing the study of their cell cycle, which is similar to that of caliciviruses (Maclachlan *et al.*,2017).

2.3.2. Taxonomy and Classification of Astrovirus

Astroviruses are classified into two genera: Mamastrovirus (MAstV), historically considered mammalian viruses, and Avastrovirus (AAstV), historically considered avian viruses (figure 2-2). Since 2008 and the advent of pan-astrovirus degenerate primers (Chu *et al*., 2008) and sequence based phylogenetics, astroviruses have been classified by their ORF2 full length amino acid sequence into three species of AAstV and 19 species of MAstV (Donato and Vijaykrishna, 2017).The International Committee on Taxonomy of Viruses (ICTV) established Astroviridae as a viral family in 1995. There have been over 50 astroviruses reported, although the ICTV officially recognizes 22 species. The genus *Avastrovirus* comprises three species: Chicken astrovirus (Avian nephritis virus types 1 - 3), Duck astrovirus (Duck astrovirus C-NGB), and Turkey

astrovirus (Turkey astrovirus 1). The genus *Mamastrovirus* includes Bovine astroviruses 1 and 2, Human astrovirus (types 1-8), Feline astrovirus 1, Porcine astrovirus 1, Mink astrovirus 1 and Ovine astrovirus 1 Figure (2-5) (ICTV ,2020; Virginia *et al*., 2021).

(unranked): Virus ; ***Realm*:** *Riboviria* ; **Kingdom:** *Orthornavirae* ; **Phylum:** *Pisuviricota* ; **Class:** *Stelpaviricetes* ; **Order:** *Stellavirales* ; **Family:** *Astroviridae* ; **Genus**: *Avastrovirus* ; Chicken astrovirus ;Duck astrovirus; Turkey astrovirus . **Genus**: *Mamastrovirus* : Bovine astrovirus 1 & 2 ;Feline astrovirus 1; Porcine astrovirus 1; Human astrovirus AstV-MLB ; Human astrovirus HAstV ;Human astrovirus HMOAstV-A ; Human astrovirus HMOAstV-B; Human astrovirus HMOAstV-C.

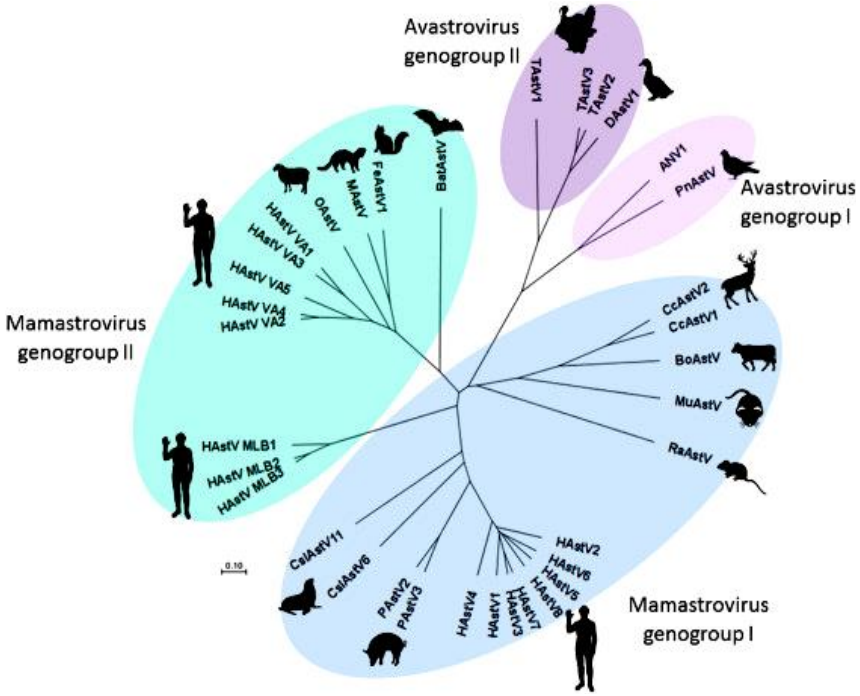

Figure 2.5: Phylogenetic classification of Astroviridae. Tree generated using capsid protein amino acid sequences.Based on Ninth Report of the International Committee on Taxonomy of Viruses, 2012 (Virginia *et al*., 2021).

2.3.3. Morphology and Structure of Astrovirus

2.3.3.1. Particles

Non-enveloped, spherical, capsid of about 35 nm with T=3 icosahedral symmetry. Surface projections are small and surface appears rough, spikes protruding from the 30 vertices. The capsid precursor protein (180 copies per particle) undergoes C-terminal cleavages by host caspases during virus maturation (Arias *et al*.,2017). Particles are assembled from the VP90 precursor protein (approximately 90 kDa), which is further processed by cellular caspases to generate the VP70 protein, losing an acidic C-terminal domain. VP34 is derived from the highly conserved N-terminal region of the polyprotein and builds up the capsid shell, while VP27/29 and VP25/26 are both derived from the variable C-terminal domain with a different form the dimeric spikes Figure (2.6**)** (Zhao *et al*.,2020).

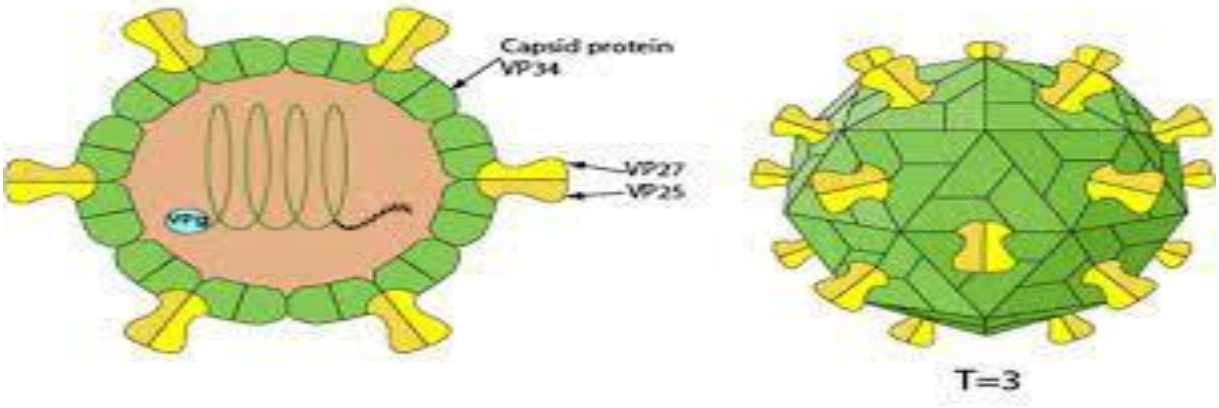

Figure2.6. particle structures of the astrovirus (Zhao *et al*.,2020).

2.3.3.2. Astrovirus Genome Organization

Astroviruses have +ssRNA genomes that are approximately 6–8 kb in length. The genome includes 5′ and 3′ untranslated regions and three open reading frames

(ORFs). The genome contains three overlapping open reading frames (ORF1a, ORF1b, and ORF2). The nonstructural proteins are translated from the genomic RNA as two large polyproteins, nsP1a and nsP1a/1b, through a translational ribosomal frameshifting. ORF1a and ORF1b encode the viral protease and polymerase respectively. ORF2 is expressed from a subgenomic RNA and encodes the VP90 capsid precursor protein. (Figure 2-7) (Cortez *et al*., 2017; Nicholas *et al*.,2019).

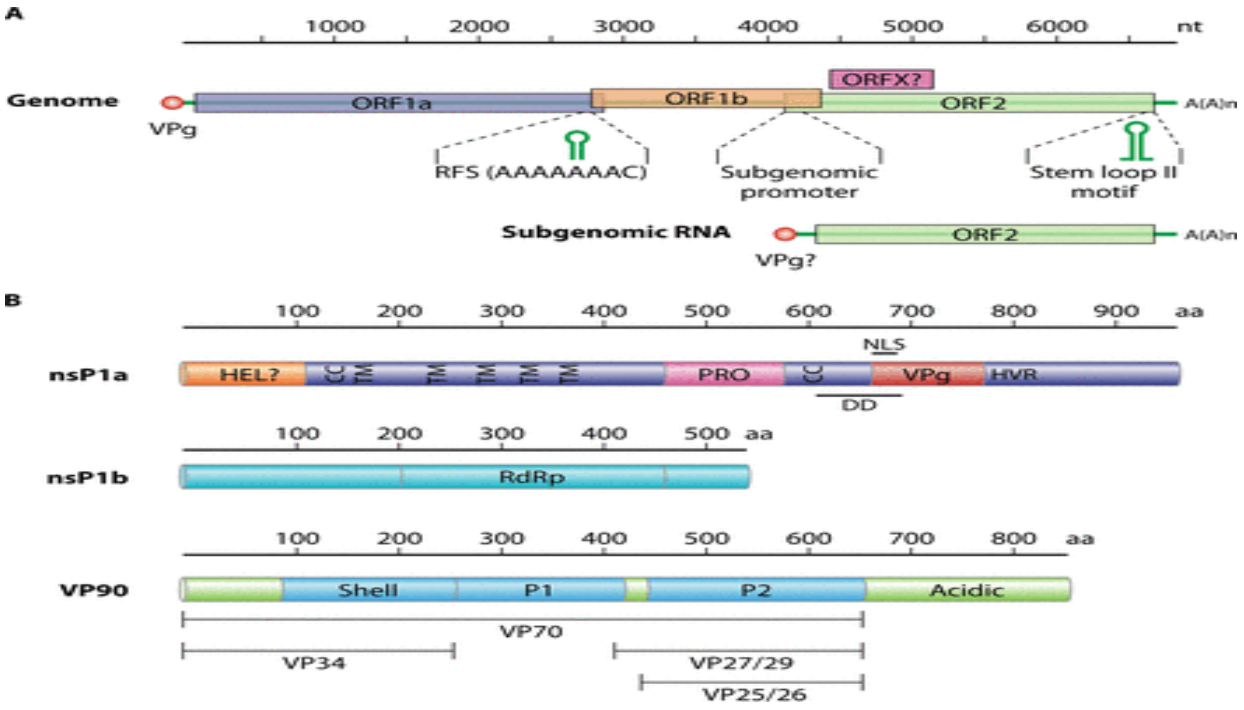

Figure 2.7: Astrovirus genomic architecture. Individual protein coding regions with known identity are designated by inset boxes. In ORF2, the darker regions indicate the hypervariable region of the capsid protein. The frameshift signal between ORF1a and ORF1b consists of a slippery sequence followed by a hairpin. There is a highly conserved hairpin at the 3′ end of the genome (Nicholas *et al*.,2019).

2.3.4. Astrovirus Tropisms and Life Cycle

Astrovirus infection begins by binding to an unidentified receptor(s) on epithelial cells in the intestine after fecal-oral transmission and enters cells via

clathrin-mediated endocytosis (Donato *et al*.,2017). After acidification of the endosome, endosome rupture, and viral uncoating, the three main structural proteins, VP25,VP27 and VP34 help the viral to attachment ,and nonstructural polyprotein translated from the VPg-linked genomic RNA. Nonstructural polyprotein nsP1a1b is expressed thanks to the RFS that exists between ORF1a and ORF1b. Cleavage of these polyproteins results in the individual nonstructural proteins required for genome replication (Marvin,2017). Negative-strand RNA is produced from the genomic strand, and transcription of the negative-strand yields the genomic and subgenomic RNA . The structural proteins encoded in the third ORF (ORF2) are expressed from the subgenomic RNA . New astrovirus particles have been observed on double membranes likely serving as the site for replication and assembly. After assembly, the progeny virions exit the cell through a non-lytic mechanism promoted by caspase activation Figure (2-8) (Marvin,2017; Janowski,2021) .

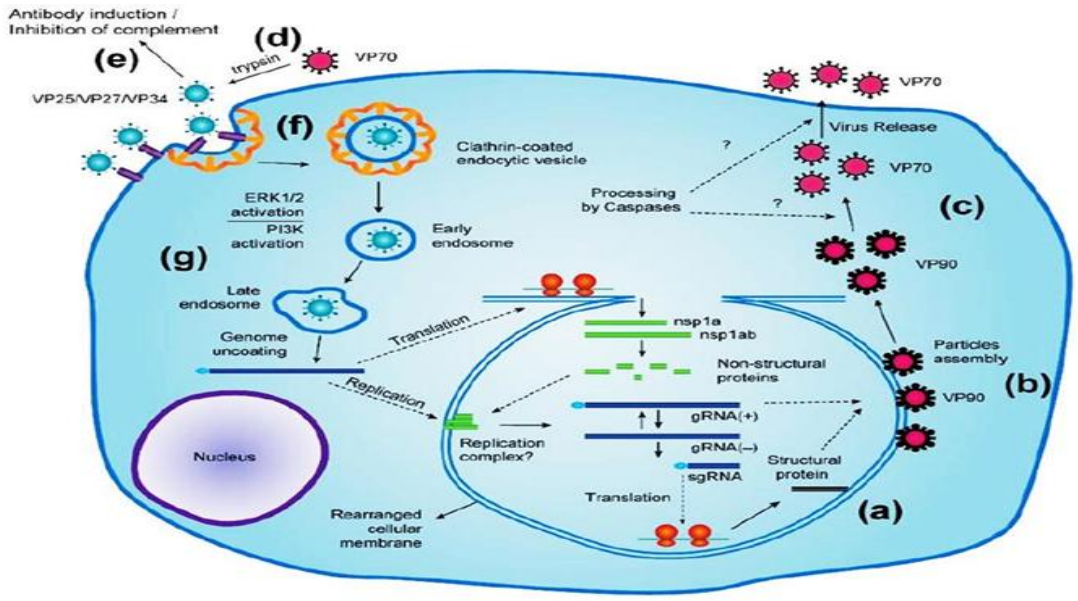

Figure 2.8: Astrovirus life cycle (Arias and DuBois, 2017).

2.3.5. Pathogenesis of Astrovirus

Despite the high prevalence of astrovirus and the advances made in the identification of novel genotypes, there is still little known about HAstV pathogenesis—especially among the different HAstV genotypes. Astroviruses cause gastroenteritis by causing destruction of the intestinal epithelium, leading to the inhibition of usual absorption mechanism, loss of secretory functions, and decrease in epithelial permeability in the intestines. Inflammatory responses were seen to not affect astrovirus pathogenesis (Johnson *et al*.,2017). Previous studies demonstrated that HAstVs increase epithelial cell permeability by disrupting cellular tight junctional complexes. Since the intestinal tract depends on tight junctions to separate the lumen from the basal lamina, the loss of integrity increases ion, solute, and water trafficking across the compartments, reducing the ability of the intestine to reabsorb water and nutrients, leading to diarrhea. Additionally, extra-gastrointestinal astrovirus-associated disease has been reported in animals and humans, which could result from the increased intestinal permeability (Vu *et al*.,2016; Johnson *et al*.,2017; Tsague *et al*.,2020).

2.3.6. Modes of Transmission of Astrovirus

Person-to-person spread by the faecal–oral route is thought to be the most common route of transmission. Food, water, and fomites have been implicated in astrovirus disease, which suggests that astroviruses persist in the environment; however, there are no confirmed cases of waterborne transmission. Astroviruses have been found in sewage, wastewater and surface water (over 50% of samples were positive for astrovirus). AstVs readily transmit between coprophagic animals such as rodents. Transmission between commercially farmed animals is extremely common and compounded by persistent viral shedding from infected animals long after the date of initial infection. This persistent viral shedding also increases the

risk of spill over into farm animals of different species. In wild animals, feces serve many roles, including marking territories, attracting mates, hunting prey, and avoiding predators. These various interactions facilitate intra-species transmission as well as open the door for cross-species exposure (Roach *et al*.,2021).

2.3.7. Immune Responses against Astrovirus

The innate immune response provides signals to recruit the adaptive immune response, which controls viral infection at later times during an infection. This response is pathogen specific and has a memory component that induces a more rapid and robust response following a second infection with the same pathogen. The adaptive immune response contains two arms: the humoral, or antibody-mediated/B cell response, and the cell-mediated response, which involves antigen-specific cytotoxic T cells. Although the findings between the current turkey model and the newly emerging mouse model yielded conflicting results, the reports showing the biphasic age distribution as well as immunocompromised individuals have formed a general conclusion that the adaptive immune system is a major component in controlling astrovirus disease (Marvin,2017).

Antibodies that recognize the spike domains on the human astrovirus spike, the domain of the capsid involved in astrovirus binding , and neutralized virus activity have been identified (York *et al*.,2016) However, the development of anti- HAstV therapies has been hampered by the gap in knowledge of neutralizing antibodies epitopes on HAstV surfaces (Bogdanoff *et al*.,2017).Mapped the neutralizing epitopes on the HAstV-2 with a neutralizing monoclonal antibody to the spike domain, which prevented spike binding to Caco2 cells (York *et al*.,2016). The solving of the crystal structures of HAstV-1 and HAstV-8 capsids and spike domains, and the HAstV-2 and TAstV-2 spike domains can advance our understanding of anti-Astrovirus antibody binding. These structures are crucial for

future studies to develop vaccines and antibody therapy prevention and treatment of astrovirus disease (Toh *et al*.,2016).The cell mediated immunity shows in Many number of reports on astrovirus detection in extra-intestinal tissues increase, how the immune systems limits spread of astrovirus to extra-intestinal tissue , some of them shows the important role for T cell-mediated control of astrovirus infection. since there are astrovirus-specific CD4+ and CD8+ T cells from astrovirus-stimulated biopsies taken from the duodena of patients that had histologically normal intestines (Dong *et al*.,2011). In addition, the chronic rotavirus and astrovirus infection in two children with T cell immunodeficiency. Considering the fact that CD4+ T cells are essential in B cell maturation and antibody specificity; it is reasonable to suspect that T cells play a role in the immune response to astrovirus infection (DuBois *et al*.,2013).

2.3.7.1 Astrovirus and TLR7

The TLR7 recognizes the single-stranded RNA viruses, vesicular stomatitis virus, Astrovirus and influenza virus. The recognition of these viruses by plasmacytoid dendritic cells and B cells through TLR7 results in their activation of costimulatory molecules and production of cytokine. The AstV infection play important role in activation of pattern recognition receptors such as (RIG-I, and TLR7) and key adaptor molecules (Interferon alpha) in the spleen and kidney, Moreover, high expression levels of interleukin (IL)-1b and IL-8 These investigations indicated that AstV infection activated host innate immune response (Wu *et al*.,2021).

2.3.7.2. Astrovirus and Interferon Gamma (IFN-γ)

The human immune system have important role for controlling and elimination the pathogenic disease and cancerous cells. In this regard, IFN-γ has a critical role in recognizing the astrovirus vial antigens and elimination it because the IFN-γ, being the central effector of cell mediated immunity, it can coordinate a plethora of

anti-microbial functions .It also can serve to amplify antigen presentation through antigen presenting cells (APCs) by enhancing antigen recognition via cognate T-cell interaction, increase the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Intermediates (RNIs) and induce anti-viral responses . Additionally, the astrovirus infected cell were destroyed by IFN-γ activity via induction of an anti-proliferative state. Immunity to several pathogens is mainly governed by IFN-γ activity. For example, the role of IFN-γ in endowing protection against many infections (Naglak *et al*.,2016).

IFN-γ can also restrict viral uncoating or entry into the host cells and confer protection against a number of viruses including astrovirus, Influenza-A, Flaviviruses, HIV-1, Ebola virus and Corona virus (Day *et al*.,2017). IFN-γ-inducible proteins can also employ components of the autophagic machinery to curb viral replication. For example, IFN-inducible GTPases utilize the microtubule-associated-protein-1-light chain-3 (LC3) protein of the autophagy pathway to arrest astrovirus replication. The concerted action of IFN inducible GTPases and GBPs leads to marked inhibition of viral growth (Dotson *et al*.,2016).

2.3.8. Laboratory Diagnostic of Astrovirus

Various methods exist to detect an astrovirus infection. Current methods include (Lum *et al*.,2016 ; Pérot *et al*.,2017)

I. Electron Microscopy (EM)

Direct EM is complicated by the fact that only a minority of virions exhibit a complete star-shaped structure, and careful searching may be necessary to distinguish between, for example, astrovirus and calicivirus ,which are similar in size.

II. Virus Isolation

Astroviruses, like other enteric viruses, can be difficult to propagate in conventional cell cultures. The first propagation of HAstVs was made possible in

human embryo kidney (HEK) cells through the use of serum medium supplemented in trypsin. Other cell lines, such as African green monkey kidney Vero cells (e.g., MA-104), were not permissive for the virus even after initial passages in HEK cells. However, similar experiments based on virus adaptation in embryonic kidney cells in the presence of trypsin have enabled the propagation of bovine and porcine astroviruses.

III. Immunodetection and Antigenic Typing

The ability to grow astroviruses has simplified the production of antisera in experimental animals, allowing the characterization of serotypes and the development of a radioimmune assay for detection of anti-MAstV *1* (serotypes HAstV 1–8) antibodies. Indirect enzyme immunoassay (EIA) that used both a monoclonal antibody directed toward the capsid of MAstV 1 for the capture, and a polyclonal antibody for the detection, was achieved in a cohort of patients with gastroenteritis, showing a sensitivity of 91% and a specificity of 96% when compared to indirect electron microscope (IEM).

IV. Molecular Diagnostics

1.Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Quantitative Reverse Transcription PCR (RT-qPCR)

RT-PCR systems that have been developed for the detection of MAstV 1(serotypes HAstV 1–8), some are targeting non-coding regions of the virus in a very sensitive and specific manner, while others are designed into conserved motives of the capsid, thereby allowing subsequent typing but with a risk of sub-optimal amplification efficiencies. Alternative to RT-PCR, nucleic acid sequence-

based amplification (NASBA) has also shown a good concordance with RT-PCR-based methods for the detection of MAstV 1 (serotypes HAstV 1–8).

2. Multiplex RT-PCR for Enteric Pathogens Panels

To meet the need for a rapid, efficient and cost-effective diagnosis, multiplex RT-PCR panels, including astroviruses and other gastrointestinal pathogens, have been developed over time. Remarkably, among commercially-available solutions, the FilmArray Gastrointestinal Panel (BioFire Diagnostics, Salt Lake City, UT, USA) allows for the simultaneous detection of 22 different enteric pathogens directly from stool specimens, with a reported sensitivity and specificity of 100% and 99.9%, respectively, for the detection of MAstV 1 (but not *MAstV 6* and *9*), and a turnaround time of around one hour.

3. Medium to High Density Detection Systems: Nanofluidic PCR and Microarrays

With progress made in miniaturization and the development of nanofluidic systems, it has now become possible to run qPCR in parallel to nanoliter-volume chambers, thereby reducing the cost per assay. For example, a microfluidic qPCR system based on multiple singleplex TaqMan qPCR assays could quantitatively detect 13 viruses, including human astroviruses, with a sensitivity as low as two copies per microliters.

4. High Throughput Sequencing (HTS)

The advent of HTS has opened the way to metagenomics, which is the parallel sequencing and subsequent description of all nucleic acid molecules present in a sample. Specifically, it represents a group of disruptive technologies over PCR or other hypothesis-driven detection methods. With a combination of random amplification of microbial genomes or transcripts and appropriate downstream data mining, deep sequencing has the ability to provide more detailed taxonomic

information than diagnostic PCRs, and may also be used for the discovery of new pathogens without any prior hypothesis .

2.4. Toll Like Receptor -7 (TLR-7)

2.4.1. Definition

TLR-7is a protein that in humans is encoded by the TLR7 gene (chromosome **Xp22.3)** . It is a member of the toll-like receptor (TLR) family and detects single stranded RNA (Leaker *et al*.,2019).

2.4.2. Location

The Toll-like Receptor 7 gene (TLR7) is located in the pseudo-autosomal region 1 of the X chromosome (p22.2). TLR7 is localized to the cell wall of endosomes where they detect nucleic acids, ssRNA (Wicherska-Pawłowska *et al*.,2021).

2.4.3. Structure

The TLR7 are contain from two distinct ligand-binding sites. Site 1, which is highly conserved between TLR7 and TLR8, recognizes nucleosides (G for TLR7 and uridine [U] for TLR8) as well as nucleoside analogs and imidazoquinoline derivatives (IQDs) and is essential for receptor dimerization. Unlike site 1, site 2 is not conserved and is spatially different between this TLRs, although site 2 in both receptors binds short oligoribonucleotides and plays an auxiliary role in receptor dimerization by enhancing the binding affinities of site 1 ligands. Thus, TLR7 recognize ssRNAs in the forms of degradation products, nucleosides, and oligoribonucleotides Figure (2-9) (Zhang *et al*.,2018).

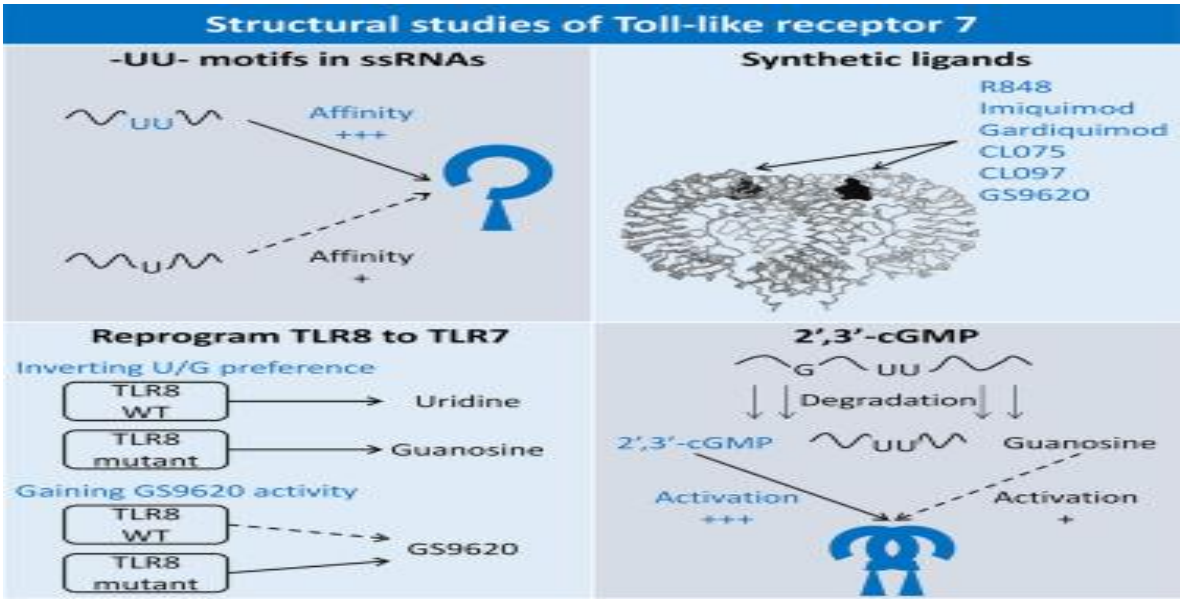

Figure 2.9. structural studies of TLR-7 (Zhang *et al*.,2018).

2.4.4. Function

Toll-like receptor (TLR) 7 are receptors for single stranded RNA that reside in the endosome. TLR7 is expressed primarily in B cells, monocytes, and plasmacytoid dendritic cells (pDCs) .The TLR-7 share some common downstream signaling, where TLR7 activation is more biased to signaling in the interferon regulatory factor (IRF) pathway, resulting in production of type I interferon (IFN) (Bender *et al*., 2020).TLR7 is well-established to bind viral ssRNA; specific examples include vesicular stomatitis virus (VSV), IAV, HIV-1, hepatitis B virus, and HCV (Gosu *et al*.,2012) Dimeric TLR7 then interacts with the ssRNA which subsequently initiates signal transduction. TLR7 contains a Toll/IL-1 receptor domain that associates with myeloid differentiation primary response gene 88 (MyD88) for signal transduction, aside from TLR3 which signals via the MyD88-independent pathway. Signaling in human immune cells by TLR7 has been documented to trigger production of pro-inflammatory cytokines including TNF-α, IL-6, IL-1β, IL-12, and IFN-α (El-Zayat *et al*.,2019). Furthermore, TLR7 is

recognizes viral double-stranded RNA (dsRNA) from, for example, reoviruses. In addition, it recognizes dsRNAs arising during replication of single-stranded RNAs (ssRNAs) of viruses, e.g., West Nile virus, RSV, or EMCV (encephalomyocarditis virus) (Mendes *et al*.,2017).

2.5. Interferon-gamma (IFN-γ)

2.5.1. Definition

Interferon gamma (IFN-γ) is a dimerized soluble cytokine that is the only member of the type II class of interferons. The IFN-γ is an extraordinarily pleotropic cytokine. It can not only heighten both the innate and adaptive immune response against pathogens and tumors, but also has the ability to maintain immune homeostasis. Since the effects of IFN-γ are cell and tissue specific, it is important to consider the recent advances in IFN-γ signaling in the context of different diseases (Januarie *et al*.,2021).

2.5.2. Location

The IFN-γ is located on the chromosome 12 (12q15). IFN-γ is secreted by T helper cells (specifically, T_h1 cells), cytotoxic T cells (T_C cells), macrophages, mucosal epithelial cells and NK cells (Liu *et al*.,2022). IFN-γ epithelial tissues, high expression of IFN-Gamma-R is detected on trophoblastic epithelium, glandular cells of stomach, ileum and colon, lung alveolar cells, salivary duct cells, renal tubular cells, and endometrial mucosa cells. Hepatocytes are weakly positive, while squamous epithelial cells are negative (Yao and Fox,2020).

2.5.3. Structure

All IFNs adopt α-helical structures with unique up-up-down-down topology , relative to other α-helix bundle proteins .The IFN-γ monomer consists of a core of six α-helices and an extended unfolded sequence in the C-terminal region. Each IFN consists of six secondary structural elements, denoted A-F, of which helices

A, C, D, and F form an anti-parallel four helix bundle Figure (2-10) (Walter ,2020).The a-helices of the Type-I IFNs are long, straight, and essentially parallel to one another . Despite considerable sequence diversity (35%–95%), all 16 IFNs adopt the same a-helical structure (Ouyang *et al*.,2012). In contrast to type-I IFNs, type-III IFNs are comprised of shorter helices that contain several kinks, which form a more compact bundle . As a result, type-III IFNs adopt structures that are more similar to the IL-10 family cytokine IL- 22 than to type-I IFNs (Deshpande *et al*.,2013; Walter ,2020).

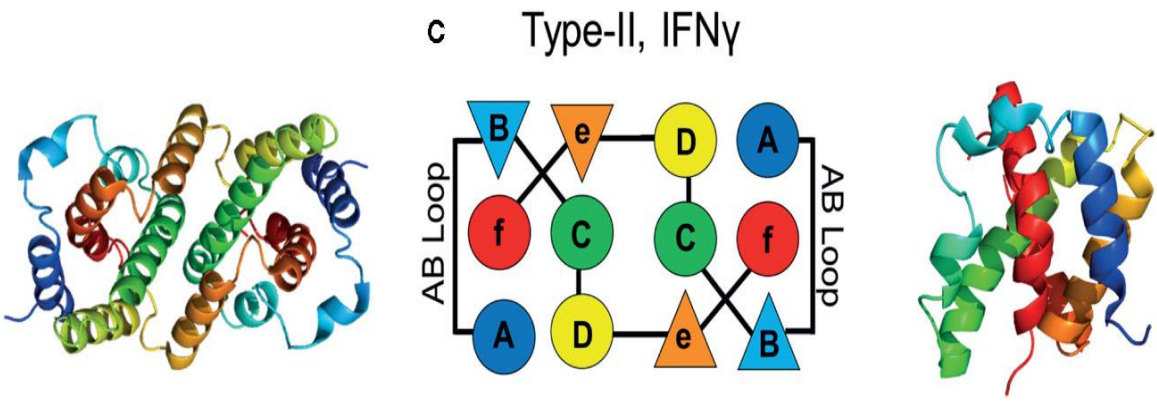

Figure 2.10.Structures of IFN-γ (C)**, pdbid = 6E3K IFNs. IFN structures are rainbow colored from the N-terminus helix A (blue) to the C-terminal helix F (red) (Walter,2020).**

2.5.4. Genome & protein

IFN-γ is a protein encoded by the IFNG gene, composed of two polypeptide chains associated in an antiparallel fashion (Alspach *et al*.,2019).In human blood, IFN-γ is present in three fractions with different molecular mass. One fraction represents the active free form of IFN-γ, while the other two are considered mature IFN-γ molecules. The fully synthetized protein is glycosylated at amino termini where the level of glycosylation determines the final weight of the defined fractions (Lilkova *et al*.,2019) Notably, it has been reported that glycosylation itself does not affect the activity of interferon, but rather prevents its degradation by proteinases. Therefore, this chemical modification increases interferons half-life

in the bloodstream and prolongs IFN-γ-mediated effects (Gordon-alonso *et al*.,2017).

2.5.5. Function

The IFN-γ is a pleiotropic type II IFN that is mainly produced by effector Th1 CD4+ T cells, cytotoxic CD8+ T cells and NK cells and to a lesser extent by other cell types, such as dendritic cells (DCs), macrophages and B cells (Liu *et al*.,2020). IFN-γ binds to the IFN-γ receptor (IFNGR), which is expressed on most cells and activates janus kinase 1 (JAK1) and JAK2 through the canonical pathway, leading to the phosphorylation of STAT1 homodimers and binding to the IFN-γ activation site (GAS) followed by subsequent gene transcribe (Billiau and Matthys,2009).
Interferon is a cytokine produced in response to viral infection and has various effects, such as regulating immunity, antiviral and antitumor activities according to the primary protein sequence, cognate receptor, gene locus, and cell type responsible for its production (Liu *et al*.,2022). The IFN-γ has a critical role in recognizing and eliminating pathogens. IFN γ, being the central effector of cell mediated immunity, can coordinate a plethora of anti-microbial functions. It can serve to amplify antigen presentation through antigen presenting cells (APCs) by enhancing antigen recognition via cognate T-cell interaction, increase the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Intermediates (RNIs) and induce anti-viral responses (Kak *et al*.,2018).

2.6. Diagnostic of TLR-7 and IFN-γ Polymorphism

2.6.1. Single Strand Conformation Polymorphism (SSCP)

SSCP analysis is a simple and sensitive technique for mutation detection and genotyping. The principle of SSCP analysis is based on the fact that single-stranded DNA has a defined conformation. Altered conformation due to a single

base change in the sequence can cause single-stranded DNA to migrate differently under no denaturing electrophoresis conditions (Haidong *et al*.,2005).

2.6.2. Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence. The term may refer to a polymorphism itself, as detected through the differing locations of restriction enzyme sites, or to a related laboratory technique by which such differences can be illustrated. In RFLP analysis, a DNA sample is digested into fragments by one or more restriction enzymes, and the resulting restriction fragments are then separated by gel electrophoresis according to their size (Chaudhary *et al.,* 2019).

2.6.3. Amplification Refractory Mutation System (ARMS)

In ARMS technique, one PCR comprises one allele-specific oligonucleotide primer at 5′-end and a common primer at 3′-end. If the presence of an amplified mutant is detected by agarose gel electrophoresis, it suggests that the target sequence contains the mutant allele. Similarly, if the result displays an absence of the amplified mutant, it indicates the presence of the normal DNA sequence on that specific point.In the same way, a normal primer at 5′-end together with a common primer at 3′-end was used in another PCR. If normal amplified product is present, it reveals the existence of a natural DNA sequence, whereas if normal amplified product is absent, then it reveals the presence of a mutant allele (Yang *et al*.,2018).

2.6.4. Sequencing of PCR Products

The term DNA sequencing refers to methods for determining the order of the nucleotides bases adenine,guanine,cytosine and thymine in a molecule of DNA.

The first DNA sequence were obtained by academic researchers, using laboratories methods based on 2- dimensional chromatography in the early 1970s. By the development of dye based sequencing method with automated analysis,DNA sequencing has become easier and faster. The knowledge of DNA sequences of genes and other parts of the genome of organisms has become indispensable for basic research studying biological processes, as well as in applied fields such as diagnostic or forensic research (Heather ,2016**)**.

CHAPTER THREE

MATERIALS & METHODS

3. Materials and Methods

3.1. Subjects

3.1.1 Patients population

This case control study is done for a one hundred-fifty specimens collected from infants and children patients with to gastroenteritis (GE) and apparently healthy persons as control group from general hospitals as well as many private clinical in Middle Euphrates provinces -Iraq. The age range of the study population was 6 months to 122 months. The specimens were collected during period from February 2021 to September 2021.

Stool swabs as well as blood from each study group of infants and children patients suffering from gastroenteritis should be enrolled, that classify into:-.

1.One hundred – fifty stool swabs as well as blood specimens from infants and children patients suffering from gastroenteritis.

2. Fifty stool swabs and blood specimens of apparently healthy persons as control group. All these specimens were submitted for viral genetic part for screening **Norovirus (NoV) and Astrovirus (AsV)** in infants and childern patients and apparently healthy control groups by Real-Time Polymerase Chain Reaction (RT-PCR) and detection the new genotypes of **NoV** and **AsV** by sequencing and phylogenetic tree . However, the second part is DNA extraction for detection SNPs of **Toll-like receptor -7 (TLR-7) and Interferon-gamma (IFN-γ) genes** polymorphism by sequencing .

3.1.2 Specimens Collection

Stool swabs as well as blood specimens were collected from patients and control by using swab for each patient for virology detection ; The flocked swab regular for Stool swab collection, according to Catalog Number 21031 (Heinz, Herenz; Germany). Each specimens are aliquot into three 1.8 cryo tube (Nunc-Kamstrup, Denmark) and stored at (-20°C) at the Virology Research Unit, College of Science, Babylon University. 5ml venous blood

were collected aseptically from all patients by using gel tubes and EDTA tubes for gating blood for DNA extraction and serum for TLR-7 & IFN-γ polymorphism & serum concentration ; then stored at (-20°C).

3.2. Study Design

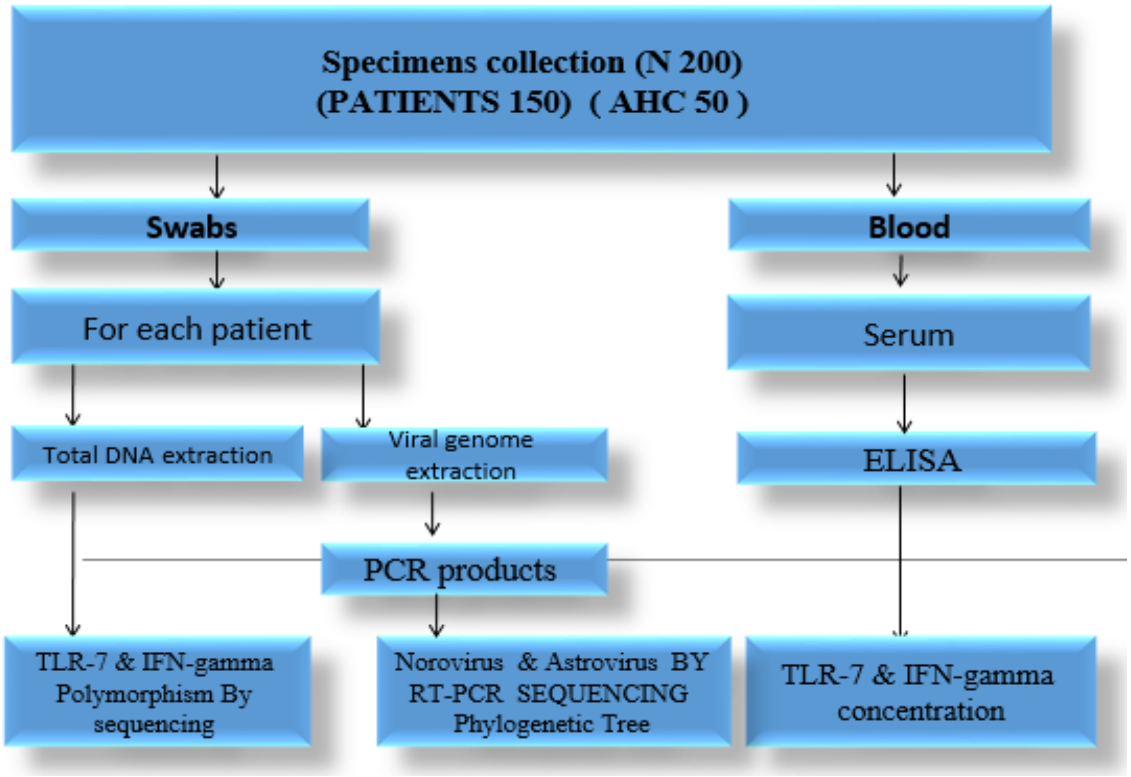

Figure 3.1: Study Design

3.3. Materials

3.3.1. Instruments and Equipment

Instruments and equipment used in this study are listed in table (3-1 , 3-2) .

Table (3.1): Instruments used in this study with their Manufacturer Company and origin

Instruments	Manufactured Company (Origin)
Centrifuge	Hettichzentrifuge, Sigma / Germany
Deep freeze -80	Bosch / Germany
Electrophoresis system	Germany
ELISA reader	Bio Tech (USA)
Fume hood	UK
Hot plate	Memmert / Germany
Incubator (model IB-909)	
Micro centrifuge	Hettichzentrifuge, Sigma / Germany
Multibiodrop	England
Real time Qiagen device Rotor Gene Q MDx	QIAGEN / Germany
Conventional PCR	
Refrigerator	Arcelik / Turkey
Sensitive balance	Sartoris / Germany
Timer with alarm	Junghans / Germany
Ultraviolet imaging device	Motic / USA
Water bath (1-A)	Memmert / Germany

Table (3.2) : Equipment used in this study with their manufacturer company and origin.

Equipment	Manufactured Company (Origin)
0.2ml PCR Tubes with Flat Caps	Extragene / Taiwan
1.5ml clear Microtubes	
Disposable plastic container	China
Disposable tips	Extragene/Taiwan

Equipment	
Falcon with different size 15ml , 50 ml	China
Flask with different size 25cm^2 , 75cm^2	
Gel loading tips	Bio Basic – Canada
Eppendorf tubes with different size 2ml, 1.5ml, 0.5 ml	
Gloves	
Micropipettes various sizes(1000,100,10) µl	Extragene/Taiwan
Plastic scraper	China
VIR-Swab	Heinz Herenz / Germany
	Copan / Italy

3.3.2 Reagents and Buffers

Reagents and buffers are used in this study are listed in table (3-2)

Table 3.2: Reagents and buffers used in this study with their Manufacturer Company and origin.

Reagents	Manufactured Company (Origin)
10X TBE DNA Sequencing Grade	Intron /S. Korea
Absolute Ethanol alcohol	Merck – Germany
Agarose E	Canada / Spain
DNA Ladder(100bp)	Intron /S. Korea
Proteinase K	Intron /S. Korea
Rad Safe Nucleic Acid staining solution	Zymo Research / USA
RNase A	

3.3.3 Kits and Marker

Kits and marker are used in this study with their Manufacturer Company and origin are listed in table (3-3).

Table 3.3: Kits and markers used in this study with their manufacturer Company and origin

Kits	Manufacturer Company/ Origin	CAT.NO
G-Spin Total DNA Extraction Kit	Intron / Korea	14001
Viral Nucleic Acid Extraction kit	Intron / Korea	17151
Human TLR-7 ELISA Kit	BT LAB \ CHINA	E0334Hu
Human IFN-γ ELISA Kit	BT LAB \ CHINA	E0105Hu
GoTaq® 1-Step RT-qPCR System	Promega \ USA	A6020

3.3.4. Contents of Patho Gene-spinTM DNA/RNA Extraction Kit

The contents of Patho Gene-spinTM DNA/RNA Extraction Kit are listed in table (3-4).

Table 3.4. Contents of Patho Gene-spinTM DNA/RNA Extraction Kit

Reagents	Amount
Lysis Buffer	(35 ml)
Binding Buffer	(30 ml)
Washing Buffer A	(30 ml)
Washing Buffer B2. Was added 40 ml of EtOH before use.	(10 ml)
Elution Buffer (20 ml).	(20 ml)
Spin Columns inserted into a collection tubes.	(2.0ml tubes) (50 columns)

3.3.5. Product Components and Storage Conditions

The product components and storage conditions of GoTaq® 1-Step RT-qPCR Master Mix were listed in table (3-5).

Table 3.5: The product components and storage conditions

GoTaq® 1-Step RT-qPCR Master Mix	**5ml**
Each system contains sufficient reagents for 500 × 20µl reactions. Includes:	
• GoTaq® qPCR Master Mix, 2X	5 × 1ml
• GoScript™ RT Mix for 1-Step RT-qPCR	225µl
• CXR Reference Dye, 30µM	200µl
• MgCl2, 25mM	750µl
• Nuclease-Free Water	2 × 13ml

3.3.6. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix.

Prepare the reaction mix (minus RNA template) by combining the GoTaq® qPCR Master Mix, GoScript™ RT Mix, PCR primers and Nuclease-Free Water as described in table (3-6).

Table 3.6: Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix.

Component	**Volume**	**Final Concentration**
GoTaq® qPCR Master Mix, 2X	10µl	1X
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	0.4µl	1X
Forward Primer (20X)	µl	50–300nM
Reverse Primer (20X)	µl	50–300nM
CXR Reference Dye (optional)	0.33µl/20µl reaction	500nM
Nuclease-Free Water	to a final volume of 20µl	

3.3.7. Kit contents of G-Spin total DNA extraction

The contents of G-Spin total DNA extraction kit are listed in table (3-7).

Table 3.7: List of reagents and buffers of G-Spin total DNA extraction kit used in this study.

Label	Contents 200 Columns
Buffer CL	90 ml
Buffer BL 1	90 ml
Buffer WA1	160 ml
Buffer WB 2	56 ml
Buffer CE 3	40 ml
Spin Column4 / Collection Tube5	200 ea
RNase A (Lyophilized powder)6	3 mg x 4 vials
Proteinase K (Lyophilized powder)6	22 g x 4 vials

3.3.8. Reagent provided of ELISA kits to evaluate TLR-7 and IFN-γ concentration levels

Reagent provided of ELISA kits to evaluate **TLR-7 and IFN-γ** concentration levels in serum patients as well as apparently healthy control groups are listed in table (3-8).

Table 3.8: List of reagents and buffers of TLR-7 and IFN-γ ELISA kit used in this study.

Components	Quantity
Standard Solution (480ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1

Components	Quantity
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated human TLR-7 Antibody Biotinylated human IFN-γ Antibody	1ml x1 1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

3.3.9.Primers of Norovirus (NoV) ; Astrovirus (AsV) ;Toll-like receptor -7 (TLR-7) and Interferon-gamma (IFN-γ) SNPs.

Primers sets are used in this study to detect the **NoV; AsV; TLR-7and IFN-γ** SNPs with their product size and source as well as origin are listed in table (3-9).

Table 3.9: Primers sets that used for detection of NoV; AsV; TLR-7and IFN-γ genes polymorphism.

Gene	Sequence (5′-3′)	Product size (bp)	Source/origin
Norovirus -GI	F-CTGCCCGAATTYGTAAATGA R-CCAACCCARCCATTRTACA	**RT-PCR**	**IDT\ USA**
Norovirus -GII	F-CNTGGGAGGGCGATCGCAA R-CCRCCNGCATRHCCRTTRTACAT	**RT-PCR**	**IDT\ USA**
Norovirus -GIII	F-CGTTTGAAGAAGTTGCAACAG R-ACGAACTCAACACGAGAGG	**RT-PCR**	**IDT\ USA**
Astrovirus	F-CAACTCAGGAAACAGGGTGT R-TCAGATGCATTGTCTTGGT	**RT-PCR**	**IDT\ USA**
TLR-7 rs3853839	F- ACTGACAAATACAGTCATGGGGTT R- GGGAGATGTCTGGTATGTGGTT	**408 bp**	**IDT\ USA**
IFN-γ rs9976971	F- GGAGGATCCCTCCTGGGG R- CATACACCCGTTCCTGTCCC	**441 bp**	**IDT\ USA**

3.4. Methods

3.4.1: Detection of Norovirus(NoV); Astrovirus (AsV) by Real Time Polymerase Chain Reaction (RT-PCR) Real-time PCR (qPCR) is based on two major processes: **Firstly, isolation** of viral genome (DNA\ 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 to achieve sensitive detection and quantification of pathogens.

3.4.1.1:Extraction of Viral Nucleic Acid from Clinical Specimens

By using specific viral DNA/RNA extraction kit (Intron/Korea); the viral genomic was extracted .

I. Principle of Viral Genomic Extraction

Patho Gene-spinTM DNA/RNA Extraction Kit is specifically designed to isolate high-quality nucleic acids from a variety of pathogen and specimen using low elution volumes that allow sensitive downstream analysis. The purified RNA/DNA is free of proteins and nucleases, and is suitable for use in downstream applications that allow pathogen detection.

Patho Gene-spinTM DNA/RNA Extraction Kit uses the chemotropic salt in lysis buffer inactivates immediately DNase/RNase to ensure isolation of intact DNA/RNA. Patho Gene-spinTM DNA/RNA Extraction Kit uses advanced silica-gel membrane technology for rapid and effective purification of DNA or RNA without organic extraction or ethanol precipitation. Furthermore, the buffering conditions are finely adjusted to provide optimum binding of the DNA/RNA to the column.

The protocol is based on the lysis buffer that effectively dissolves and denatures virus envelope and capsid protein without additional enzyme

treatment to easily elute viral gene. The binding buffer added after the elution helps to attach genes only to silica-gel membrane, and two different washing buffers efficiently remove proteins and other contaminants to get high purity viral gene. Swab and blood specimens were used same protocol.

II. Specimens preparation:

Stool swabs were in 3 ml liquid viral transport media tube (UTM),each specimen was aliquot into three cryotube containing 1000 µl of the sample which stored at (-20ºc) until genome extraction. After that, required part of specimens were taken and centrifuged at 10000 rpm for 5 minutes, discarded the supernatant except 100 µl of the solution was left to be used in re-suspension of the pellet for RNA/DNA extraction.

III. Assay Procedure:

The procedure was carried out in accordance with the manufacturer's instruction with some modification as following:

1. Three hundred (300) µl from cell-culture supernatant or blood plasma was transferred into 1.5ml micro centrifuge tube.
2. A 500 µl Lysis buffer was added, then the lysate mixed by vortex for (25sec). Mixture was incubated at room temperature for (15 min). DNase (20) µl (modification).
3. Six hundred µl from binding buffer was added, and completely mix well by gently vortex. This step is conducive efficient passage of cell lysates through a column and to increase binding onto column resins and important for effective deproteinization.
4. The lysates of cell was placed in a spin column that provided 2m collection tube.
5. Loaded lysates on the column and centrifuged for 2 min at 13,000 rpm.
6. Discarded solution in collection tube and place the spin column back in the same (2ml) collection tube.

7. A 500 µl of washing buffer A was added to spin column and centrifuged for 2 min at 13,000 rpm.
8. The solution was discarded in collection tube and places the spin column back in the same (2ml) collection tube and centrifuged for 2 min at 13,000 rpm and then Discarded solution.
9. Five hundred µl of washing buffer B was added to the spin column and centrifuged for 1min at 13,000 rpm.
10. The solution was discarded in collection tube and places the spin column back in the same (2ml) collection tube. Centrifuged for 1min at 13,000 rpm. It is important to dry the membrane since residual ethanol may interfere with downstream reactions.
11. Placed spin column in an RNase-free (1.5ml) microcentrifuge tube; then 50 µl of Elution buffer was added directly onto the membrane and was incubated at RT for 2min, then centrifuged for 2min at 13,000 rpm.
12. At this stage the supernatant was containing viral genome (RNA).

3.4.1.2: Estimation of the Extracted RNA and DNA Concentration and Purity

After extraction of viral RNA from samples ; the concentration of RNA yield and purity are measured by using Mlite bio drop (England) , by Appling 5 µl of the extracted RNA in the instrument curette. Extracted with purity in between (1.7-1.9) at absorption wave length 260/280 was included in this study, otherwise; RNA/DNA extraction of the sample was repeated.

3.4.1.3. GoTaq® 1-Step RT-qPCR Protocol

I.Description

GoTaq®1-Step RT-qPCR System(a,b) combines GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix in a single-step real-time amplification reaction. The system, which is optimized for RT-qPCR, contains a proprietary fluorescent DNA-binding dye, BRYT Green® Dye. The system enables detection of RNA expression levels using a one-step RT-

qPCR method, combining GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix in a single step real-time amplification reaction.

The GoScript™ RT Mix for 1-Step RT-qPCR (50X) includes optimized amounts of GoScript™ Reverse Transcriptase, RNasin® Plus RNase Inhibitor and additives to enhance single-step reactions.The GoTaq® qPCR Master Mix is a simple-to-use, stabilized2Xformulation that includes all components for qPCR except template, primers and water. This formulation, which includes a proprietary dsDNA-binding dye, a low level of carboxyl-X-rhodamine (CXR) reference dye (identical to ROX™ dye), GoTaq® Hot Start Polymerase, MgCl2, dNTPs and a proprietary reaction buffer, produces optimal results in qPCR experiments. A separate tube of CXR Reference. Dye is included for use with instruments that require a higher level of reference dye than that in the GoTaq® qPCR Master Mix.

II. General Considerations

i. qPCR Primers

Optimize the primer concentrations for each primer combination. Primer concentrations can range from 50nM to 300nM; perform titrations to ensure optimal results. As a general rule, a concentration of 200nM for each PCR primer is a recommended starting point.

ii. RNA Template

The amount of RNA required to detect the target of interest depends on several factors, primarily the abundance of that RNA target in each sample. As a starting point to detect RNA at unknown expression levels, we recommend using 100ng/ml of total RNA template per reaction. A high-copy-number RNA transcript may be detected in as little as 500ng/ml, while a low-copy-number RNA transcript may require more than 100ng/ml. Up to 100ng/ml of RNA can be used in each reaction. For optimal results, the RNA template should be purified to remove genomic DNA contamination. This is

particularly important when using amplification targets within a single exon to avoid amplifying any contaminating genomic DNA.

iii. BRYT Green® Dye

The BRYT Green® Dye in the GoTaq® qPCR Master Mix has spectral properties similar to those of SYBR® Green I: excitation at 493nm and emission at 530nm. Use the instrument optical settings established for SYBR® Green I assays with GoTaq® qPCR Master Mix.

iv. CXR Reference Dye and Instrument Considerations

The GoTaq® qPCR Master Mix contains a reference dye, carboxy-X-rhodamine (CXR), which is identical to ROX™ and allows GoTaq® qPCR Master Mix to be used directly on most instruments that perform passive reference normalization, e.g., from Applied Bio systems.

III. GoTaq® 1-Step RT-qPCR Protocol

1.Materials to Be Supplied by the User

- real-time PCR instrument and related equipment (i.e. Optical-grade PCR plates and appropriate plate covers)
- sterile, aerosol-resistant pipette tips.
- nuclease-free pipettes dedicated to pre-amplification work
- RNA template.
- Qpcr primers.

2. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix

The final reaction volume in this protocol is 20µl. The volumes given here may be scaled for larger or smaller reaction volumes.

1. The GoTaq® qPCR Master Mix and Nuclease-Free Water were thawed at Real time PCR.
2. The GoTaq® qPCR Master Mix for 3–5 seconds to mixVortexed at low speed to avoid aeration.
3. Determined the number of reactions to be set up, including negative control reactions. Added 1 or 2 reactions to this number to compensate for

pipetting error. While this approach does require using a small amount of extra reagent, it ensured that you will have enough reaction mixed for all samples.

4. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix (continued)

1. Prepared the reaction mix (minus RNA template) by combining the GoTaq® qPCR Master Mix, GoScript™ RT Mix, PCR primers and Nuclease-Free Water as described in Table (3-6). The RNA template was added. Vortexed briefly to mix.

Note: The primer concentrations should be optimized for each primer combination.

2. The appropriate volume of reaction mix to each PCR tube or well of an optical-grade PCR plate.
3. The RNA template was sealed (or water for the no-template control reactions) to the appropriate wells of the reaction plate.
4. The tubes or optical plate was sealed, and centrifuged briefly to collect the contents of the wells at the bottom. Protected from extended light exposure or elevated temperatures. The samples are ready for thermal cycling.

4.Thermal Cycling

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results as shown Table (3-10).

Table 3.10: Standard Cycling Conditions

Step	Cycles	Temperature	Time
Reverse transcription	1	at37°C	15 minutes
Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation	1	95°C	10 minutes
Denaturation		95°C	10 seconds
Annealing and data collection	40	60°C	30 seconds
Extension		72°C	30 seconds

Use the instrument optical settings established for SYBR® Green I assays with GoTaq® qPCR Master Mix.

3.5. Detection of TLR-7 (rs5743557) and IFN-γ (rs3853839) SNPs by Sequencing

Total DNA for SNPs of **TLR-7 (rs5743557) and IFN-γ (rs3853839)** polymorphism were extracted from peripheral blood of patients using sanger sequencing technique.

3.5.1. Principles of Assay

Is based on two major processes: isolation of total DNA from specimens and Polymerase chain reaction (PCR) is allows the amplification of a target region from a DNA template by using specific oligonucleotides .Finally, the PCR products of **TLR-7 (rs5743557) and IFN-γ (rs3853839)** to detected SNPs were sent to macro gene \ KOREA to detection the variation of **TLR-7 (rs5743557) and IFN-γ (rs3853839)** SNPs.

3.5.2. Extraction of Total DNA from Clinical Samples

The G-spin™ Total DNA Extraction Mini Kit is suitable for use with deferent swabs and fresh or frozen whole blood and blood which has been treated with EDTA. Pre-separation of leukocytes is not necessary • Purification does not require phenol/chloroform extraction or EtOH precipitation, and provides the simplest protocols. DNA is eluted in Buffer GE , TE (10:1), 10mM Tris (pH 7.5 ~ 8) or water, is prepared for direct addition to PCR or other enzymatic reactions. Alternatively, it can be safely stored at (–20°C) for later use. The purified DNA is protein-free, nucleases-free and does not include other contaminants or inhibitors. G-spin™ Total DNA Extraction Mini Kit is optimized for extraction of (20-30kb) DNA fragments and able to extract up to 50 kb fragments.

All reagents required for the total DNA extraction were provided with DNA extraction kit (G-Spin total DNA Extraction kit, Cat .No. 14001 Intron / Korea).

3.5.3. Assay Procedure

The procedure is carried out in accordance with the manufacturer's instruction as following

I. **Extraction of Total DNA From Swabs**

1. A 300 µl of blood were placed into a (1.5 ml) micro-centrifuge tube.
2. Added (400 µl) of CL Buffer, (20 µl) of proteinase K solution and (40 µl) of RNase A into sample tube and mixed by vortexing vigorously. Then incubated the lysate at (56°C) for 30 min.
3. A 1.5 ml tube were centrifuged briefly (to remove drops from the inside of the lid.
4. Added (400 µl) of Buffer BL into the lysate, and mixed well by gently inverting 5 - 6 times. After mixing, was incubated the lysate at (70°C) for 5 min.
5. A 1.5 ml tube was centrifuged briefly to remove drops from the inside of the lid.
6. A 400 µl of absolute ethanol was added into the lysate, and mixed well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, the (1.5 ml) tube was to remove drops from inside of the lid.
7. A 800 µl of the mixture from step 7 was applied carefully (to the Spin Column (in a 2 ml Collection Tube) without wetting the rim. Closed the cap was closed and centrifuged at 13,000 rpm for 1 min. Then the filtrate was discarded and placed the spin column in a 2 ml collection tube (reused).
8. step 8 was repeated by applied up to 600 µl of the remaining mixture from step 7 to the spin column. The filtrate was discarded and placed the spin column in a new (2 ml) collection tube.

9. The mixture from step 7 was carefully applied to the spin column (in a 2 ml collection tube) without wetting the rim, then the cap was closed , and centrifuged at 13,000 rpm for 1 min. The filtrate was discarded and placed the spin column in a new (2 ml) collection tube (additionally supplied).
10. Seven hundred µl of Buffer WA was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. The flow-through was discarded and reused the collection tube.
11. After then (700 µl) of Buffer WB was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. The flow-through was added and placed the column into a new (2.0 ml) collection tube (additionally supplied), then gained centrifuged for additional (1 min) to dry the membrane. The flow-through was discarded and collection tube altogether.
12. The spin column placed into a new (1.5 ml) tube (not supplied), and added (30 - 100 µl) of buffer CE directly onto the membrane. Then incubated for 1 min at room temperature and then centrifuged for 1 min at 13,000 rpm to elute.

3.5.4. Measurement of Concentration and Purity of Extracted DNA

The DNA quantity and purity was determined using a spectrophotometer (Nano drop) at the absorbance at 260nm and 280nm respectively. The concentration of DNA was estimated at ng/Ml and the purity calculated as 260/280 ratio, when the DNA solution is pure the ratio ranged 1.8-2.

3.6. Detection of TLR-7 (rs5743557) and IFN-γ (rs3853839) Genes Polymorphism By Polymerase Chain Reaction (PCR)

3.6.1 Primers Pairs Dilution

The primers source were from Bioneer primers are commonly transported in a lyophilized state. The units of a lyophilized primer are given as a mass, in Pico moles. To create a stock of primers, one would reconstitute the primer in sterile, nuclease-free water. The company supplies the amount of sterile,

nuclease-free water to be added to each primer to obtain master stock (100Pmol/ml) that will be used again to obtain working stock. As following: The tube was spin down before opening the cap, then the desired amount of water was added according to the oligos manufacturer to obtain a 100 pmol/µl (Master Stock). Vortex properly for re-suspend the primers evenly. Then 10µl of the master stock was transferred to a 0.2ml Eppendorf tube that contains 90µl of sterile, nuclease-free water (Working Stock). The master stock is stored at -20 C° and the working stock is stored at -20 C°. Finally, the working stock was thawed on ice and vortex before using in PCR and then stored at -20 C°.

3.6.2. PCR Experiments

PCR amplification was done using conventional thermal cycler (Biometra - Germany) as follows: Template DNA (about 2 µl) was added into PCR master mix tubes. Forward and reverse primers were added 1.5 µl into PCR master mix tubes (for each one). Distilled water was added to PCR Premix tubes to a total volume of 25 µl as the table (3-11).

Table 3.11: Recommended volumes and concentration for applying PCR into AccuPower® PCR tubes.

No.	Content of PCR Reaction Mixture	Volume/ µl
1	Master mix	10 µl
2	Forward primers (each one of snps)	1.5 µl
3	Reverse primers (each one of snps)	1.5 µl
4	Template DNA	5 µl
5	Nuclease free water	5 µl
Total		**25 µl**

3.6.3. Thermal Cycles Condition

Reactions were placed in a thermal cycler (Biometra-Germany) that had been preheated to 94°C and beforehand set up to the desired cyclic conditions. The target regions of **TLR-7 (rs5743557) and IFN-γ (rs3853839)** polymorphism were amplified using specific primers according to mention conditions in table (3-12).

Table 3.12: Amplification Conditions of TLR-7 (rs5743557) and IFN-γ (rs3853839) Genes in Patients with Gastroenteritis .

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles	Hold
TLR-7 rs5743557	95C^0 /5 min	95C^0 / 30 sec	58 C^0 /30 sec	72 C^0 /30 sec	72 C^0 /10min	40	4 C^0
IFN-γ rs3853839	95C^0 /5 min	95C^0 / 1 min	60 C^0 /1min	72 C^0 / 2min	72 C^0 /5min	40	4 C^0

PCR products of target regions **TLR-7 (rs5743557) and IFN-γ (rs3853839)** polymorphism were electrophoresed on 1.5% agarose at 75 V for 1h and visualized by safe stain. Photos were taken using gel documentation system.

3.7. Agarose Gel Electrophoresis Technique

The agarose gel electrophoresis was perfumed according to the method of Robinson and Lafleche (2000). This technique was used to detect viral genomic ; genomic DNA extracts, and PCR products.

3.7.1 Preparation of Solutions and Buffers

I. Loading Buffer

The buffer was prepared from 0.25 % Bromophenol blue and 40% sucrose and stored at 4° C (Sambrook and Rushell, 2006).

II. TBE Buffer (1X)

To prepare 500 ml of 1X TBE buffer, 50 ml of TBE (10X) stock solution was mixed with 450 ml of deionized water. The pH value was adjusted to 8 with concentrated HCl or 0.5 M tris base solution. Then the volume was completed to 500ml with deionized water.

3.7.2 Gel Electrophoresis Protocol

1. Device setup: The casting gates were sited on the ends of the gel tray and locked in place firmly against casting tray. This was done by engaging the "claws" of the gate in the recess of the side wall of the tray. The comb was sited into the slots of the gel tray, (1.0 mm above the base of gel casting tray) so that the sample wells are near the cathode.

2. Gel dissolving: 1g of agarose was dissolved in 100ml of 0.5 X TBE solution by melting to 100°C to prepare 1% agarose gel for migrated genomic DNA extracts. Whereas, 1% or/and 2% agarose gel was prepared in 1X TBE buffer for migrated PCR products.

3. Gel casting: After agarose gel dissolving completely, it let to cooling to approximately 60°C and 2-3 µl of the safe stain stock solution was added, then slowly pour the agarose into the gel- casting tray, and any air bubbles were removed. The comb was positioned at approximately 1.5 cm from one edge of the gel. The agarose was allowed to solidify at room temperature at least 30 min. After that, the claws were disengaged from the gel tray and the comb was separated gently. Then the gel was placed in the gel tank in such a way that the wells should be on end with the cathode. 1X TBE buffer (depending the purpose) was added to the buffer tank until it was about 5 mm above the top of the gel.

4. Loading the samples: Each 5µl of the genomic DNA sample was mixed with 3µl loading dye briefly and loaded into the wells. Whereas, the PCR products were loaded without loading dye because of the PCR master mix contained loading dye.

5. Gel electrophoresis conditions: After sample loading the electric field was turned on at 5 V/cm (75V) for 60-120 min until bromophenol blue dye reached at the end edge of the gel.

6. The gel was photographed using gel documentation system (Cleaver Scientific - UK).

3.8. Sequencing of Studied Markers

3.8.1. Nucleic Acids Sequencing of PCR Amplicons

The resolved PCR amplicons were commercially sequenced from both directions, forward and reverse directions, following the instruction manual of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Biosystem) sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, the virtual positions, and other details of the retrieved PCR fragments were identified.

3.8.2. Interpretation of sequencing data

The sequencing results of the PCR products of the targeted samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in their corresponding position within the referring genome. The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. Each detected variant within the genome sequences was annotated by SnapGene Viewer ver. 4.0.4 (https://www.snapg ene.com).

3.8.3. Translation of Nucleic acid variations into Amino acid residues

The amino acid sequences of the targeted ORF1a gene-encoded nonstructural protein were retrieved from the NCBI server (http://www.ncbi.nlm.nih.gov). The retrieved protein ID number of the nonstructural protein was QAA77572.1 for the human-infecting astrovirus. This protein was made of 920 amino acid residues. The observed nucleic acid variants in the coding portions were translated into a reading frame corresponding to the referring amino acid residues in the encoded protein using the Expasy online program (http://web.expasy.org/translate/). Multiple amino acid sequence alignment was conducted between the referring amino acid sequences and their observed mutated counterpart using the "align" script of the BioEdit server.

3.8.4. Comprehensive Phylogenetic Tree Construction

A specific comprehensive tree was constructed in this study according to the neighbor-joining protocol described by (Bunyan and Salem, 2022). The observed variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server (Hamad *et al*. 2022). Then, a full inclusive tree, including the observed variant, was built by the neighbor-joining method and visualized as a circular cladogram using the iTOL suit (Letunic and Bork, 2019). The sequences of each classified phylogenetic group in the comprehensive tree were colored appropriately.

3.9: Evaluation of **TLR-7** and **IFN-γ** Concentration in Blood Serum of Patients and Control.

The concentration of **TLR-7** and **IFN-γ** in the serum of patients with EG were evaluated by enzyme linked immunosorbent assay (ELISA).

I. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human TLR-7 and IFN-γ antibodies. TLR-7 and IFN-

γ presents in the sample were added and binds to antibodies coated on the wells. And then biotinylated Human TLR-7 and IFN-γ Antibodies were added and binds to TLR-7 and IFN-γ in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated TLR-7 and IFN-γ Antibodies. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human TLR-7 and IFN-γ. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

II. Assay Procedure

1. All reagents were prepared, standard solutions and samples as instructed. Bring all reagents to room temperature before used. The assay was performed at room temperature.
2. Determined the number of strips required for the assay. Inserted the strips in the frames for used. The unused strips should be stored at 2-8°C.
3. Added 50µl standard was added to standard well. **Note**: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Forty µl of sample was added to sample wells and then added 10µl anti-**TLR-7** (or **IFN-γ**) antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated at 60 minutes at 37°C.
5. The sealer was removed and washed the plate for 5 times with wash buffer. Soaked wells with at least 0.35 ml wash buffer for 30 seconds for each wash. For automated washing, aspirated or decanted each well and washed for 5 times with wash buffer. Blotted the plate onto paper towels or other absorbent material.
6. Added 50µl substrate solution A to each well and then added 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. 50µl from stop solution was added to each well, the blue color was changed into yellow immediately.
8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after added the stop solution.

III. Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

IV. Typical Data This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.

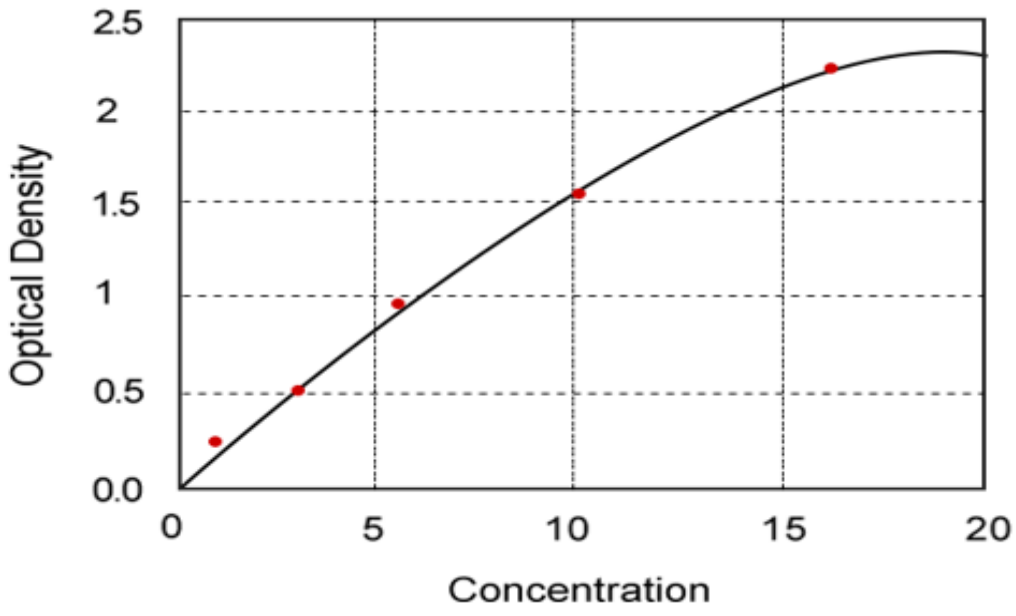

Figure 3.2. Standard curve

3.10. Statistical Analysis

Statistical analysis was carried out using SPSS version 23, where data were expressed as the Means, Standard deviation, one –sample T Test, one–way ANOVA and the Chi–Square test, and correlation, were used to find the

association between the categorical variables, P-value ($P \leq 0.05$) was considered statistically significant.

CHAPTER FOUR

THE RESULTS

4. The Results

4.1: Distribution of Patients with Gastroenteritis (GE) and Apparently Healthy Control (AHC) Groups According to Their Age

Table (4-1) shows the mean age groups of the study population. The mean age of the patients with GE was (43.56±**8.31** months) was more than the mean age of the AHC (40.6± **10.96** months). There are non-significant statistical differences (p=0.42) between pateints with gastroenteritis and Apparently healthy control.

Table 4.1 : Distribution of Patients with GE and AHC according to their Age.

Study groups	No.	Mean of age (Months)	S. D	S. E	Range		(P-value)
					Minimum	Maximum	
GE	150	43.56	8.31	2.304	6 months	120 months	P=0.42 N S (P>0.05)
AHC	50	40.6	10.96	4.59	8months	120 months	
Total	200						

4.2.Distribution of Patients with GE and AHC According to Age Stratum

Figure (4-1) shows the age stratum of the study population groups. The age group 6-44 months , 45-83 months and 84-122 of GE and AHC,were 47% ,16% , 9% and 16% , 7% and 6% ,respectively.

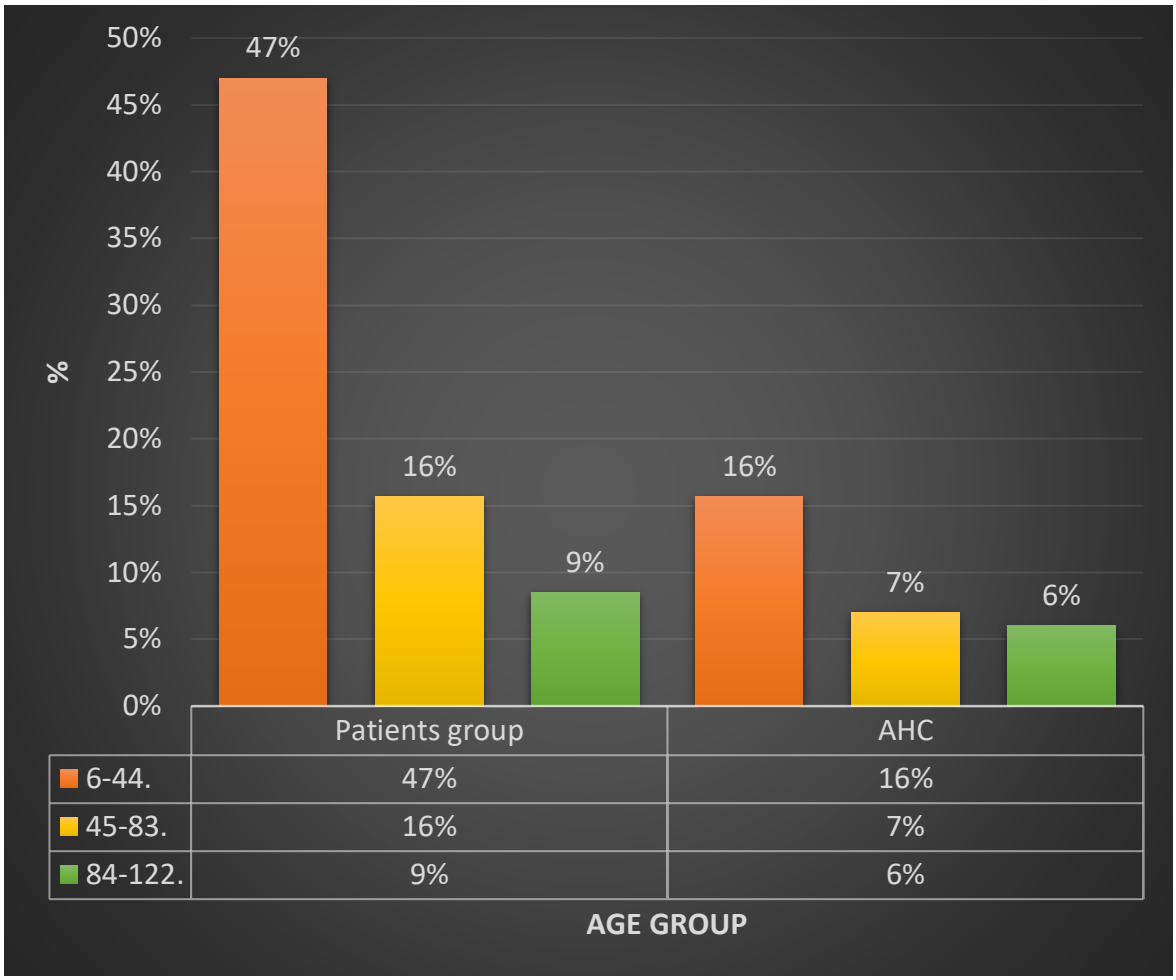

Figure 4. 1: Age Groups of the Study Population.

4.3: Distribution of Patients with GE and AHC According to Their sex

sex distribution is represented in Figure (4-2). Fifty-eight percent (58%) (116 out of 200) of the study population were male, while female represented 42% (84 out of 200).

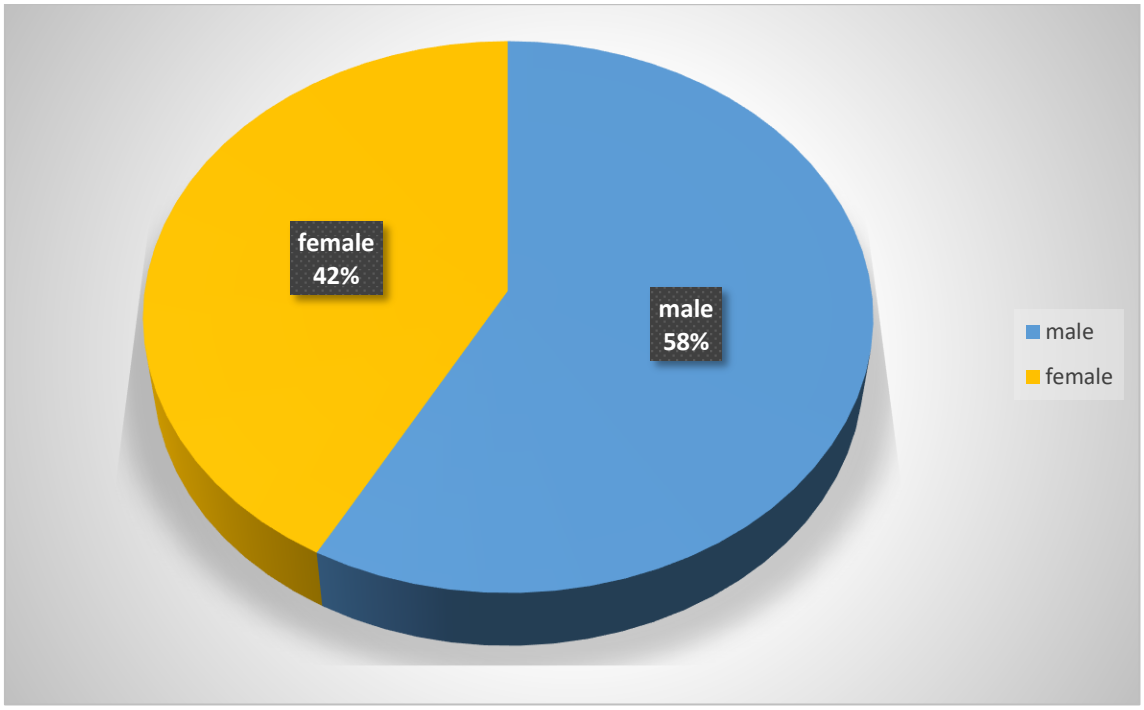

Figure 4.2: sex Distribution of the Study Population.

4.4. Clinical Characteristics of the Study Population

The most important clinical characteristics of the study population are shown in table 4-2. Sixty-five (43.4%) of patients was present with stomach cramping, while stomach ache ; Loss of appetite and Body aches accounted for 30% ; 23.3% and 3.3% ,respectively of gastroenteritis illness.

Half of children (50%) had a hospital stay for more than 3 days , and about half of them had a medical history especially nausea, vomiting , stomach ache ,loss of appetite, body aches, fever , low-grade fever or chills, headache, and muscle aches 20 (13.3 %) , 40(26.7%) ,30 (20%) ,28 (18.7%), 25(16.7%),15(10%),10 (6.7%), 13 (8.7%) ,5 (3.3%) and 4 (2.6%),respectively as shown in Table (4-2).

Table 4.2: Clinical Characteristics of infant and young children patients

Variables	No.(%)
No. days prior to admission ≤ 3 days 4-14 Days > 14 Days	 75 (50%) 47 (31.3 %) 28 (18.7%)
Diagnosis Stomach Cramping Stomach Ache Loss of Appetite. Body Aches.	 65 (43.4%) 45 (30%) 35 (23.3%) 5 (3.3%)
Length of Hospital Stay 1-3 days > 3 days Variables	 65 (43.3%) 80 (53.3%) 5 (3.3%)
Medical history None Nausea. Vomiting. Stomach Ache. Loss of Appetite. Body Aches. Fever. Low-Grade Fever or Chills,	 30 (20%) 20 (13.3 %) 40 (26.7%) 30 (20%) 28 (18.7%) 25 (16.7%) 15 (10%) 10 (6.7%) 13 (8.7%) 5 (3.3%) 4 (2.6%)

4.5. Detection of Norovirus (NoV) and Astrovirus (AsV) by Real-Time Polymerase Chain Reaction Technique (RT.PCR)

4.5.1. Extraction Nucleic Acid by Specific Viral DNA/RNA Extraction Kit

Out of 150 stool swabs specimens involved in this study 51.3% (77 out of 150 cases) were found to have a viral infection more than 48.7%(73 out of 150 cases) patients who did not show have a viral genome as shown in Figures (4-4). While, no viral nucleic acid was detected among all the examined apparently healthy specimens (50) as control group . There were statistically significant differences (p = 0.03) between patients with the viral genome and those without the viral genome Table (4-3).

Table 4.3 : Percentage of Viral Genome Extraction of Patients with GE and AHC Groups.

Viral Genome For Norovirus		Study Groups		
		AHC No. (50)	GE No. (150)	Chi-Square (P-value)
Positive	N	0	77	
	%	0%	51.3 %	
Negative	N	50	73	P=0.03
	%	100%	48.7%	S.
Total	N	50	150	(P<0.05)
	%	100%	100%	

4.5.2. Detection of Norovirus Genotypes By qRT.PCR

The analysis of norovirus genotypes results was made by using a Real Time PCR analyzer (Rotor-gene Q MDX /thermal cycler integrated with a system for fluorescence detection and a dedicated software).

The total positive result of Norovirus (GI , GII&GIII) according to qRT-PCR shows 37.6% (29 out of 77 cases) as positive less than 62.4% (48 out of 77 cases) as negative, as shown in Table (4-4) as well as Figures (4-5-A, B, C, D & E) . Statistically significant differences (p = 0.03) among patients group.

Table 4.4: Percentage of *Norovirus* Genotypes Positive Signals in Patients with GE by Using qRT.PCR Technique.

NoV Genotypes	No.	%	P value
Positive	**29**	**37.6%**	**P=0.03**
Negative	**48**	**62.4 %**	**Sign**
Total	**77**	**100**	**>0.05**

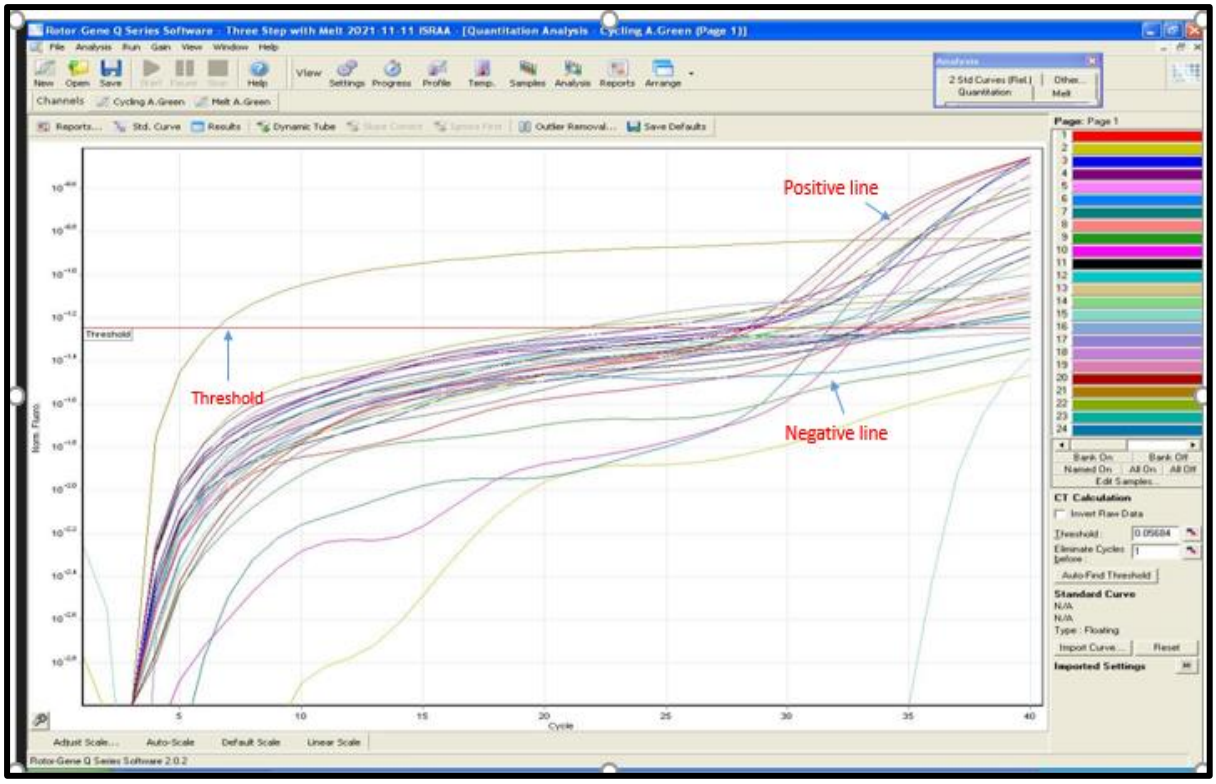

Figure 4.4 A: Detection of Norovirus G1 by RT-PCR

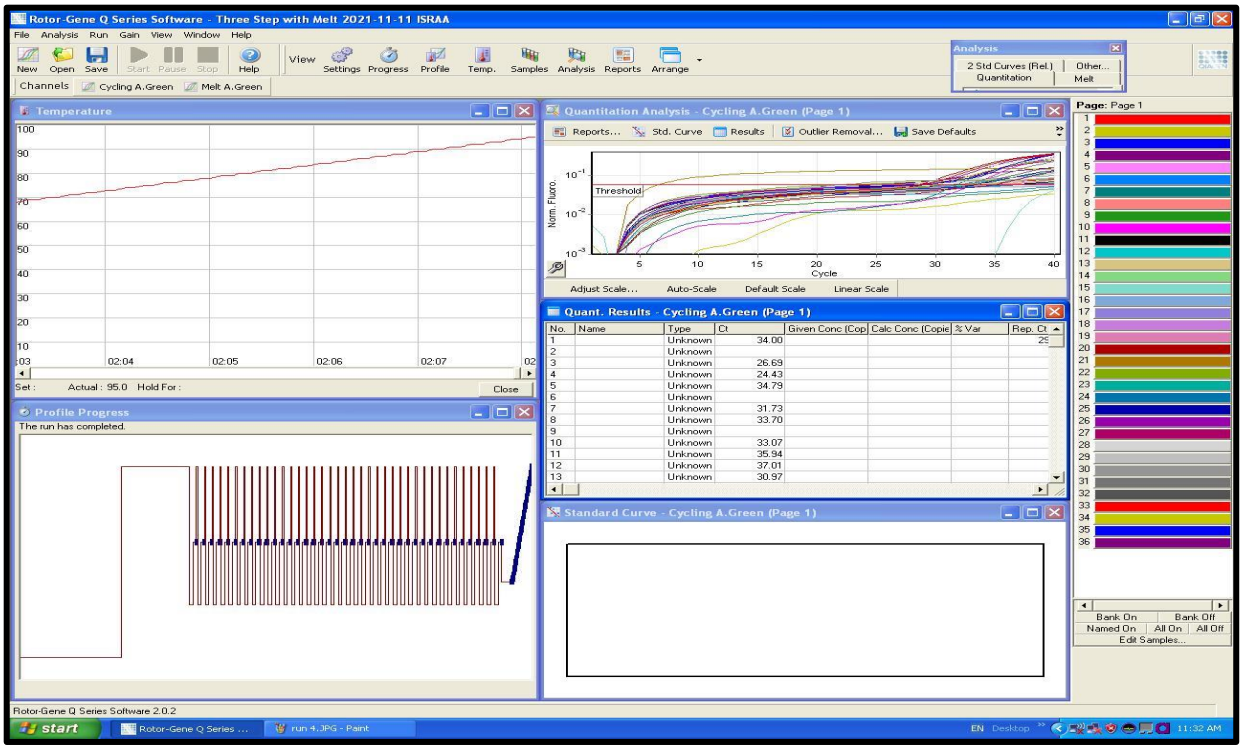

Figure 4.4.B: Quantitative and standard curve for detection of Norovirus by RT-PCR.

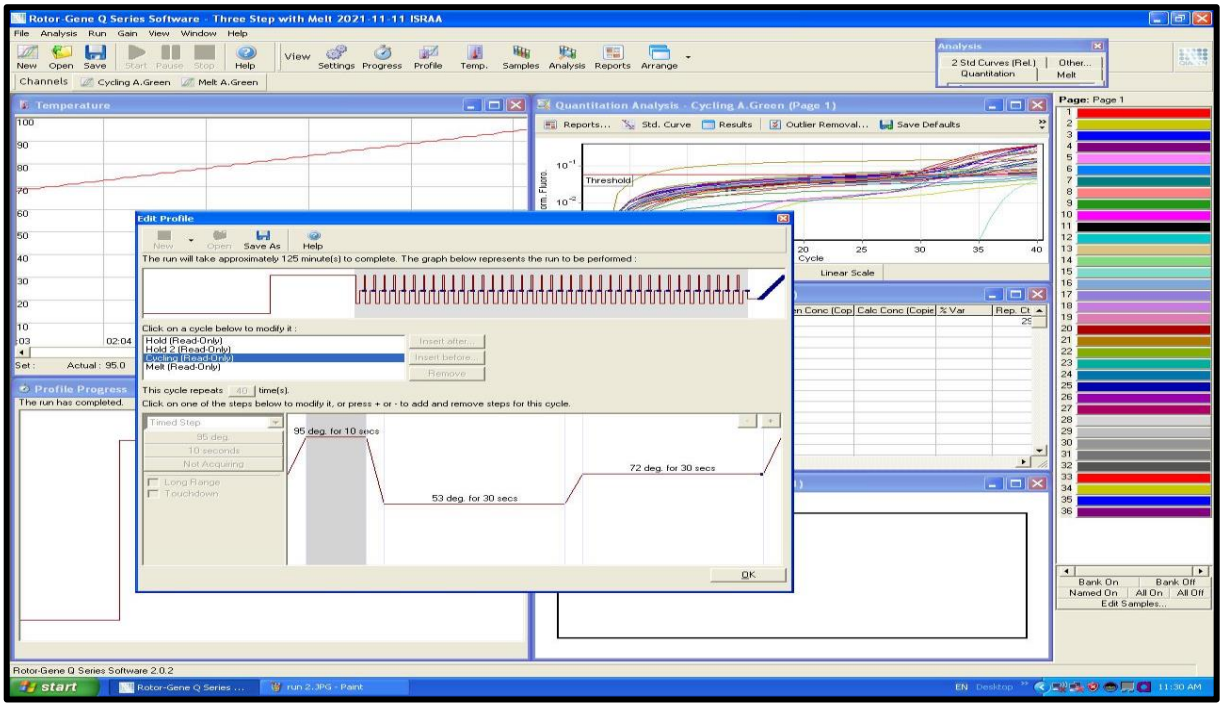

Figure 4.4.C: Quantitative & melting curve for detection of Norovirus G1 by RT-PCR

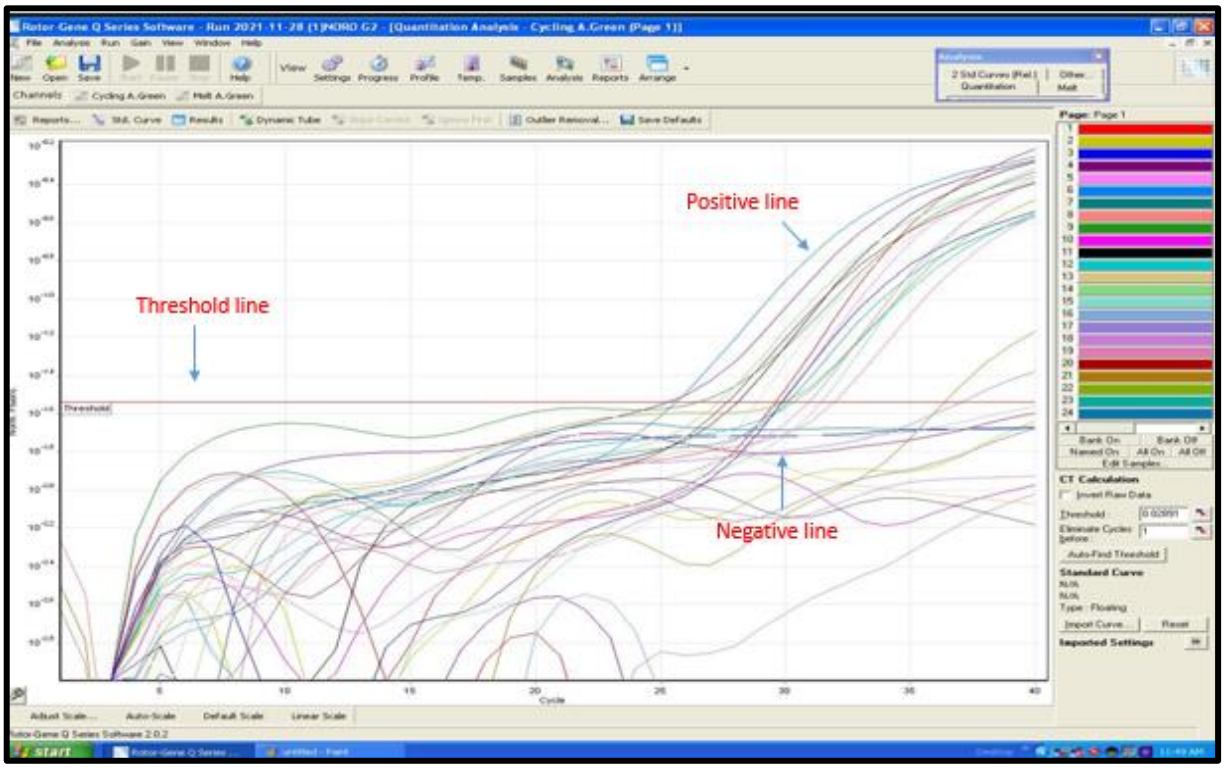

Figure 4.4.D: Detection of Norovirus G2 by RT-PCR

4.5.2.1.The Results of NoV in the Patients With GE According to the Age Stratum.

In gastroenteritis, the most commonly affected age group infected with NoV was (3-36 months) which constituted 19.5% (15 out of 77 cases), while the age group (37-72 months) was constituted 11.6% (9 out of 77 cases), followed by 6.5 % (5 out of 77 cases) in age group (73 -120 months).

Statistical comparison of these age strata revealed significant differences ($p < 0.02$) Table (4-5).

Table 4.5. Frequency of NoV RT-PCR Signal Among The Patients With GE According to the Age group.

	Months	Norovirus			P value
		No.	Positive	Negative	
Age group	3-36	44	15	29	Anova test P=0.02 Sign. (P<0.00)
		57.1%	19.5%	37.6%	
	37-72	21	9	12	
		27.2%	11.6%	15.7%	
	73-120	12	5	7	
		15.7%	6.5%	9.1%	
Total		77	29	48	
		100%	37.6%	62.4%	

4.5.2.2.Sequencing of Human Norovirus Genotypes of Clinical Isolates

One specific PCR fragment partially covering the coding regions of the VP1 gene (343 bp) in human-infecting noroviruses was amplified in this study **Figure (4.6)**. The amplified PCR fragments were directly exposed to Sanger sequencing experiments to assess the pattern of genetic polymorphism in the clinical viral samples.

All the observed nucleic acid variants was translated to their corresponding amino acid sequences using the Expasy translate server. A specific comprehensive tree was built to assess the accurate genotyping of the observed variants and their phylogenetic distribution. After positioning the 343 bp amplicons' sequences within the genomic sequences of the human norovirus, the details of its sequences was highlighted, and the total length of the amplified amplicons was also determined Table (4-6).

Table 4.6. The position and length of the 343 bp PCR amplicons that used to partially amplify the coding portions of the VP1 gene within human norovirus genomic sequences (GenBank acc. no. EU310927.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
VP1 gene Nucleic acid sequences of the viral VP1 gene	*CGTGGGAGGGCGATCGCAATCTGGCTCCCAGCTTTGTG AATGAAGATGGCGTCGAGTGACGCCAGCCCATCTGATG GGTCCACAGCCAACCTCGTCCCAGAGGTCAACAATGAG GTTATGGCTTTGGAGCCCGTTGTTGGTGCCGCAATTGCG GCACCTGTAGCGGGCCAACAAAATGTAATTGACCCCTGG ATTAGAAATAATTTTGTACAAGCCCCTGGTGGAGAGTTC ACAGTATCCCCTAGAAACGCTCCAGGTGAAATACTATGG AGCGCGCCCTTGGGCCCTGATCTGAATCCCTACCTTTCTC ATTTGGCCAGAATGTACAATGGTTATGCAGGTG**	343 bp

*refers to forward primer placed in the forward direction
**refers to reverse primer placed in the reverse complement direction

4.5.2.3.Sequences Alignment Fragment Results of VP1 Gene (343 bp) in Human-infecting noroviruses

Interestingly, the alignment results of the 343 bp samples revealed the presence of four Nuclotide variations represented by four Nuclotide substitutions

in four loci in the analyzed samples in comparison with the most similar referring reference nucleic acid sequences (GenBank acc. no. EU310927.1) Figure (4-7). The present results indicated the presence of four Nuclotide variants in the investigated samples, namely 56G>A, 147A>T, 231C>T, and 288T>C. Results from the direct Nuclotide translation of the 56G>A indicated that this variant showed a missense effect on the protein, namely p.6S>N.

Results of translations was also indicated that 147A>T, 231C>T, and 288T>C of the detected variants was translated to p.36A=, p.64F=, and p.83P= respectively Figure (4-7). These translated variants were silent (synonymous) variations that occupied specific distributions in the investigated samples. It was inferred from the tree that our investigated samples were divided into two genotypes within the same norovirus sequences. The majority of samples were suited within the GII clade, while both S3 and S4 was suited within the GIII clade.

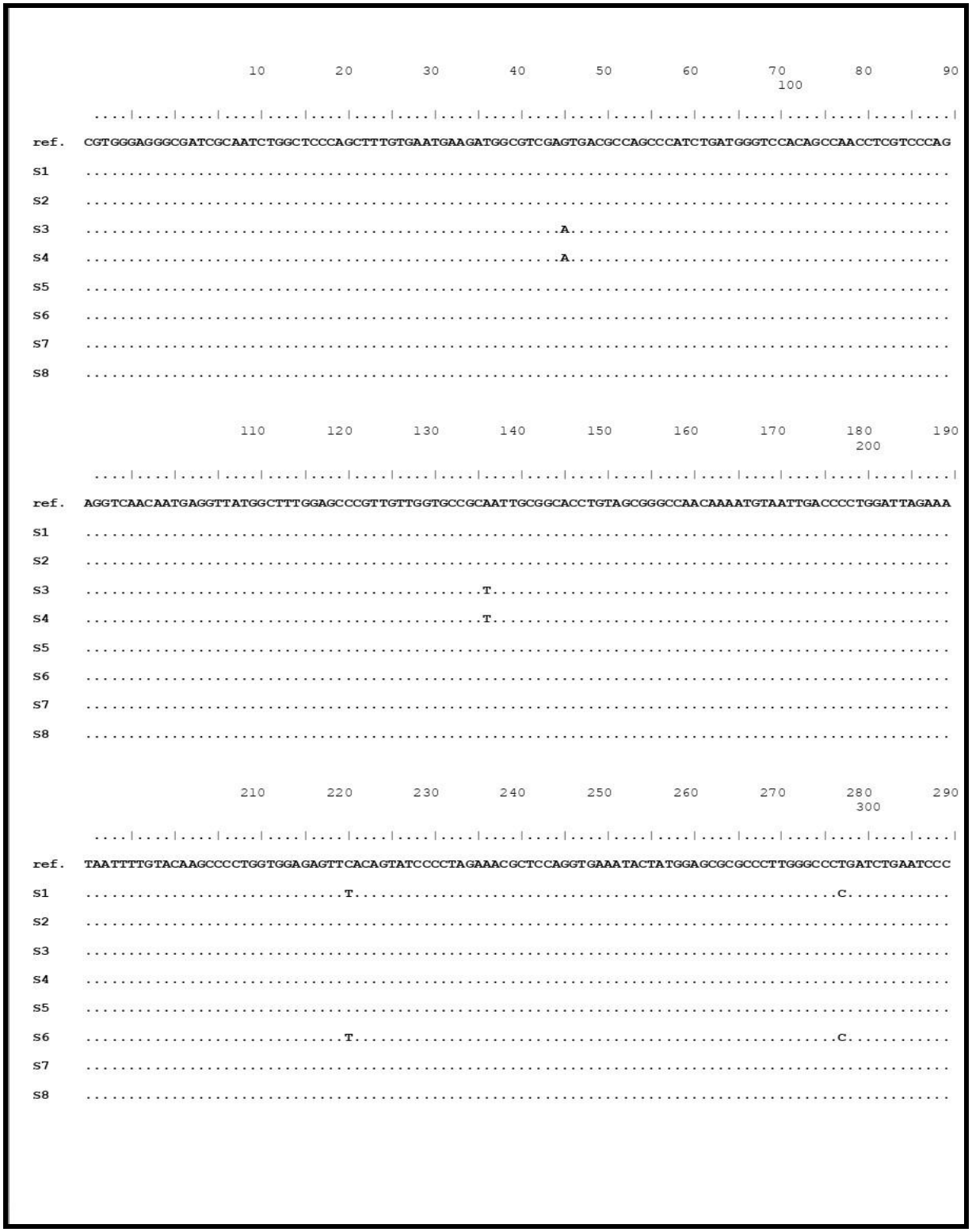

Figure. 4.5. Nucleic acid sequences alignment of eight samples with their corresponding reference sequences of the 343 bp amplicons of the VP1 genetic sequences. The symbol "ref" refers to the NCBI referring sequence (GenBank acc. no. EU310927.1), and the letter "S#" refers to the sample number

In addition, current results indicated the presence of four Nuclotide variants observed in the investigated samples, namely 86C>T, 131G>A, and 239G>A detected in several investigated clinical samples. To confirm these variations, the sequencing chromatograms of the investigated samples, as well as their detailed annotations, were verified and documented, and the chromatograms of their sequences were shown according to their positions in the PCR amplicons.

The presence of each one of these variants was confirmed in its original chromatograms and the absence of any possible technical error was also confirmed Figure (4.8).

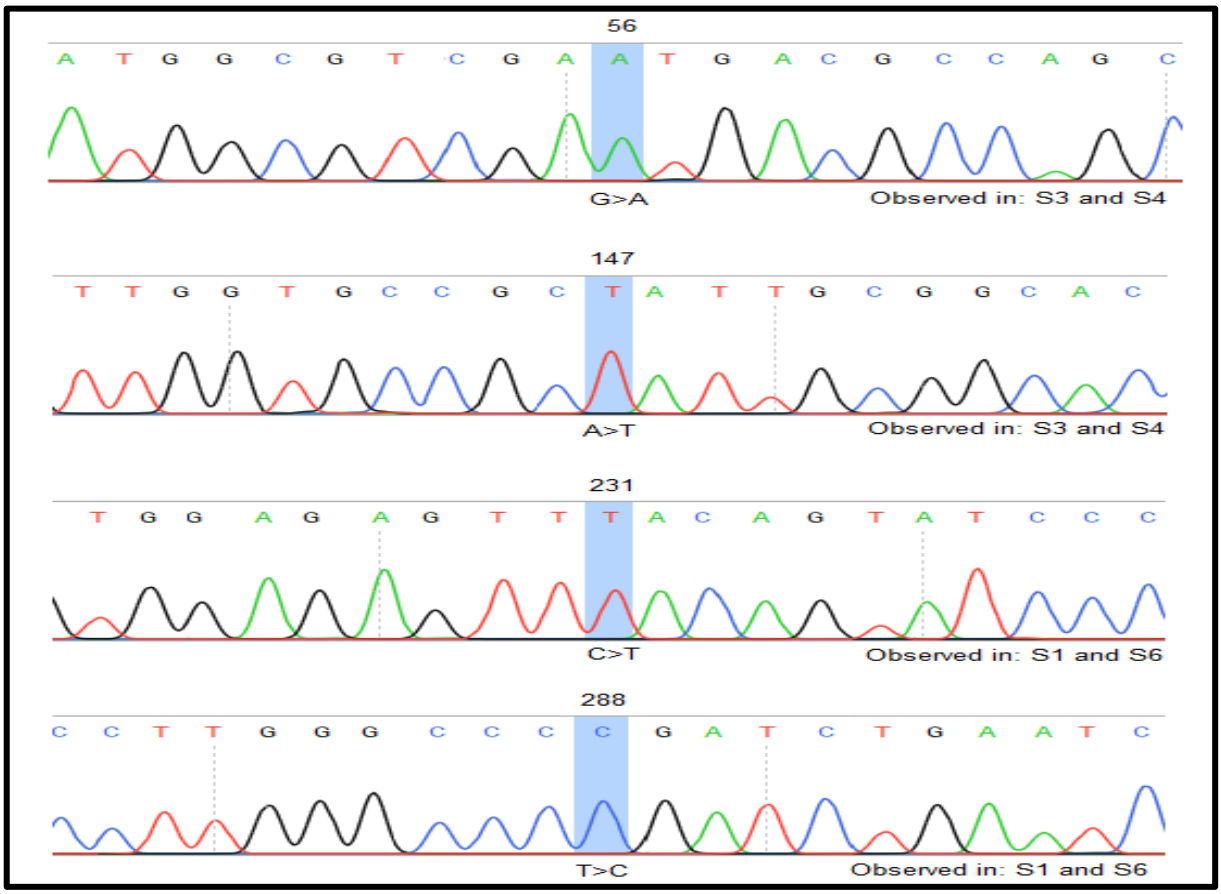

Figure 4.6. The chromatogram of the investigated human norovirus. The clarity of the observed peaks refers to the strict contamination-free technical parameters followed to validate each variant in the present samples. The letter "S" refers to the code of the investigated samples in this study. The identified variations were respectively arranged according to the names of the investigated samples (S1 to S8).

The observed nucleic acid variations were further analyzed to identify whether such substitutions induce possible alteration in their corresponding positions in the major capsid protein. All nucleic acid sequences of S1 to S8 were translated to their corresponding amino acid sequences using the Expasy translate suite. Results from the direct nucleic acid translation of the 56G>A indicated that this variant showed a missense effect on the protein, namely p.6S>N.

Whereas the amino acid alignment of these amino acid sequences with their references showed that three of these variants (147A>T, 231C>T, and 288T>C) exhibited silent effects on the major capsid protein within the size of the amplified loci Table (4.7) and **Figure** (4-9 a).

These synonymous (silent) variants was detected in various samples and exemplified in the entire major capsid protein sequences, namely p.36A=, p.64F=, and p.83P= Table (4.7) and **Figure** (4-9 b).

However, these amino acid alterations may be developed by the invading viruses as an adaptation to drugs that are directed toward its targeted major capsid protein (Legnardi *et al.*, 2020). To summarize all the results obtained from the sequenced 343 bp fragments, the exact positions of the observed mutation was described in Table (4.7).

Table 4.7. The pattern of the observed mutation in the 343 bp of the VP1 gene amplicons in comparison with the NCBI referring sequences (GenBank acc. EU310927.1).

No.	Sample	Native	Allele	Position in the PCR fragment	Position in the reference sequences	Type of variant	Variant summary
1.	S3, S4	G	A	56	5101	Missense (6S)	p.6S>N
2.	S3, S4	A	T	147	5192	Silent (36A)	p.36A=
3.	S1, S6	C	T	231	5276	Silent (64F)	p.64F=
4.	S1, S6	T	C	288	5333	Silent (83P)	p.83P=

The identified variations were respectively arranged according to the names of the investigated samples (S1 to S8). The letter "p." refer to the "protein" position in which the variation was detected. The symbol > and = refer to the missense and silent effect of a variant respectively.

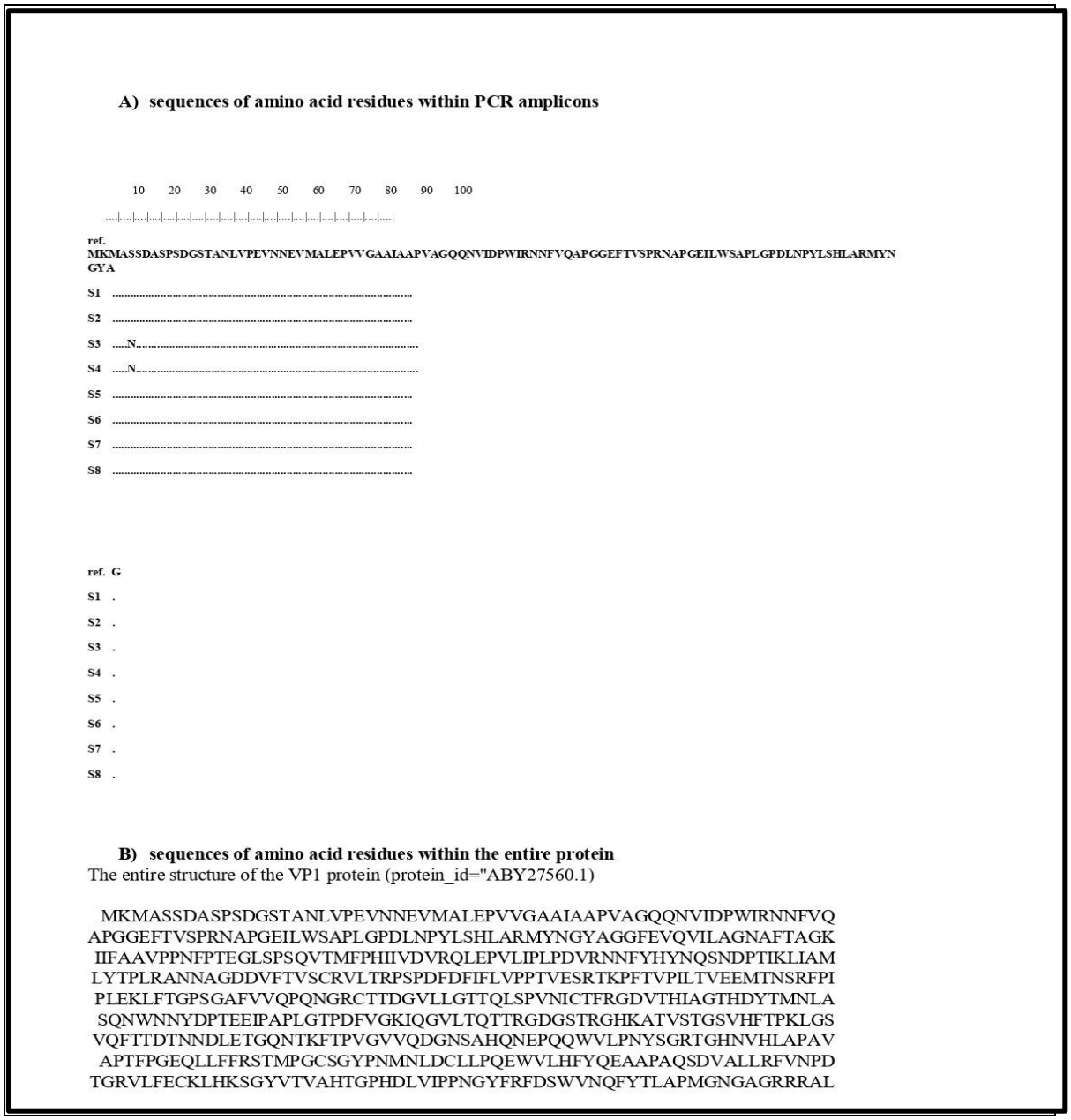

Figure 4.7.Amino acid residues alignment of the detected variations of the VP1-encoded major capsid protein within the investigated human-infecting norovirus samples. A) The amino acid substitutions are highlighted according to their corresponding positions within the amplified 343 bp locus. B) The amino acid substitutions are highlighted according to their corresponding positions within the entire protein. The grey highlights refer to the amplified region of the VP1-encoded glycoprotein. The cyan colors refer to the amino acid non-synonymous variations in the entire protein sequence.

4.5.2.4. Phylogenetic of Norovirus Isolates from Patients with GE

To give a phylogenetic understanding of the actual distances between these investigated samples and the most relative reference strains of human norovirus, a comprehensive phylogenetic tree was generated in the present study according to nucleic acid variations observed in the amplified 343 bp of the VP1 gene amplicons. This phylogenetic tree contained S1 to S8 samples alongside other relative Nuclotide sequences of human norovirus sequences.

Within this tree, investigated the local sample were samples were incorporated alongside relative sequences to constitute the majority of the incorporated sequences within the generated cladogram. The total number of the aligned nucleic acid sequences in this comprehensive tree was forty-one. Two types of cladograms were generated to explain two different representations of the incorporated human norovirus sequences, a rectangular cladogram **Figure (4-10 a**), and a circular cladogram **Figure (4-10 b**).

In both cases of the constructed cladogram, the investigated samples were clustered into five phylogenetic clades within the human norovirus sequences. The most interesting fact observed in our investigated viral isolates is correlated with the positioning of our investigated samples into two related important genotypes within the human norovirus, these serotypes are genotype II (GII) and genotype III (GIII). Within the GII, six investigated samples (S1, S2, S5, S6, S7, and S8) were incorporated to constitute one large clade of GII that was made of twenty-nine samples. Within this major clade, our samples slightly deviated into different positions. This sort of slight diversity was reflected by the slight evolutionary effects of the observed nucleic acid substitutions of 231C>T and 288T>C in inducing tilts in the incorporated tree. Thus, these variations were only minor deviations within the same genotype. Furthermore, the aggregation of all investigated viral samples with each other may refer to the presence of only close patterns of the phylogenetic distribution of these sequences. The current observation of this tree has confirmed sequencing reactions because it explained the actual neighbour-joining-based positioning in such observed variations.

Slight phylogenetic distances was observed between the samples of GIII and the nearest serotypes of the same human noroviruses. Within the GIII clade, both S3 and S4 were incorporated. The reason behind the different positioning of both samples was attributed to the detection of the 56G>A and 147A>T. Both variants caused a dramatic conversion of their positioning from the GII clade to GIII clade. This observation indicates a considerable evolutionary roles for 56G>A and 147A>T in changing the genotype of the investigated norovirus sequences.

Thus, the currently observed nucleic acid variations was noticeable distributions within the same viral genotype in altering the current positioning of the investigated viral samples.

Next to the clade of GII, human norovirus samples of GI were suited. This observation indicated the human norovirus GI represents the closest genotype to the samples of GII according to VP1 sequences. However, the VP1-based GI sequences are less frequently deposited in the NCBI database since the clade of GI was made of only two samples of two phylogenetic distances.

Besides the clade of GI, the clades of GV and GIV were respectively suited with distinct phylogenetic positions from both GII and GIII. These data indicated that the VP1 gene sequences showed closer phylogenetic positions between GII and GIII than that found between the other incorporated genotypes.

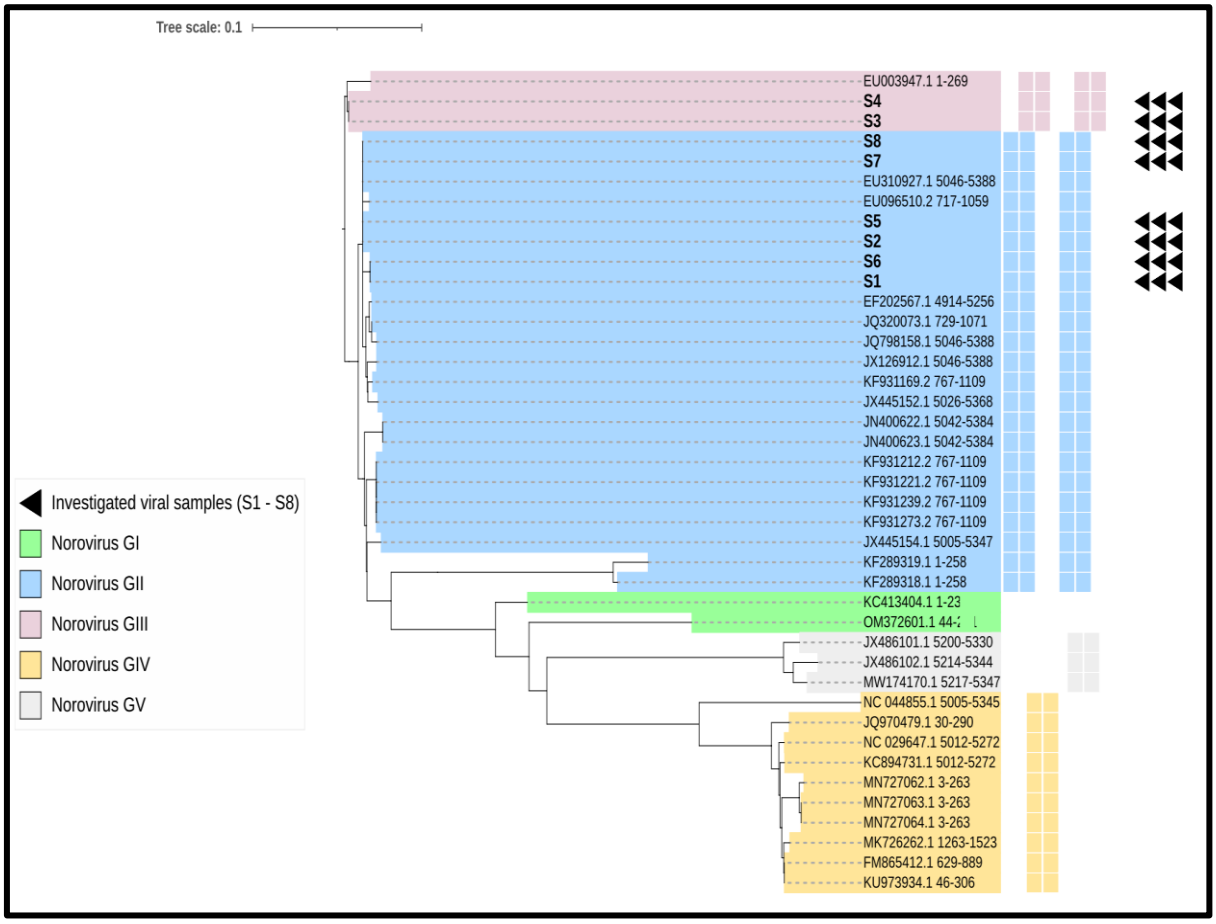

Figure 4.8 The comprehensive rectangular cladogram phylogenetic tree of genetic variants of the VP1 gene fragment of eight human-infecting norovirus samples. The black-colored triangle refers to the analyzed viral variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0.1" at the top portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "S#" refers to the code of the investigated samples.

Noteworthy, the utilization of the VP1 gene sequences in this study has given a remarkable confirmation for the presence of the accurate identification of the actual genotype of this viral organism. It was observed that the investigated viral samples occupied the same positions within the major clade of GII with slight phylogenetic distances. This indicated the presence of high genetic homology among the viral sequences of these strains within the major clade of GII. The other related clades of the other genotypes were found to occupy variable phylogenetic positions away from the major clade of GII sequences.

4.5.2.5. Sample sequence were Submitted in NCBI and the Accession Number of nucleotide sequences of Norovirus GII

SUB11565544 Seq1 ON678619
SUB11565544 Seq2 ON678620
SUB11565544 Seq3 ON678621
SUB11565544 Seq4 ON678622
SUB11565544 Seq5 ON678623
SUB11565544 Seq6 ON678624

4.5.2.6. Sample was submitted in NCBI and the accession number of nucleotide sequences of Norovirus GIII

SUB11565548 Seq1 ON678617
SUB11565548 Seq2 ON678618

4.5.3. Detection and Qualtitative of Astrovirus (AsV) Genome By qRT.PCR:

The analysis of AsV results was made by using a Real Time PCR analyzer (Rotor-gene Q MDX /thermal cycler integrated with a system for fluorescence detection and a dedicated software). The total positive result of AsV according to qRT-PCR shows 18.1% (14 out of 77 cases) as positive less than 81.9% (63 out of 77 cases) as negative, as shown in Table (4-8) as well as Figures (4-11-A & B) . Statistically significant differences (p = 0.04) among patients group.

Table 4.8. Percentage of AsV Positive Signals in Patients with GE by Using qRT.PCR Technique.

AsV Genome	No.	%	P value
Positive	**14**	18.1	**P=0.04 Sign >0.05**
Negative	63	81.9	
Total	77	100	

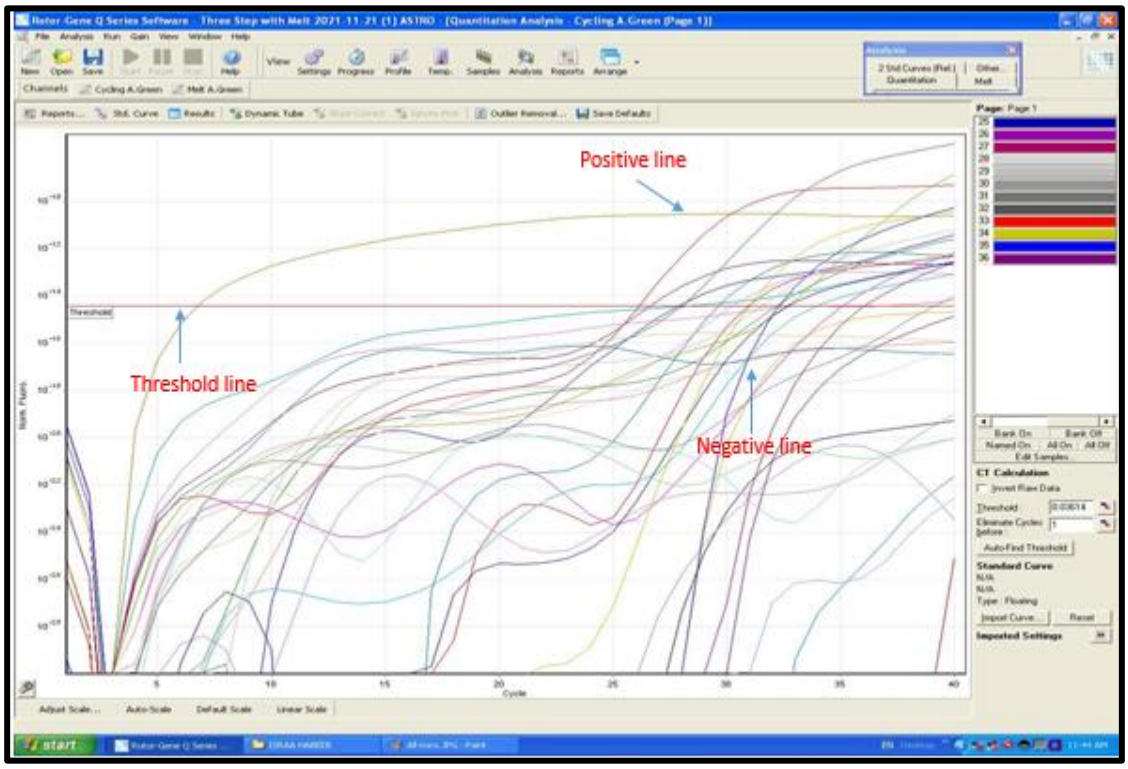

Figure 4.9 . A: Detection of Astrovirus by RT-PCR

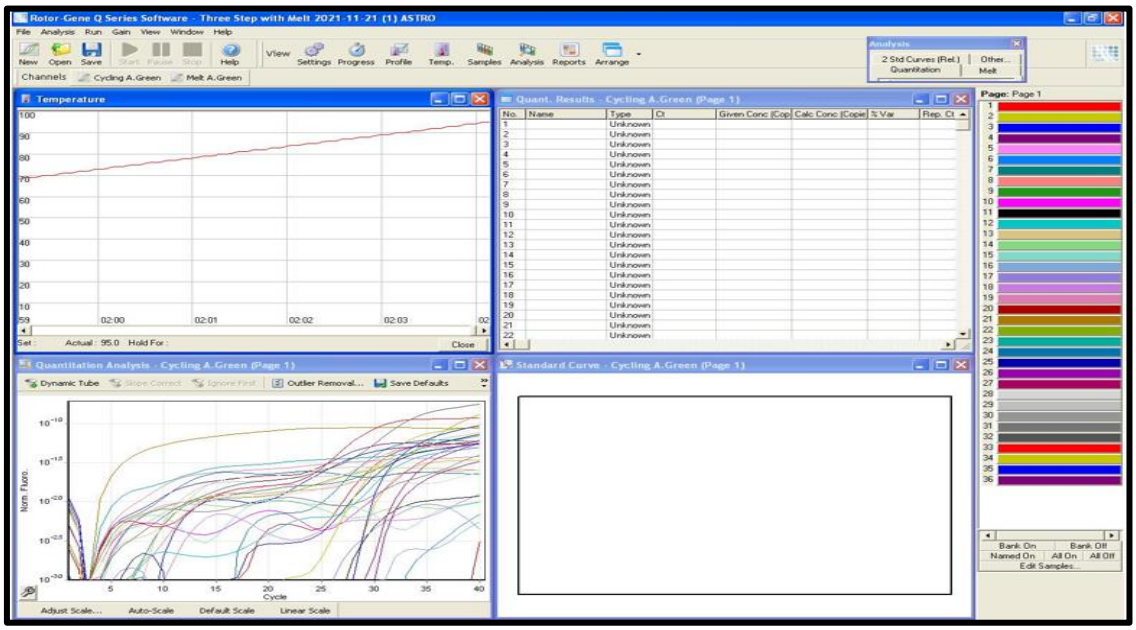

Figure 4.9.B: Qualtitive & melting curve for detection of by RT-PCR

4.5.3.1.The Results of Astrovirus in the Patients With GE According to the Age group.

In gastroenteritis, the most commonly affected age group infected with AsV was (3-36 months) which constituted 9.1% (7 out of 77 cases), while the age group (37-72 months) was constituted 5.1% (4 out of 77 cases), followed by 3.9% (3 out of 77 cases) in age group (73 – 120 months). Statistical comparison of these age strata revealed significant differences ($p < 0.05$) Table (4-9).

Table 4.9. Frequency of AsV RT-PCR Signal Among The Patients With GE According to the Age Stratum.

	Months	AsV			P value
		No.	Positive	Negative	
Age group	3-36	44 57.1%	7 9.1%	37 48.1 %	Anova test P=0.02 Sign. (P<0.00)
	37-72	21 27.2%	4 5.1%	17 22.1%	
	73-120	12 15.7%	3 3.9%	9 11.7%	
Total		77 100%	14 18.1%	63 81.9%	

4.5.3.2: Sequencing of Human AsV of Clinical Isolates

One specific PCR fragment partially covering the coding regions of the ORF1 gene (289 bp) in human-infecting astrovirus was amplified in this study **Figure (4-11)**. The amplified PCR fragments were directly exposed to Sanger sequencing

experiments to assess the pattern of genetic polymorphism in the clinical viral samples.

All the observed Nuclotide variants were translated to their corresponding amino acid sequences using the Expasy translate server. A specific comprehensive tree was built to assess the accurate genotyping of the observed variants and their phylogenetic distribution. After positioning the 289 bp amplicons' sequences within the genomic sequences of the human astrovirus, the details of its sequences were highlighted, and the total length of the amplified amplicons was also determined Table (4-10).

Table 4.10: The position and length of the 289 bp PCR amplicons that used to partially amplify the coding portions of the ORF1a gene within human astrovirus genomic sequences (GenBank acc. no. MH933759.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
ORF1a gene Nucleic acid sequences of the viral ORF1a gene	*CGTCATTATTCGTTGTTATACTAACCTGTAGATTCATCC GTATGGCAACGGTTTTTATTGGCACCAGATTCGAGGTCC GTGATGCCAATGGGAAGGTTGTGGCTACTGTACCAACCA GAATTAAAAATGTGGCATTTGACTTTTTCCAGAAGCTAA AACAGTCAGGGGTGAGGGTTGGAGTCAATGAATTTGTTG TTATAAAGCCAGGTGCATTATGTGTCATAGACACCCCTG AAGGGAAAGGGACAGGTTTCTTTTCTGGCAACGACATAG TAACAGCAGCACATGT**	289 bp

*refers to forward primer placed in the forward direction
**refers to reverse primer placed in the reverse complement direction

4.5.3.3.Sequences Alignment Fragment Results of ORF Gene (289 bp) in Human-infecting Astroviruses

Interestingly, the alignment results of the 289 bp samples reveale the presence of four Nuclotide variations represented by four Nuclotide substitutions in three loci in the analyzed samples in comparison with the most similar referring reference nucleic acid sequences (GenBank acc. no. MH933759.1) Figure (4-

13). The present results indicate the presence of three Nuclotide variants in the investigated samples, namely 86C>T, 131G>A, and 243G>A detected in several investigated clinical samples.

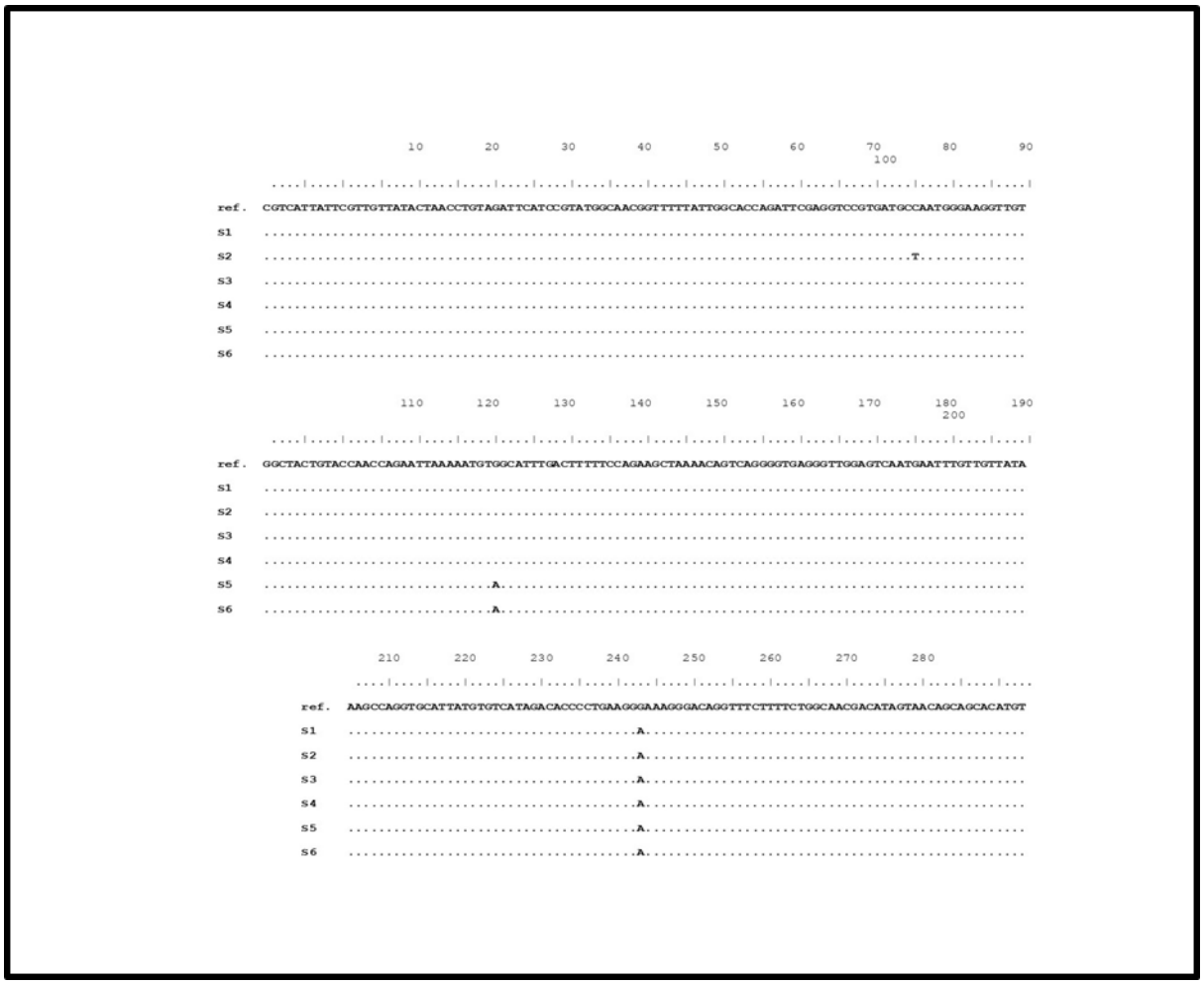

Figure 4.10: Nucleic acid sequences alignment of six samples with their corresponding reference sequences of the 289 bp amplicons of the ORF1a genetic sequences. The symbol "ref" refers to the NCBI referring sequence (GenBank acc. no. MH933759.1), letter "S#" refers to the sample number.

In addition, current results indicate the presence of three nucleic acid variants in the investigated samples, namely 86C>T, 131G>A and 239G>A detected in several investigated clinical samples.

To confirm these variations, the sequencing chromatograms of the investigated samples, as well as their detailed annotations, were verified and documented, and the chromatograms of their sequences were shown according to their

positions in the PCR amplicons. The presence of each one of these variants was confirmed in its original chromatograms and the absence of any possible technical error was also confirmed Figure (4-14).

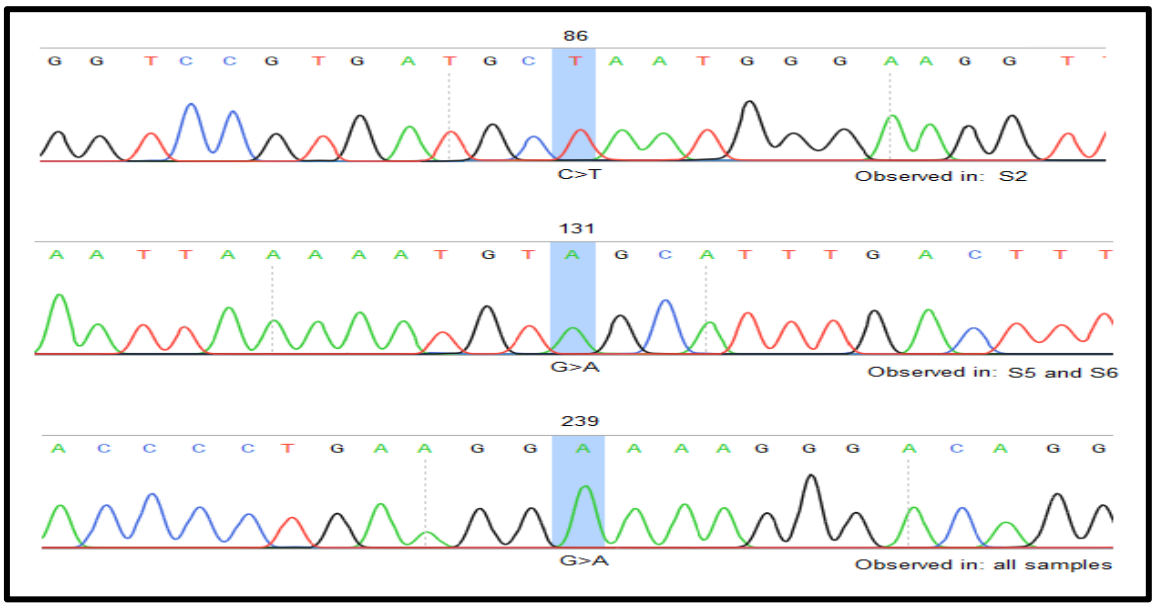

Figure 4.11 The chromatogram of the investigated human astrovirus. The clarity of the observed peaks refers to the strict contamination-free technical parameters followed to validate each variant in the present samples. The letter "S" refers to the code of the investigated samples in this study. The identified variations were respectively arranged according to the names of the investigated samples (S1 to S6).

The observed nucleic acid variations was further analyzed to identify whether such substitutions induce possible alteration in their corresponding positions in the major capsid protein.

All nucleic acid sequences of S1 to S6 was translated to their corresponding amino acid sequences using the Expasy translate suite. Amino acid alignment of these amino acid sequences with their references showed that nine variants exhibited silent effects on the nonstructural protein within the size of the amplified loci Figure (4-15 a).

These synonymous (silent) variants was detected in various samples and exemplified in the entire nonstructural protein sequences, namely p.364A=, p.409V=, and p.445A= Table (4.11) and Figure (4-15 b).

To summarize all the results obtained from the sequenced 289 bp fragments, the exact positions of the observed mutation was described in Table (4.11).

Table 4.11. The pattern of the observed mutation in the 289 bp of the ORF1a gene amplicons in comparison with the NCBI referring sequences (GenBank acc. MH933759.1).

No.	Sample	Native	Allele	Position in the PCR fragment	Position in the reference sequences	Type of variant	Variant summary
1.	S2	C	T	86	1260	Silent (364A)	p.364A=
2.	S5, S6	G	A	131	1305	Silent (409V)	p.409V=
3.	S1 – S6	G	A	239	1413	Silent (445A)	p.445A=

The identified variations were respectively arranged according to the names of the investigated samples (S1 to S6). The letter "p." refer to the "protein" position in which the variation was detected.

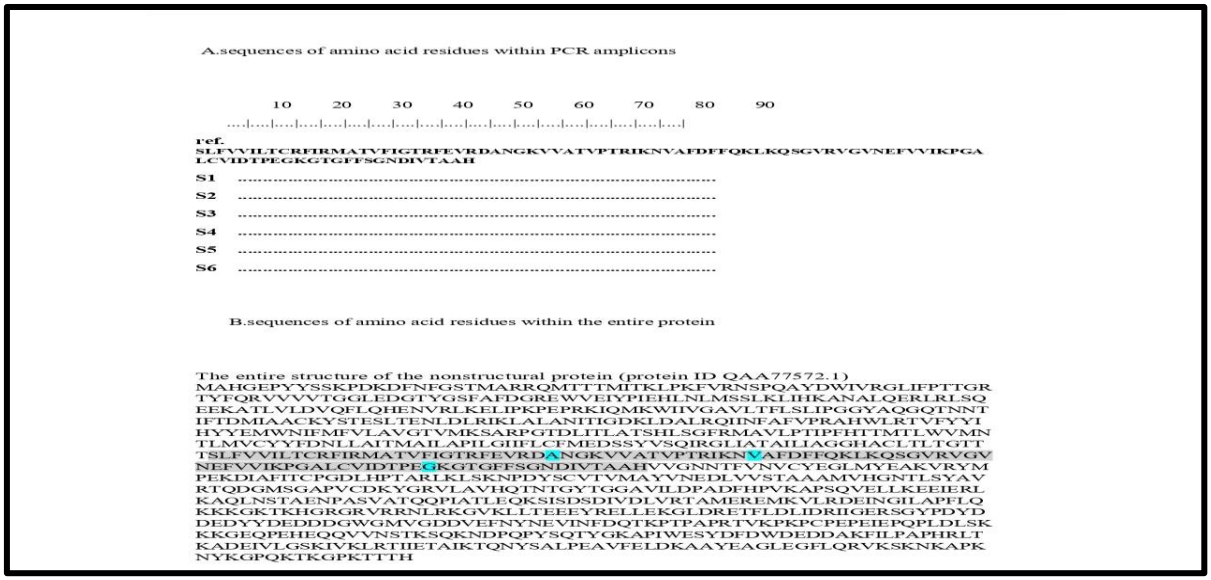

Figure 4.12: Amino acid residues alignment of the detected variations of the ORF1a-encoded nonstructural protein within the investigated human-infecting astrovirus samples. A) The amino acid substitutions are highlighted according to their corresponding positions within the amplified 289 bp locus. B) The amino acid substitutions are highlighted according to their corresponding positions within the entire protein. The grey highlights refer to the amplified region of the ORF1a-encoded glycoprotein. The cyan colors refer to the amino acid synonymous variations in the alignment chart.

4.5.3.4. Phylogenetic of Astrovirus Isolates from Patients with GE

To give a phylogenetic understanding of the actual distances between these investigated samples and the most relative reference strains of human astrovirus, a comprehensive phylogenetic tree was generated in the present study according to nucleic acid variations observed in the amplified 289 bp of the ORF gene amplicons. This phylogenetic tree contained S1 to S6 samples alongside other relative nucleic acid sequences of human astrovirus sequences.

Within this tree, the local samples were incorporated alongside relative sequences to constitute the majority of the incorporated sequences within the generated cladogram. The total number of the aligned nucleic acid sequences in this comprehensive tree was forty-one. Two types of cladograms were generated to explain two different representations of the incorporated human norovirus sequences, a rectangular cladogram **Figure (4-16 a)**, and a circular cladogram **Figure (4-16 b)**.

In both cases of the constructed cladogram, the investigated samples were clustered into eight phylogenetic clades within the human astrovirus sequences. The most interesting fact observed in our investigated viral isolates is correlated with the positioning of our investigated samples and their neighbour sequences into eight related important serotypes within the human astrovirus, these serotypes are ranged from serotype-1 to serotype-8. Within the serotype-3, all investigated samples (S1 to S6) were incorporated to constitute one large clade of serotype-3 that was made of 16 samples. Within this major clade, our samples slightly deviated into different positions. This sort of slight diversity was reflected by the potential evolutionary effects of the observed nucleic acid substitutions in inducing such tilts in the incorporated tree. Despite the noticeable alterations these nucleic acid substitutions (86C>T, 131G>A, and 239G>A) made within the serotype-3, these variations were only minor deviations within the same serotype. Furthermore, the aggregation of all investigated viral samples with each other may refer to the presence of only close patterns of the phylogenetic distribution of these sequences. The current

observation of this tree has confirmed sequencing reactions because it explained the actual neighbour-joining-based positioning in such observed variations. Distinct phylogenetic distances were observed between the samples of serotype-3 and the nearest serotypes of the same human astroviruses. Thus, the currently observed nucleic acid variations were only a minor tilt within the same viral serotype without taking and noticeable evolutionary effect in altering the current positioning of the investigated S1 – S6 samples.This finding strongly suggested that these (S1 – S6) samples may represent known genetic variations of the serotype-3 within the human astrovirus sequences.

Next to the clade of serotype-3, human astrovirus samples of serotype-1 were suited. This observation indicated the human astrovirus serotype-1 represents the closest serotype to the samples of serotype-3 according to ORF1a sequences. The clade of serotype-1 was made of 30 samples of variable phylogenetic distances. Besides the clade of serotype-1, the clades of serotype-2, serotype-7, and serotype-6 were respectively suited with distinct phylogenetic positions from both serotype-3 and serotype-1. On the other portion of the tree, the viral sequences of serotype-5, serotype-8, and serotype-4 were respectively located in other distinct phylogenetic distances. These data indicated that the ORF1a gene sequences showed closer phylogenetic positions between serotype-3 and serotype-1 than that found between the other incorporated serotypes. This tree showed that the most diverse clades among all observed clades were represented by serotype-3 and serotype-1. Within both serotypes, a higher number of human astrovirus sequences were incorporated with various distances. Our investigated samples represented a special type of serotype-3, while the other incorporated sequences of serotype-3 were found in close phylogenetic positions to some of the samples of serotype-1 within the same tree.

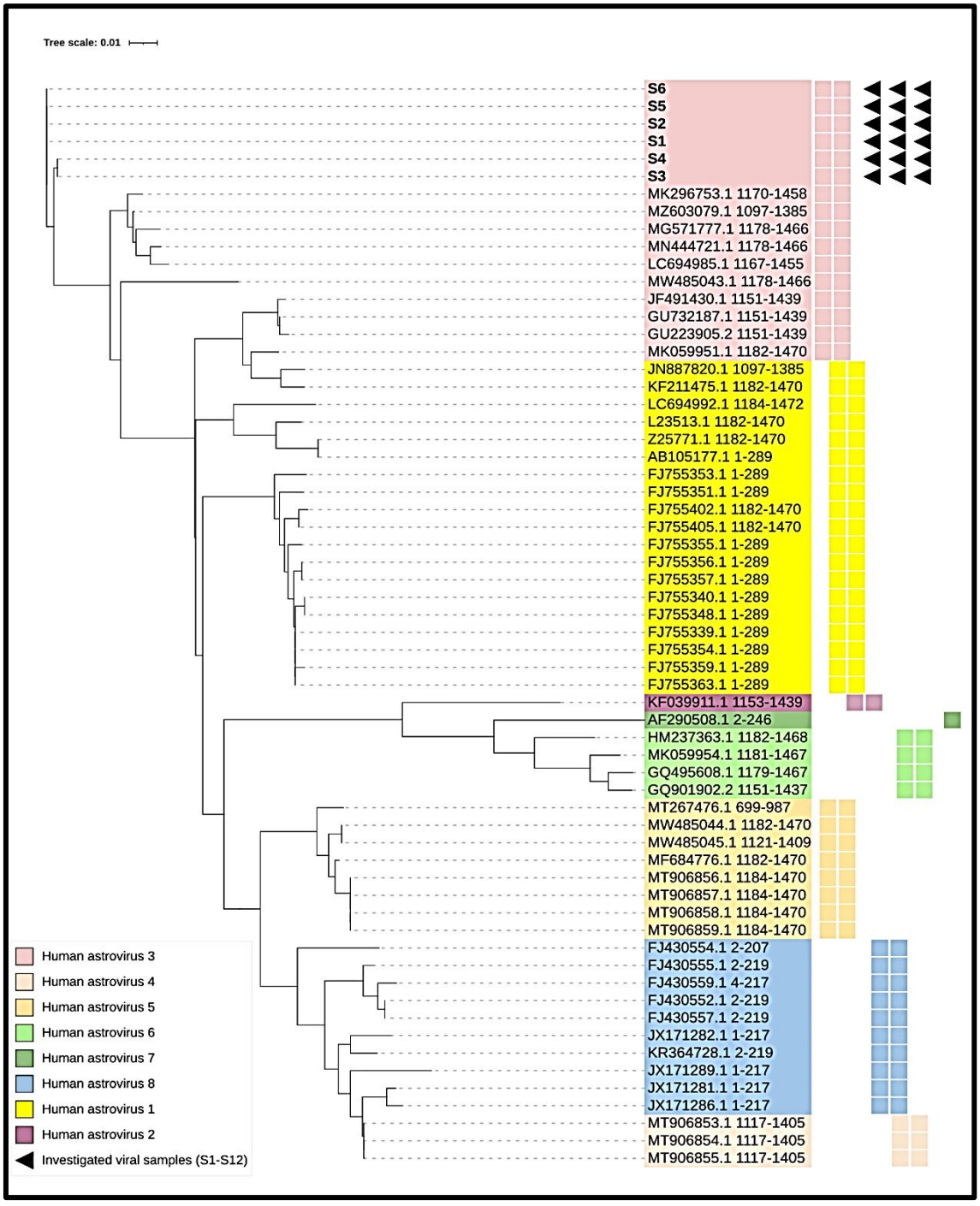

Figure 4.13 The comprehensive rectangular cladogram phylogenetic tree of genetic variants of the ORF1a gene fragment of six human-infecting astrovirus samples. The black-colored triangle refers to the analyzed viral variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0.01" at the top portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "S#" refers to the code of the investigated samples

Noteworthy, the utilization of the ORF1a gene sequences in this study has given a noticeable confirmation for the presence of the precise identification of the actual serotype of this viral organism. It was found that the investigated viral

samples occupied the same positions within the major clade of serotype-3 with extremely phylogenetic distances. This indicated the presence of high genetic homology among the viral sequences of these strains within the major clade of serotype-3.

The other related clades of the other serotypes was found to occupy variable phylogenetic positions away from major clade serotype-3 sequences. This data may have shown no potential phylogenetic effect of the observed nucleic acid variations on inducing remarkable alterations in the investigated viral strains.

However, this ORF1a gene-based comprehensive tree has provide an inclusive tool for the high ability of such genetic fragments to efficiently identify viral serotypes using the ORF1a genetic fragment. This, in turn, gives a further indication of the ability of the currently utilized ORF1a gene-specific primers to describe the investigated human astroviruses and their accurate phylogenetic positions.

4.5.3.5. Sample was submitted in NCBI and the accession number of nucleotide sequences of Astrovirus

BankIt2589912 Seq1 **ON669284**
BankIt2589912 Seq2 **ON669285**
BankIt2589912 Seq3 **ON669286**
BankIt2589912 Seq4 **ON669287**
BankIt2589912 Seq5 **ON669288**
BankIt2589912 Seq6 **ON669289**

4.6.The Results of Gene Polymorphism of Toll-like receptor -7 (TLR-7 rs3853839) and Interferon-gamma (IFN-γ rs9976971) SNPs

4.6.1. Extraction Total Genome DNA from the Stool Swabs

By using specific Total genome DNA extraction kit (G-Spin total DNA Extraction kit, Intron / Korea) the genomic DNA Figure (4-17) was extracted ,purifying and migrated using agarose gel from the stool swabs specimens of patients with GE as well as apparently healthy control groups as a first step to amplify the target region of **TLR-7 rs3853839 and IFN-γ rs9976971** genes.

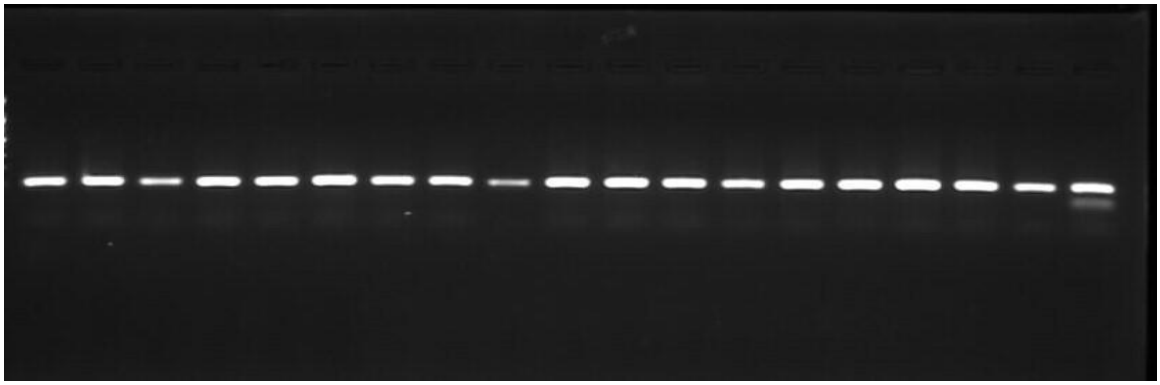

Figure 4.14: The electrophoresis pattern of gnomic DNA extracted from stool swabs samples of GE patients and healthy control groups ; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (5 µl in each well), stained with red safe solution.

4.6.2.Genotyping of TLR-7 (rs3853839) Gene in GE and AHC

For TLR-7 rs3853839 genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal condition as mentioned in the table (3-7). The results revealed that the presence a single band (408 bp) of the target sequence of TLR-7 rs3853839 gene in agarose gel Figure (4-18)

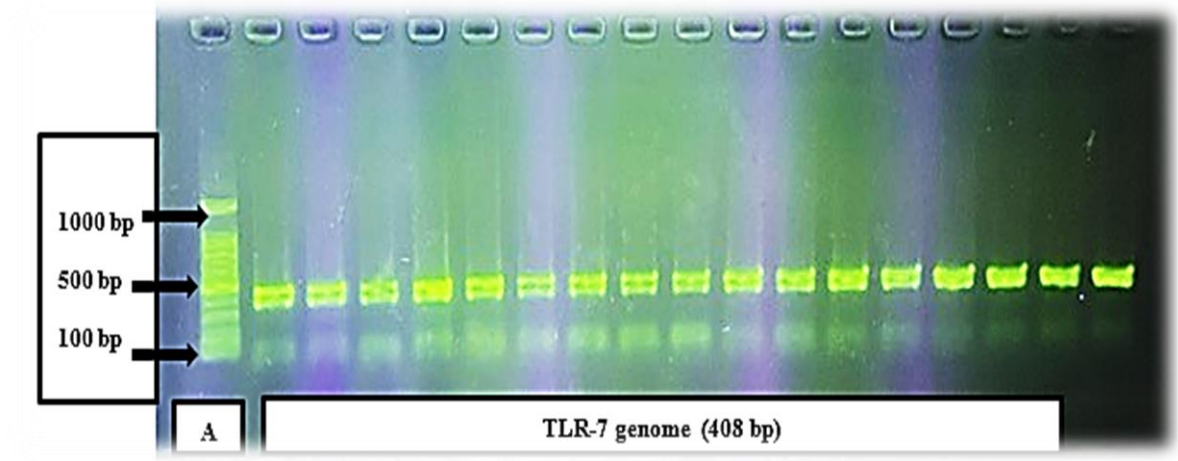

Figure 4.15: Agarose gel electrophoresis of an amplified product patterns of TLR-7 rs3853839 exon5 region. Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (5 µl in each well), stained with red safe solution.

Table (4-12) show the percentage of a single band (408 bp) of the target sequence of **TLR-7 rs3853839** gene. The positive result, according to PCR amplification of a single band (408 bp) of **TLR-7 rs3853839**gene in women patients with GE and AHC were 30% (45 of 150 cases) and 10% (5 of 50 cases),

respectively . While, the negative results were in patients with GE and AHC were 70% (105 of 150 cases) and 90% (45 of 50 cases), respectively as shown the Table (4-12).

Table 4.12: Percentage of TLR-7 rs3853839 signals in patients with GE and AHC groups by PCR technique.

TLR-7 rs3853839 gene band	**GE No.(%)**	**AHC No.(%)**
Heterozygote	45 (30%)	5 (10%)
Homozygote	105 (70%)	45 (90%)
Total	150 (100%)	100%) 50

4.6.2.1.Genotyping of TLR-7 rs3853839 Among Study Groups.

The results show that DNA polymorphism distribution were DNA polymorphism distributions according to CA ; AT ; **TA** and GA genotypes of **TLR-7 rs3853839** polymorphism were respectively 62.2% (28 out of 45 cases) ; 31.1% (14 out of 45 cases); 6.7% (3 out of 45 cases) and 0% (0 out of 45 cases) in the GE patient group and was found GA genotype 100 % (5 out of 5 cases) in the control group. In addition , was found just transversion mutation in GE patients , while in control group just transition mutation in **TLR-7 rs3853839** gene **Table (4-13).**

Table 4.13: Comparison between patient with GE and HC on percentages of TLR-7 rs3853839 gene polymorphism.

Polymorphism of TLR-7 rs3853839 gene	Type of Mutation	Study group		OR [Patients]	OR [Control]	*P value*	95% C.I for OR [Patients]	
		HC NO.(50)	GE NO.(150)				*lower*	*Upper*
C\A	Transversion	0.0%	62.2%	0.7	1.1	**0.08**	0.79	0.95
A\T	Transversion	0.0%	31.1%	0.8	1.3	**0.001**	0.85	0.95
T\A	Transversion	0.0%	6.7%	0.7	1.1	**0.08**	0.79	0.95
G\ A	Transtion	100%	0.00%	0.7	1.2	**0.01**	0.90	0.99

4.6.2.2.Sequences Alignment Fragment Results of TLR-7 rs3853839 Gene, Exon-5 Region by Bio Edit Program Version 7.2.5

The sequencing results show that many SNPs between the one resolved haplotypes and between the **TLR-7 rs3853839**, exon5 for Primer3 [plus] reference sequences. The results appeared in the presence of nine SNPs Figure (4-18). Reveal that which located at positions 22; 341; 354 a substitution mutation A→C ; positions 8; 361 substitution A→ T ; position 18 substitution T→ A; and last one at position 113 substitution C→ A Figure (4-19) according to the reference sequence alignment of the human **TLR-7 rs3853839** gene.

```
          10        20        30        40        50        60        70        80        90       100
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
reference
ACTGACAAATACAGTCATGGGGTTGGGGATGCTGTTTAGACAGCTGAAAATAAGACCTGAATTGTTTATTTTTAAAATGTTGCAAAAG
AGAGGCAGCAAA
IS24      ................A...A.....................................................................
IS25      ....................................................................................
IS29      ....................................................................................
IS48      ....................................................................................
IS53      .......T............................................................................
IS55      ....................................................................................

          110       120       130       140       150       160       170       180       190       200
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
reference
TGGGAATTTTTAATTCTGATTCTTGGTATGTTTTAGAACAATGATTTGTTCTTTCTTATACTTTCAGGTGTTTCCAATGTGGACACTGAA
GAGACAAATT
IS24      ....................................................................................
IS25      ....................................................................................
IS29      ....................................................................................
IS48      ...........C........................................................................
IS53      ....................................................................................
IS55      ....................................................................................

          210       220       230       240       250       260       270       280       290       300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
```

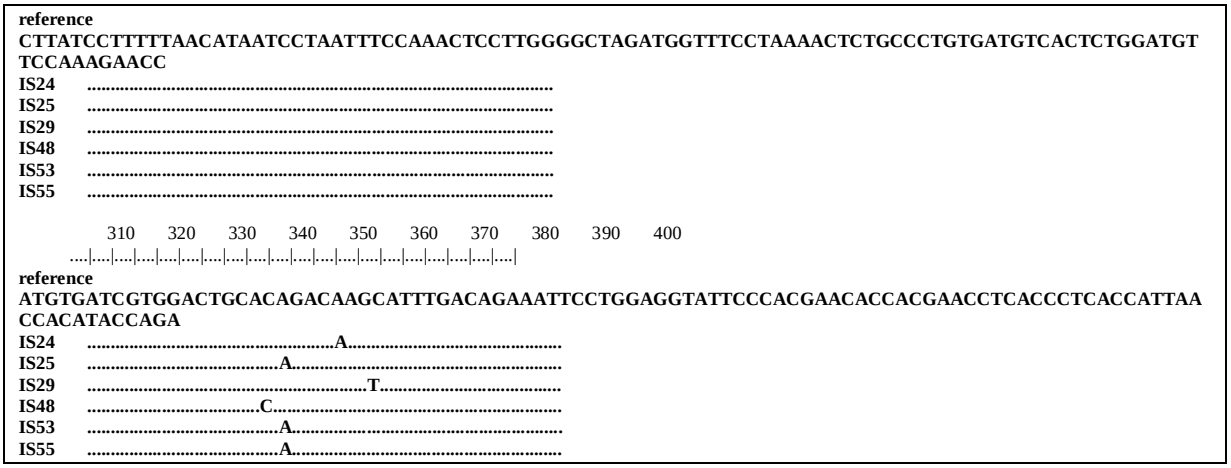

Figure 4.16: Sequences alignment fragment results of TLR-7 rs3853839 gene, Exon-5 region by Bio Edit program version 7.2.5

The recording six new recording in gene bank NCBI & American bank Unde ACCESSION NUMBERS :

LC715217

LC715218

LC715219

LC715220

LC715221

LC715222

4.6.3.Genotyping of IFN-γ rs9976971 Gene in GE and AHC

For **IFN-γ rs9976971** genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal condition as mentioned in the table (3-7). The results reveal that the presence a single band (441 bp) of the target sequence of **IFN-γ rs9976971** gene in agarose gel Figure (4-20).

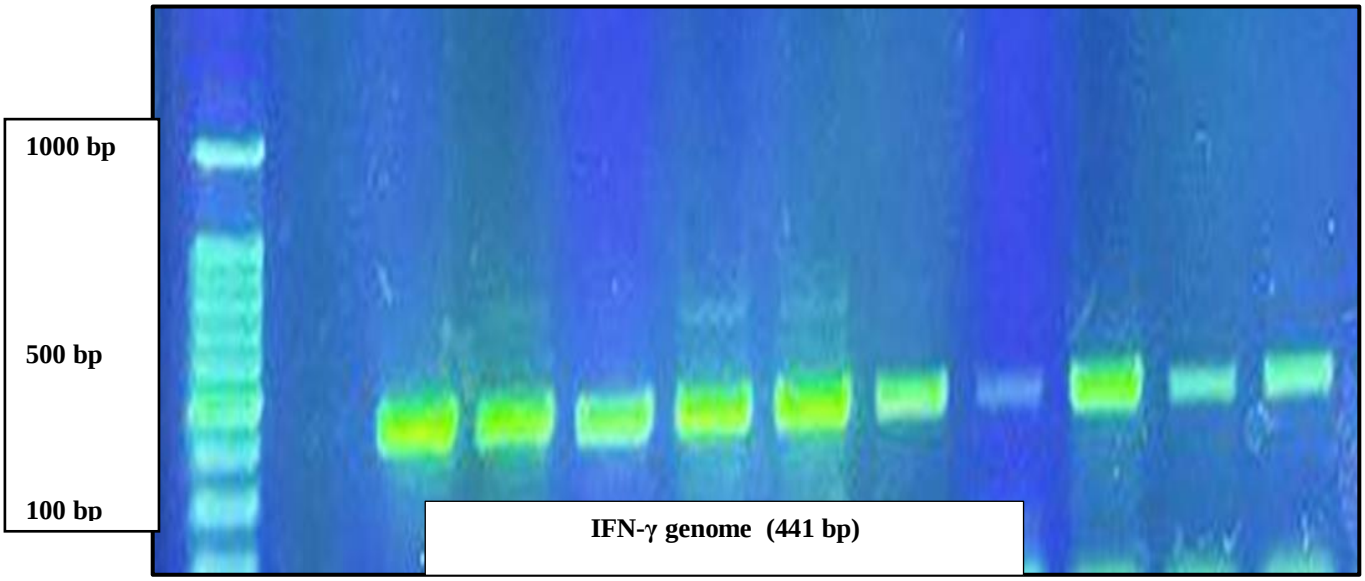

Figure 4.17: Agarose gel electrophoresis of an amplified product patterns of IFN-γ rs9976971exon5 region. Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (5 µl in each well), stained with red safe solution.

Table (4-14) shows the percentage of a single band (441 bp) of the target sequence of **IFN-γ rs9976971** gene. The positive result, according to PCR amplification of a single band (441 bp) of **IFN-γ rs9976971**gene in patients with GE and AHC were 21.3% (32 of 150 cases) and 8% (4 of 50 cases), respectively . While, the negative results were in patients with GE and AHC was 78.7% (118 of 150 cases) and 92% (46 of 50 cases), respectively as shown the Table (4-14).

Table 4.14: Percentage of IFN-γ rs9976971 signals in patients with GE and AHC groups by PCR technique.

IFN-γ rs9976971 gene band	GE No.(%)	AHC No.(%)
Heterozygote	32 (21.3%)	5 (8%)
Homozygote	118 (78.7%)	45 (92%)
Total	150 (100%)	50(100%)

4.6.3.1.Genotyping of IFN-γ rs9976971 Among Study Groups

The results showed that DNA polymorphism distribution were DNA polymorphism distributions according to GA ; CT ; **AT** and AG genotypes of **IFN-γ rs9976971** polymorphism were respectively 53.1% (17 out of 32 cases) ; 21.9% (7 out of 32 cases); 15.6% (5 out of 45 cases) and 9.3% (3 out of 32 cases) in the GE patient group .

While, polymorphism distributions according to GA and AG genotypes of **IFN-γ rs9976971**polymorphism were 60%(3 out of 5 cases) and 40% (2 out of 5 cases) ; respectively in the control group. In addition , was found just transversion mutation in GE patients , while in control group just transition mutation in **IFN-γ rs9976971** gene **Table (4-15)**. The frequency of transversion mutation more than the transition mutation (A\G).

Table 4.15: Comparison between patient with GE and HC on percentages of IFN-γ rs9976971 expressed gene polymorphism

Polymorphism of IFN-γ gene	Type of Mutation	Study group		OR [Patients]	OR [Control]	*P value*	95% C.I for OR [Patients]	
		HC NO.(50)	GE NO.(150)				*lower*	*Upper*
G\A	Transtion	60%	53.1%	0.8	1.7	**0.001**	0.83	0.99
C\T	Transversion	0.0%	21.9%	0.7	1.4	**0.008**	0.77	0.97
A\T	Transversion	0.0%	15.6%	0.6	1.3	**0.006**	0.80	0.96
A\G	Transtion	40%	9.3%	0.8	1.1	**0.003**	0.88	0.95

4.6.3.2.Sequences Alignment Fragment Results of IFN-γ rs9976971 Gene, Exon-5 Region by Bio Edit Program Version 7.2.5

The sequencing results show that many SNPs between the one resolved haplotypes and between the **IFN-γ rs9976971**, exon5 for Primer3 ^{plus} reference sequences.

The results appeared in the presence of ninteen SNPs Figure (4-6). Which revealed that which located at positions 20; 21;74 a substitution mutation G→A ; positions 48 substitution C→ T ; position 19 substitution A→ T; and last one at position 405 substitution A→ G Figure (4-21) according to the reference sequence alignment of the human **IFN-γ rs9976971** gene.

```
    10       20       30       40       50       60       70       80       90       100
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
reference
GGAGGATCCCTCCTGGGGCGGAAGGAGCAGTTGCGCTGCCCCCAGCTCAGCGCCTCTTCCTCCTGCGGGACAAGCGGCGCTTATCGC
ATACGGCTAGGCC
IS21    ..........................................................A.....................................
IS22    ................A.....................T.....................................................
IS23    ................A....A.....................................................................
IS24    ................A.....................................................................

              110      120      130      140      150      160      170      180      190      200
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
reference
CCCTCGCCAGGGCCCCTAACCTCTGCACAGTCTGGGATTCCTGGACGTGGATGGGTACTGGCAGCGCACGGTCGTGCCTGTCGTGTA
CTGAACCAGGGAG
IS21    ..........................................................................................
IS22    ..........................................................................................
IS23    ..........................................................................................
IS24    ..........................................................................................

              210      220      230      240      250      260      270      280      290      300
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
reference
CTCCCCGAAGGCGCGAACCAGGGTTGAATTGCACTCCGCGCTCCCCCAGCAAAGCCCCTCGCCCCGACCTGGAGCCGAGTCCTCCCG
GCAGGGCTCCCTT
IS21    ..........................................................................................
IS22    ..........................................................................................
IS23    ..........................................................................................
IS24    ..........................................................................................

              310      320      330      340      350      360      370      380      390      400
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
reference
CTGTGATTGACCCTGAGCCTGCGTTCGCGCTGACGACGGGGACTGCGGGGGTCTCGTGGTGGGAATTGTGGGCGCTGACATAGGAGA
GGCGCCTGCTGGG
IS21    ..........................................................................................
IS22    ..........................................................................................
IS23    ..........................................................................................
IS24    ..........................................................................................

              410      420      430      440
        ....|....|....|....|....|....|....|....|.
reference  CGCTAGGACGCAGGACCCCTTGGGACAGGAACGGGTGTATG
IS21    ....G.....................................
IS22    .........................................
IS23    .........................................
```

Figure 4.18. Sequences Alignment Fragment Results of IFN-γ rs9976971Gene, Exon-5 Region by Bio Edit program version 7.2.5.

The recording four new recording in gene bank NCBI & American bank Unde

ACCESSION NUMBERS :

LC715223 ; LC715224 ; LC715225 ; LC715226

4.7. Evaluation of Serum TLR-7 and IFN-γ concentration By ELISA Among Study Population

Table (4-16) shows the mean of serum **TLR-7** concentration for AHC and patients with GE groups was **20.60 ± 1.0**pg./ml and **67.28 ± 2.0**pg./ml ,respectively. Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum **TLR-7** concentration among these study groups (Table 4-16) .

Table 4.16: Results of serum TLR-7concentration by ELISA for AHC and patients with GE.

TLR-7	Control (pg/ml)	Case (pg/ml)
Mean± SE	**20.60 ± 1.0**	**67.28 ± 2.0**
LSD	**5.43**	
P value	***P*<0.05 (0.001) ***	

While, the table (4-17) shows the mean of serum **IFN-γ** concentration for AHC and patients with GE groups were 19.66± 1.78 pg./ml and 52.9 ±1.9 pg./ml ,respectively. Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum **IFN-γ** concentration among these study groups (Table 4-17).

Table 4.17: Results of serum IFN-γ concentration by ELISA for AHC and patients with GE.

IFN-γ	AHC (pg/ml)	GE (pg/ml)
Mean± SE	**19.66± 1.78**	**52.9 ±1.9**
LSD	**3.59**	
P value	***P*<0.05 (0.039) ***	

4.8. Spearman's Rho Statistical Testing of Age, gender, Norovirus (NoV) ; Astrovirus (AsV) and SNPs of TLR-7and IFN-γ To Evaluate The Studied Markers In Study Population

A strong positive relationship (with highly significant correlation) was found between Nov; Ast and SNP TLR-7 (rs5743557) in GE ($r = 0.986$, $P = 0.007$).

Similarly, there is a strong positive relationship (with highly significant correlation) between NoV; AsV and IFN-γ (rs3853839) in GE (r = 0.984 , P = 0.008). A strong positive relationship (with highly significant correlation) was found between Nov; Ast and Sign and symptoms in patients with GE (r = 0.970, P = 0.002). In addition, A strong positive relationship (with highly significant correlation) was found between NoV; AsV and SNPs of TLR-7 (rs5743557) and IFN-γ (rs3853839) according to ages patients who have GE infection (r=0.855, P= 0.001) ; (r=0.788, P= 0.009) and (r=0.739, P= 0.004), respectively. However, there are no significant correlations among NoV; AsV and SNPs of TLR-7 (rs5743557) and IFN-γ (rs3853839) according to the gender of study population (and as illustrated in Table 4 -18).

Table 4. 18: Spearman's Rho Statistical Testing of Age, Gender, NoV ;AsV and SNPs of TLR-7 (rs5743557) and IFN-γ (rs3853839) To Evaluate The Studied Markers In infants and children with gastroenteritis infection.

Spearman's rho		**Age groups** (Months)	**TLR-7 rs5743557**	**IFN-γ rs3853839**	**NoV & AsV**	**Sign and symptoms**
NoV & AsV	R	0.855**	0.986**	0.984**		0.970
	P	0.001	0.007	0.0008		0.002
TLR-7 rs5743557	R	0.788**				
	P	0.009				
IFN-γ rs3853839	R	0.739**				
	P	0.004				
Gender	R	0.166	-0.149	0.123	0.145	
	P	0.249	0.477	0.512	0.034	

Conclusions

The Following Conclusions are obtained from the Present Study

1. Noroviruses and astroviruses have become the most important cause of viral gastroenteritis in infants and children.

2. NoV-GII ; NoV-GIII strains and AsV- serotype3 remained the predominant genotype and major pathogen causing diarrhea in Iraqi childhood. In contrast, NoV-G-I had experienced minor pathogen causing diarrhea in Iraqi childhood.

3. The circulation of different human NoV genotypes (GII& GIII) and AsV-serotype3 in patients older than 12 years of age, suggesting that preventive measures should be taken against norovirus and astrovirus infection in older patients.

4.Our study indicated that TLR-7 and **IFN-γ** polymorphism may be associated with GE risk in the Iraqi childhood. But, the exact role and effects of TLR-7 and **IFN-γ** polymorphism in GE is not fully identified.

5.The serum levels and production rate of TLR-7 and **IFN-γ** significantly increase in childhood with GE in compare childhood heathy control group. It may be concluded that TLR-7 and **IFN-γ** acts as a risk factor in the pathogenesis of virial gastroenteritis.

Recommendations

The Recommendations of these Study Dependent on Current Results are

1. Further prospective studies are required with a large number of cases are needed to validate the results of the current study which may lead to a better understanding of the role of NoV and AsV in childhood patients with gastroenteritis.

2. To study the complete hole genome sequence and the detection the new genotypes and strains prevalent in Iraq and their differences from the global prevalence and importance in childhood patients with gastroenteritis.

3. Continuous monitoring norovirus genotypes and astrovirus serotypes circulating in pediatric population is needed for current vaccine development.

4. Continued systematic surveillance to evaluate norovirus and astrovirus association with diarrhea and other diseases is needed to assist with epidemiological surveillance and disease burden in people of all the age groups.

5.Discussion

5.1. Viral Infection; Symptoms; Signs and Gastroenteritis

Gastroenteritis is the second leading cause of pediatrics infections worldwide, resulting in at least two million hospitalizations annually. Enteric viruses are among the most important pathogens associated with diarrheal illnesses in adults and children. These cause a great endemic burden and management difficulty even in advance healthcare systems (Gholam *et al*.,2019).Rotavirus and NoV, followed by AstV, AdV, SaV, and others. These agents are transmitted to the host via various routes such as faeco–oral, person-to-person, and by fomites (Hasan *et al*.,2021). Steyer *et al*., (2016) suggested that tests for viral agents such as rotaviruses (in winter), noroviruses genogroup II, adenoviruses trait 40/41, and astroviruses are to be excluded primarily. GE is more common in infants and children under 3 years with the highest incidence in these age periods. nausea and vomiting are the most common associated symptoms. Lack of exclusive breastfeeding and contamination of weaning foods may be risk factors. Infections may be the primary cause of AGE among the children studied. The current results shows that the symptoms of infant and young children patients distributed between acute to severe , where the diagnosis of disease depending on the Stomach Cramping was 65 cases (43.4%), in addition the most symptoms of disease was Nausea at 40 cases (26.7%), as shown in table (4.2), these finding was consistent by Jassas *et al*., (2018) who mentioned that the clinical symptoms of gastroenteritis disease can cause several signs and symptoms including vomiting, nausea, abdominal pain, and, most importantly, diarrhea. Gastroenteritis can be acute, chronic, or recurrent, in addition The largest portions of gastroenteritis cases are due to viral infections. The complications including severe dehydration and shock, metabolic acidosis, severe electrolyte imbalance, convulsions, and severe immunocompromised may all occur with late or missed treatment. Physicians should be alert to any child complaining of flu-like symptoms along with repetitive diarrhea and vomiting. Other major causative

agents of infectious gastroenteritis are bacterial, followed by parasitic infections (Gholam *et al*.,2019). The clinical manifestations of viral gastroenteritis are due to the effects that the viruses, along with specific cytotoxins, have on the enterocytes of the intestine. The virus uses the enterocyte to replicate, leading to interference with brush border enzyme production, which in turn leads to malabsorption and osmotic diarrhea. Additionally, viral toxins lead to direct damage and cell lysis of enterocytes and intestinal villa, causing a transudate loss of fluid into the intestine. The loss of cell function can lead to electrolyte abnormalities which are caused by the loss of transporter functionality. That can lead to acid-base disturbances as well. The virus is then shed through feces, and occasionally in the vomitus. Peak viral load within the stool is anywhere between 24 to 48 hours after symptomatology. Some studies show viral shedding lasting for several weeks past symptomatology (Jenkins *et al*.,2021).

5.2. Distribution of Patients with Gastroenteritis (GE) and Apparently Healthy Control (AHC) Groups According to Their Age

Generally, acute gastroenteritis infections are mostly reported in <1 year of age children (Abdel-Rahman *et al*.,2021). The current result was showed that the average age of sick children with gastroenteritis was 43.56 months, meaning that children aged 3 years and under were the most affected by the infection compared to older ages, and this is consistent with what was mentioned by El-Shabrawi *et al*., (2015); Khalil *et al*., (2015) and Abdel-Rahman *et al*.,(2021). This study results showed that the average age of infection with gastroenteritis at age 3 year and less was higher than the age after 3 years. This may be due to the fact that the digestive system may be fully developed and functional, but the immature anatomical and functional features of the early intestine predispose to it abnormal bacterial (bacterial or viral) colonization in the intestine, the latter may interfere with function, the immune and neurological development of the digestive system, which makes microorganisms capable of causing disease in abundance at this age specifically (Indrio *et al*.,2022). Previous report that indicated that an

adventurous stage when most children progress from crawling to walking, which is usually characterized by falling and standing as they practice. They also have a common habit of putting dirt into their mouth without the knowledge of their mother. Furthermore, when children are together, the probability of spreading infection is high. Infants and toddlers usually wipe off saliva from their mouth with their hands and use the same hands to wipe or rub their noses and eyes, transferring the same hands to their toys and touching other children. The virus is then transmitted from child to child via bodily contact, toys, and other fomites (Siqueira *et al*.,2017). Detection may be lower in older children be due to protective immunity. If children, especially in this area, are exposed at a younger age, they may have protective immunity and, therefore, the infection rate can be lower It is observed in older children (Khumela *et al*.,2021).While, when the age progressed beyond 3 or 4 years, the disease began to decline, reaching the primary stage, and this is consistent with what was mentioned by the researchers Doll *et al*.,(2018) and Hungerford *et al*.,(2018) who found that the decrease in the disease is due to an increase health care facilities represented in providing vaccines for some group against certain pathogens, possibly due to improvements in hygiene conditions, and other public health interventions.

5.3. Distribution of Patients with GE and AHC According to sex.

Gastroenteritis are one of the infectious disease causes by many viruses, and the prevalence of this disease was shown higher in male when its epidemiology is studied by sex, the males are exposed to the disease more compared to female children (Khan, 2021). In the current study, the incidence of infection in males was higher than females, reaching 58% for males, while it was 42% for females; This agrees with a previous study by Tsague *et al*., (2020) who showed that the infection rate higher in males (11.4%) than females (8.6%)**.** In addition, these finding consistent with a study done by Hussein *et al*., (2018) who concerning the sex, a study revealed a high number of male's patients than females' patients

in Diyala. These may be because males excreted large amounts of microorganisms especially viruses in their feces than females' children.In contrast, other studies have been conducted in Iraq (in Basra) by Thwiny *et al*., (2015) and in China by Qi *et al*.,(2018) who showed that the infection in female more than male. Furthermore, Obili *et al*., (2020) who explained that females have a higher infection than males due to the different composition of the digestive system and the proximity of the stool opening from the urine opening, which may increase the incidence of infectious diseases that may be caused by bacteria or viruses. Das *et al*., (2018) have shown that there is a slight decline in these gender differences in the recent years, this is may be depending on the type infections that them get it in this age. The rate of infection in our study in male to female children was (1.3: 1), these findings are incompatible with study by Ali *et al*., (2021). Another studies which were in anti-parallel to with the reported ratios of 1.5: 1 from Bahrain and 1: 2.4 from India (Kahn *et al*., 2012). This result may be due to the vulnerability of males that probably to be admitted to hospitals than female children. A slight decline in these gender differences in the recent years, this is may be depending on the type infections that them get it in this age.

5.4. Detection of Norovirus (NoV) and Astrovirus (AsV) by Real-Time Polymerase Chain Reaction Technique (RT.PCR).

5.4.1. Viral nucleic acid in study populations

Although at least 25 different bacteria and protozoa can cause diarrhea, more than 75% of cases found to be caused by viruses. Viruses that can cause gastroenteritis include rotavirus,norovirus, enteric adenovirus, human astrovirus, and Sapporo virus. The traditional methods for virus discovery such as filtration, tissue culture, electron microscopy and serology were powerful techniques for the detection of viruses. However, due to their limitations, the traditional techniques were replaced by molecular techniques such as polymerase chain reaction (PCR) and DNA sequencing (Sanger method). Out of 150 stool swabs specimens involved

in this study 51.3% were found to have a viral infection more than 48.7% patients who did not show have a viral genome. A previous study found that Qiagen kits tend to extract a high proportion of human nucleic acids, which could explain the lower viral proportion reported in the present study (Zhang *et al*., 2018). Despite this difference, all the viruses present in the mock or clinical samples could be detected with all the methods evaluated herein.

A diarrheal stool sample is the standard specimen type required by diagnostic laboratories to test for gastroenteritis pathogens. In routine practice, a much larger proportion of patients would not submit a stool specimen for laboratory testing (Zhuo *et al*., 2018).Therefore, some factors that may reduce the rate of viral genome diagnosis, including what the researchers, Schrader *et al*., (2012) and Kim *et al*., (2013) referred to when diagnosing the virus with this technique, which is the presence of some ions and salts in the samples that contain the virus, as is the case with samples stool and other fetal fluids, which are potential inhibitors of RT-qPCR, especially that viral genes of the type RNA, which are more affected by the presence of substances that affect the diagnosis.The diagnostic methods used to detect viruses in stool samples are sequence-dependent molecular amplification techniques such as PCR, which cannot identify a pool of viruses and completely new viruses in clinical samples. Therefore, a novel approach that is sequence independent such as viral metagenomics approach using NGS is desirable and should be developed for viral diagnosis and to overcome the unresolved cases of gastroenteritis (Mo *et al*.,2015; Oude *et al*.,2016).

5.4.2. Detection of Human Norovirus (HNoV) By RT-PCR.

Human Norovirus (HNoV) is the leading cause of nonbacterial foodborne outbreaks of gastroenteritis. Individuals who are asymptomatically infected may act as reservoirs to facilitate transmission of NoV. Human noroviruses (HuNoV) are associated with 18% of diarrheal diseases worldwide and cause 200,000

deaths among children every year, mostly in developing countries (Atmar *et al.*,2018). In our study, we detected HuNoV RNA(GI ; GII&GIII) according to qRT-PCR in 37.6% % of studied cases. This overall frequency is slightly more than data reported by Kirby *et al.*,(2010) with Anfruns-Estrada *et al.*,(2020) with differences being probably due in part to differences in the date of sample collection and to the fact that our study included cases infected by a higher diversity of genotypes. While all patients included in that previous study were infected by GII.4 or GII.3 strains. In addition, these result, consistent with study of Mohammad *et al.*, (2020) who found 35% (15 out of 43) in stool samples from patients with gastroenteritis had mixed infection with one or more enteric viruses. In contrast, these result disagreement with Timurkan *et al.*, (2017) Rönnelid *et al.*, (2020) and Sun *et al.*, (2022) who found 86 (20.1%) of the 427; 20% and 1.82% (13/713) in stool samples from patients with gastroenteritis, respectively. Furthermore, Gelaw *et al.*, (2019) who detection of noroviruses (NoVs) 13.2% in stool samples by RT-PCR. Dey *et al.*, (2014) who detection rate of 19.5% of Noroviruses in the children in this study was less than some viruses and higher than the other viruses those previously reported. Li *et al.*, (2021) was from 2011 to 2018, noroviruses were detected in 16.5% of specimens from children with diarrhea. These differences might be due to the varying study periods, as well as the geographical distribution of the studied population. In addition, the vaccine that was given to children to a decline in these viruses associated gastroenteritis and a subsequent increase in norovirus associated gastroenteritis as observed (Enweronu-Laryea *et al.*,2018). The high norovirus prevalence corresponds well with other study in high- and middle-income countries where norovirus has become the most common etiology in severe childhood diarrhea (Bucardo *et al.*, 2014). Other studies from Malawi and Tanzania have shown lower prevalence of norovirus compared to this study, but used other detection methods that might not be directly comparable (Platts-Mills *et al.*, 2017; Iturriza-Gomara *et al.*, 2019).

Quantitative RT-PCR is one of the important techniques that have been applied for the detection of enteric viruses associated with gastroenteritis. This test has enabled rapid, accurate, and simultaneous detection of enteric viruses with enhanced sensitivity and specificity (Siah *et al*.,2014; Zhang *et al*.,2015). By implementing real-time PCR as a diagnostic technique for fecal viruses, many more and different viruses are detected making the performance of these techniques worthwhile. So, the detection of virus particles in children has made the real-time PCR assay was very important because the examination has a high sensitivity and specificity towards diagnosing viral particles in samples. It has now become difficult to determine the fraction of gastroenteritis cases that, in fact, can be attributed to the types of many viruses that related to the gastroenteritis disease (Corcoran *et al*.,2014).The opinion of authors of current study, the differences in percentages of NoV detection among the present as well as these studies could be attributed to the period of sample collection (seasons); site of infection, genetic as well as environmental factors, sample size, the quality & sensitivity of the techniques used in these studies to viral genome extraction. However, most of the studies done in this respect have included a small numbers of gastroenteritis cases and for better revealing of the importance of NoV in childhood suffering from acute gastroenteritis, this may need enrollment of large case-control studies.

5.4.2.1. The Results of *Norovirus* in the Patients with GE According to the Age group.

Noroviruses are increasingly being recognized as one of the more important viral agents of childhood gastroenteritis disease worldwide (Mans,2019).

In current study, was found in gastroenteritis, the most commonly affected age group infected with NoV was (3-36 months) which constituted 19.5% (15 out of 77 cases), while the age stratum (37-72 months) was constituted 11.6% (9 out of 77 cases), followed by 6.5 % (5 out of 77 cases) in age group (73 -120 months).

These results was consistent with Dey *et al*., (2014) and Benninga *et al*., (2016) who found the global prevalence of norovirus gastroenteritis conducted between 2008 and 2014 estimated norovirus to be responsible for 18% (95%) of all acute gastroenteritis cases in children < 5 years.

The findings from this study showed the importance of Norovirus in children under 5 years hospitalized with acute gastroenteritis and identified children ≤3 years to be the most vulnerable to a host of Norovirus strains. Benninga *et al*.,(2016) which indicated that infants and most young children have low immunity against bacterial and viral infections associated with the gastrointestinal tract, such as *E. coli* and Norovirus, and therefore the average age of infection is close to obtained in our results above. Although noroviruses can cause gastroenteritis in all age groups, the course of this infection in childhood is more serious than in adults due to the insufficient development of the immune system in young children. In various studies on the relationship between age and genotype, evidence of such a relationship has not been detected (Timurkan *et al*., 2017; Lopman and Grassly,2016; Mans,2019). The results of current study may be explained by two reasons. the immune system in children aged under 2 years was still not perfect; had lower consumption of breast feeding which may provide protection against infection and these children have poor health awareness.

5.4.2.2. Sequencing of Human Norovirus Genotypes of Clinical Isolates

Noroviruses are divided into six genogroups (GI–GVI) and human strains are grouped into GI, GII, and GIV. Although Norovirus causes economical and public-health problems, a thorough study of Norovirus is hampered by the lack of cell culture systems or animal models. Noroviruses are indirectly transmitted to humans from contaminated food and water sources and can also be transmitted directly from person to person. Extensively diverse NoV genotypes within genogroup I and genogroup II were reported from previous studies in Ethiopia

(Gelaw *et al*.,2022). In the current results, the most interesting fact observed in our investigated viral isolates is correlated with the positioning of our investigated samples into two related important genotypes within the human norovirus, these serotypes are genotype II (GII) and genotype III (GIII). Within the GII, six investigated samples (S1, S2, S5, S6, S7, and S8) were incorporated to constitute one large clade of GII that was made of twenty-nine samples. These results compatible with the study of Timurkan *et al*., (2017) who found GII noroviruses, especially the GII.4 strains, are the predominant cause of gastroenteritis, both in outbreaks and in sporadic cases worldwide. The prevalence and outbreak activity of the GI genogroup, which is the second most predominant genogroup, are limited, and reports of GIV around the world are also rare. NoV is prone to gene recombination, resulting in new variants or genotypes. Therefore, the investigation of genotypic distribution is of great significance for epidemiological studies of viruses and the development and application of vaccines. The NoVs types were GII17, GII12, GII21, GII2, and GII4, indicating that the GII types of diarrhea circulating are diverse, and continuous surveillance of GII types of diarrhea is necessary (Sun *et al*., 2022).

Norovirus VP1 consists of an S domain that forms a scaffold enveloping the viral RNA and a P domain composed of subdomains P1 and P2 (Beier *et al*.,2014). The S domain has the most stable conserved sequence. The P1 domain is less variable than P2 domain and the P2 domain is the most exposed and the most variable in structure (Kim *et al*.,2019).in addition Differentiation of VP1 genes among the predominant genotypes determined by sequence-based typing methods may help improve the understanding on the epidemiology and evolution of NoVs (Lee *et al*.,2021). The results showed that Detection of GI and GII Norovirus strains in swabs of blood and stool samples obtained from infants and children with gastroenteritis, and we analyzed them using sequencing analysis, and this in turn may help to provide some important information that in turn discover the most important pathogenic strains of Norovirus that are prevalent

among these groups. This is consistent with what was indicated by the researcher Lu *et al* ,(2020), who discovered the same pathological strains for samples of some university students.In the current study we showed the presence of heterogeneous nucleotides in the diagnosis of Norovirus, and that the most common type is GII. These result agreement with study by Verhoef *et al.*, (2015), GII often associated with person-to-person transmission contact of a person with other types of transmission, whereas non-GII.4 genotypes, such as GII.3, are more often associated with transmission via food (Verhoef *et al.*,2015). The increasing the number of samples extracted may increasing the probability of detection and genetic sequencing of the virus, but processing time, cost and resources also increase accordingly. This finding could be related to different biological properties and/or host responses between GI and GII strains during infection, but differences between sensitivities of GI and GII RTqPCR assays used for screening could also partially explain it. Indeed, the proportion of cases with a negative RTqPCR result in stool was also significantly higher for GI than GII cases (38.6% vs. 20.6%, respectively), suggesting that performance of RTqPCR assays or viral load in stool could differ between the two genogroups. Evidence of the association between viral load and genogroup is limited, but some data indicate that shedding may be higher for GII genotypes (Cheung *et al.*,2019 ; Anfruns-Estrada *et al.*,2020).The present study has the following limitations. Case number is very low but it is from a single center. The positivity rates and genetic diversities of the different enteric viruses observed in this smaller study might be enlightening but not conclusive.

5.4.3. Detection of Human Astrovirus (HAstV) By RT-PCR.

Gastroenteritis (GE) is one of the most common disease around the world. Acute viral gastroenteritis is one of the most common infectious diseases worldwide. Human astrovirus is currently recognized as a major cause of sporadic gastroenteritis in both children and adults (Wu *et al.*,2020). The detection rate of

HAstV in childhood with GE was 18.1% in the present study, which is different to that previously reported in, shanghai (5.22%), Thailand (2.6%), Asian Russia (2.8%) and Germany (5.0%), the mean incidence worldwide of 11.0% and Guangzhou, China (3%) (Johnson *et al*.,2017; Kumthip, *et al*.,2018; Zhirakovskaia *et al*.,2018; Jacobsen *et al*.,2018; Lu *et al*.,2021; Luo *et al*.,2021). Iraqi studies have shown low single infection prevalence for astrovirus. This exemplified with Al-Sadawi *et al*.,(2017) study in Alnajaf/ Iraq which recorded 3/200 (10.4%) chromate-graphic immunoassay- positive Astrovirus results; Ali *et al*., (2016) in Baghdad/ Iraq in which out of the 465 samples analyzed only 10.1% were immunochromatographic assay positive astrovirus results ; Mitab, (2013) in Almuthana/ Iraq study which recorded 39/335 (11.6%) multiplex rRT-PCR technique positive results for Astrovirus and AlShuwaikh, 2016 study in Baghdad/ Iraq along with Hussein, *et al*., (2018) study in Dyala/ Iraq that gave zero (0%) results for Astrovirus by the immunochromatographic assay from 188 and 160 samples with Acute Diarrhea respectively.The highest incidence rate in this study has matched only with Mitab, (2013) study, this may be explained the high accuracy and sensitivity of the RT-qPCR assay in the detection of these viruses and this matched with what mentioned in other previous studies (Logan *et al*.,2007). In comparison to other global molecular based studies from Italy (Biscaro *et al*.,2018), Germany (Jacobsen *et al*.,2018), Lebanon (Zaraket *et al*.,2017) and Egypt (Ahmed *et al*.,2011). The prevalence of HAstV in this study is the highest, telling that this virus exists, flowing and contributing expressively to gastroenteritis in different Iraqi provinces. Vu *et al*., (2016) and Tsague *et al*., (2020) were detected HAstV in 11.7% and (10.3%) of 506 fecal samples from infants on admission for acute gastroenteritis, respectively. In addition, the frequency value obtained in this study is higher than the frequency of 7.0% for HAstVs in diarrheal stool samples obtained in a study in South Africa among children aged 7–12 months (Nadan *et al*., 2019). By contrast, the percentage in the present study is lower than that reported in children under 5 year old in Lagos,

Nigeria (Ayolabi *et al*.,2012), where a prevalence of 40.4% was reported (Ayolabi *et al*.,2012). The effects of differences in social factors and geographical locations could probably have resulted in this variation. The positive rate of HAstV can differ with respect to time and has tended to decrease in the last decade. Analysis of the monthly positive rate by year did not show distinct seasonal variations in HAstV infection. Cumulative positive rates revealed a relatively high HAstV incidence from March to July and a relatively low incidence from August to October; however, definitive conclusions cannot be drawn because of the small number of positive samples (Kim *et al*.,2019).

This study has several limitations. only samples that tested positive with the commercially available RT-PCR assay were genotyped; thus, types that did not test positive with this assay were probably not detected. The company claims that this PCR assay can detect HAstV Types 1–8, but this assay may not detect HAstVs whose genomes have variations at primer-binding sites. The detection of HAstV infection in hospitalized children with acute diarrhea in mid-Euphrates provinces Hospitals was performed exclusively on symptomatic patients, but not in children without acute diarrhea (asymptomatic). The stool samples were collected at only one site and it would be of interest to perform a similar study at several others sites in same provinces. The lack of some data from patients, such as those having pets, did not allow the identification of these specific risk factors associated with Astrovirus infection.

5.4.3.1. The Results of *Astrovirus* in the Patients With GE According to the Age Stratum.

HAstVs are considered to cause infections mainly in children younger than five years **(**Biscaro *et al*.,2018 ; Varela *et al*.,2019). In gastroenteritis, the most commonly affected age stratum infected with AsV was (3-36 months) which constituted 9.1% (7 out of 77 cases), while the age stratum (37-72 months) was constituted 5.1% (4 out of 77 cases), followed by 3.9% (3 out of 77 cases) in age

stratum (73 – 120 months).These results compatible with Kim *et al*.,(2019) and Varela *et al*.,(2019) studies, who found 38.8% of HAstV-positive patients were older than five years, which suggests that HAstV infection may also occur in adolescents and adults. The higher percentage of patients over five years in this study could be attributed to the fact that we collected stool samples from patients of all ages with diarrhea symptoms. Vu *et al*., (2019) Most novel astroviruses were found in children <2-year-old (30/39 children, 77%, $p = 0.01$).In addition ,AstV-1 is the most frequently isolated type worldwide, in this study the sequenced strain has typed as AstV-1 serotype in a patient with a diarrheal infection aged 7 months old and this matched with Guix *et al*., (2002)which demonstrated that most infections with AstV-1 and AstV-3 occurred in children younger than 2 years old. The high incidence of Astrovirus relies on the age of patients, analytical approaches used and the season of isolates assembly in which HAstV infectious rate is more common among those younger than 2 and in temperate regions in the cold weather period beside of using RTqPCR assays that provide high speed, sensitivity and reproducibility and reduction of contamination risk in the diagnostic of HAstVs (Saied *et al*.,2013).We thought, this phenomenon in this study may be explained by Astrovirus is common and spreads through food and water. In fact, the virus may continue to be shed for up to a month after infection, although intermittently and at low levels.

5.4.3.2. Sequencing of Human Astrovirus Genotypes of Clinical Isolates

Currently, the genotype of HAstV has been classified into classic HAstV (HAstV1–HAstV8), novel HAstV-MLB (MLB1–MLB3), and novel HAstV-VA/HMO (VA1–VA5). The classic HAstV accounts for 2.9–5.0% of acute gastroenteritis in children, and classic HAstV1 remains the predominant genotype. Although the novel HAstV-MLB and HAstV-VA/HMO were initially detected in children with gastroenteritis, the definite association between these novel HAstV genotypes and gastroenteritis has not yet been established

(Wohlgemuth *et al*.,2019; Vu *et al*.,2020). On the other hand, the novel HAstV-MLB and HAstV-VA have been increasingly reported to associate with central nervous system infection in humans, particularly, in immunocompromised individuals (Koukou *et al*.,2019). The current results of comprehensive circular cladogram phylogenetic tree of genetic variants of the ORF1a gene fragment of six human-infecting astrovirus samples. The prevalence of HAstV infection has been sequentially reported from around the world with a wide range of variability from 0 to 29.7% (Vu *et al*.,2017). The prevalence varies from study to study depending on the study population and geographical region that the study has been conducted. For instance, in South America, the prevalence of HAstV infection in Brazil was reported with low infection rate at 0.8% (Amara *et al*.,2015). , whereas in North America a prevalence was reported at 3.5% in Mexico (Romo-Saenz *et al*.,2020). In Africa, a prevalence of HAstV infection was reported at 7.0% in South Africa (Nadan *et al*.,2019). , 9.9% in Kenya and Gambia, 10.3% in Congo, 11–14% in Egypt (Zaki *et al*.,2020). , and 19.4% in Nigeria. In Europe, a prevalence of HAstV infection was reported at 2.5% in Netherland and 3% in Italy. In Asia, a prevalence of HAstV infection was reported at 1.6–2.8% in China, 1.9% in Korea, 2.6% in Taiwan, 2.4–16.4% in Japan, and 1.4–3.1% in Thailand (Wei *et al*.,2021). Some of HAstV-infected cases were found to be co-infected with two or three other enteric viruses. This observation is similar to what we have reported previously during 2011–2016 at 57.4% in the same geographical area (Kumthip *et al*.,2018) suggesting that gastroenteritis in children in Thailand are infected with a wide variety of enteric viruses and require more attention on the situation. Nevertheless, high rate of co-infection with other enteric viruses is not uncommon, it has also been reported elsewhere, such as 38.2% in Nigeria(Arowolo *et al*.,2020) and 71% in Germany(Jacobsen *et al*.,2018). The infection rate abruptly increased to 2.2% in children with the age of > 6–12 months old and continued increasing in the older age groups, and reached highest rate in children with the age of > 48–60 months old.

The data imply that at the age of < 1–6 months old, the children might be protected by maternal antibodies acquired in utero or via breast feeding and these maternal antibodies declines after > 6–12 months old. As a result, the infection rates went up to 2.2%, 2.6%, and 2.7% in children with the age of > 6–12, > 12–18, and > 18–24 months old, respectively. In addition, the infection rates increased further approximately two folds to 4.1%, 3.8%, and 4.8% in children with the ages of > 24–36, > 36–48, and > 48–60 months old. The infection rates increased approximately two folds in children with the age of > 24–60 months old compared to those observed in children with the age of > 6–24 months old is probably related to the hygienic fed pattern in younger children compared to relatively unhygienic behavior or activities at the playground of older children. Apparently, older children may have much more chances to expose to and being infected with HAstV as well as many other enteric viruses (Wei *et al*.,2021). Six genotypes were found to circulate during 2015–2016, with HAstV-1 being predominant, followed by HAstV-5, HAstV4, HAstV-2, HAstV-8, and HAstV-3. Except for HAstV-1a and 1b, all other HAstVs are of a single subtype/lineage. HAstV5\4\2\8\3 have not been reported in Shanghai.The detection of these genotypes in this study indicates that multiple HAstVs were circulating in Shanghai. It would be interesting to monitor the frequency of these genotypes in the Shanghai population in the future. Like other recent studies (Bitencurt *et al*., 2019; Kim *et al*., 2019; Wu *et al*., 2020), HAstV-6 and -7 were not detected. Notably, the peak of HAstV-induced cases in 2016 was almost five times higher than in the preceding year. A rapid rise in HAstV-4 cases was observed during the high HAstV peak season from December 2015 to April 2016. Furthermore, HAstV-4- positive samples showed a high viral load. Multiple factors might have contributed to this result. Compared with the 2015 data, both the case number and detected genotypes of HAstV infections had increased over the same period last year, and HAstV-4 in particular had the most obvious change. The HAstV4 strains observed in this study may be a variant because they exhibited a

characteristically high stool shedding. In addition to clarifying the relationship between this variant and the severity of the disease, it is also necessary to study its infectiousness. As is well known, norovirus infections are a leading cause of acute gastroenteritis worldwide in persons of all age groups. Interestingly, the cases of norovirus infections in January 2016 were almost five times lower than in January 2015 during the same period based on SDCSS data (Gong *et al.*, 2018). In contrast, the incidence of rotavirus infection was similar. There may be some underlying connection between the decrease in the number of norovirus cases and the increase in HAstV cases. Phylogenetic analyses of the RdRp (ORF1b) nucleotide sequences of HAstV strains detected in this study revealed that these strains share high nucleotide sequence identities with global strains reported from different remote countries and continents, demonstrating a worldwide circulation of HAstV strains that occurs continuously and has an impact on epidemiology and evolution of HAstV strains.A combined analysis of ORF1b and ORF2 regions that allow to differentiate the polymerase genotype, capsid genotype and polymerase/capsid genotype was performed in this study. Of note, a putative recombinant HAstV polymerase genotype 1/HAstV capsid genotype 2 was identified, suggesting the possible occurrence of a recombination event. More details could be obtained on the recombination event if SimPlot analysis was performed; unfortunately, the requirement for SimPlot is a long sequence of more than 1000 bp, which is not the case for our amplified strains. There is increasing evidence of recombination events in astrovirus which contribute to genetic variability and imply cross species transmission or zoonotic potential. Putative recombination sites within ORF1b and ORF2 have been reported previously. It is common for ssRNA viruses to exchange genome fragments in highly conserved regions, increasing the prevalence of the virus and affecting its phylogenetic grouping and vaccine development. The recombination of strains is an open door to mutations that may mislead future epidemiological investigations and compromise vaccine development (Nadan *et al.*,2019; Nadan *et al.*,2021).

Limitations of this study included the lack of seasonal distribution data of classic human astrovirus due to lockdown restrictions during the study period, and the absence of novel astrovirus strain data.

5.5. The Results of Gene Polymorphism of Toll-like receptor -7 (TLR-7) and Interferon-gamma (IFN-γ) SNPs.

5.5.1. Genotyping of TLR-7 Gene in GE and AHC

Small-molecule agonists of TLRs are currently used for detection the severity of many viruses since have important role against viruses (Dowling and Mansell,2016). One important example has been the use of TLR7 and TLR9 agonists in combination with detection severity of many viruses (Offersen *et al*.,2016; Tsai *et al*.,2017). Toll-like receptors (TLRs) including TLR3, TLR7 and TLR8 are essential for activation of the antiviral response upon viral infection, and can sense ssRNA or dsRNA in the cytosol (Jensen *et al*.,2012) .Studies have shown an increased viral infection of *Norovirus* in children with TLR7 (McCartney *et al*.,2008; Jensen *et al*.,2012), This explains the percentage of TLR-7 identification in our results, where DNA polymorphism distributions according to CA ; AT ; **TA** and GA genotypes of **TLR-7 rs3853839** polymorphism were respectively 62.2% ; 31.1%; 6.7% and 0% in the GE patient group and was found GA genotype 100 % in the control group. The results appeared in the presence of ninteen SNPs. Reveal that which located at positions 22; 341; 354 a substitution mutation A→C ; positions 8; 361 substitution A→ T ; position 18 substitution T→ A; and last one at position 113 substitution C→ A.

Li *et al*.,(2021) polymorphism of rs3853839 is in the 3' untranslated region (UTR) of the TLR7 gene and leads to changes in hsa-miR-298, which is a typical miRNA binding change of 3' UTR. Similarly, Mukherjee & Tripathi (2019) stated that *TLR7* SNPs (rs3853839 & rs179008) had significant relationship with Dengue virus infection. Also Zhang *et al*., (2020), in Chinese

Han patients, SNPs at TLR7 were investigated for their relevance in HIV-1 infection and prognosis. TLR7 SNP (rs3853839) was also found to be substantially linked to chikungunya virus infection in Indians (Dutta & Tripathi, 2017). In Chinese patients, the TLR7 SNP (rs3853839) has also been linked to HCV persistence and predisposition to enterovirus-71-mediated hand, foot and mouth infection (Yue *et al.*, 2014). All of these investigations found that the TLR7 SNP (rs3853839) plays a role in viral infection and pathogenesis in different ethnic groups. The significant elevation of TLR-7 mRNA transcripts in individuals carrying the G mutant allele, as well as the higher level of G allele-containing TLR7 mRNA in heterozygous participants supported a physiological role for TLR7 SNP (rs3853839) in the adjustment of TLR7 mRNA expression, according to a study on systemic lupus erythematosus patients (Shen *et al.*, 2010). Also, Raafat *et al.*, (2018) reported that TLR signaling can be regulated by miRNAs through direct effects on expression or by modulation of downstream regulators, adaptor molecules, and cytokines. The TLR7 SNP (rs3853839) has the potential to impact miRNAs binding and as a result, TLR7 mRNA expression and/or sensitivity. The wild C allele features a binding site for miR-3148 (miR-3148), which causes fast transcript breakdown and reduces TLR7 mRNA levels. Similarly, Shen *et al.*, (2010) concluded the link between the risk allele G of the TLR7 SNP (rs3853839) and higher TLR7 transcript expression. The GG genotype was shown to be substantially more common among severe/critically sick patients in the current investigation.

5.5.2. Genotyping of IFN-γ Gene in GE and AHC

In this study, we demonstrate that HAstV-1 induces IFN-γ at 21.3% (32 out of 150 cases) while in apparently healthy group shows at 8% (5 out of 50 cases) this increasing in the percentage of IFN-γ in patients with Astrovirus in contrast to

the control group It means that the virus induce the IFN-γ production that lead to the limitation from infection these findings was consistent with the researcher Guix *et al* (2015) who demonstrated that the production of IFN, which limits astrovirus replication in vitro and is important for the clearance of astrovirus in vivo. In the current finding , we diagnosed IFN-γ and its role in reducing the severity of infection with the astro virus, and the importance of choosing this type of interferon was for its important role in reducing viral infection, as it is specialized for viruses, and this is consistent with what was mentioned by previous studies that mentioned type II IFNs (IFN-γ) are responsible for regulating and activating the immune response induced in virtually all cell types upon recognition of viral components, especially nucleic acids, by cytoplasmic and endosomal receptors, and type II interferon is induced by cytokines such as IL-12, and its expression is restricted to immune cells such as T cells and NK cells (Marvin *et al*.,2016; Carsetti *et al*.,2020). Other study have indicated that the importance Clinical study of the relationship between interferon-gamma and astrovirus infection, extending extensively ranging from asymptomatic infections to fatal encephalitis, so the study of this type of interferon plays a major role in knowing the body's ability to end viral infection and reduce its symptoms that may affect the health of infected children, as they are at the beginning of the formation of the immune system and its functional and physiological completion (Cortez *et al*.,2017). By analogy, IFN-γ is a potent activator of macrophages, and clinical and experimental evidence showed that IFN-γ plays a key role in antimicrobial activities that regulate mycobacterial infection. In recent years, IFN-γ +874 A>T gene polymorphism has been widely studied for potential association with increased/reduced/no risk of PTB, but the findings were inconsistent, and still the precise association between the IFN-γ +874 A>T SNP and PTB risk is inconclusive. This inconclusive finding warranted further studies with large sample sizes for accurate estimation of the association between the IFN-γ +874 A>T gene polymorphism and PTB disease. Hence, in order

provide a precise conclusion against the above-mentioned controversial results, a meta-analysis is thought to be needed to achieve more reliable and accurate statistical evidence on the association between IFN-γ +874 A>T SNP and PTB susceptibility. The pooled ORs generated from a large sample size and sufficient statistical power from various studies have the power to reduce the random errors (Areeshi *et al*.,2021). IFN-γ polymorphism correlated with some of the clinical features of gastroenteritis may be related to factors including race, immune state perfect , type of viral infection DNA or RNA or genetic or clinical heterogeneity.

5.6. Evaluation of Serum TLR-7 and IFN-γ concentration By ELISA Among Study Population

Various inflammatory cytokines and interferon's are considered to have an important role to regulate IL-10-producing B cells or B10 cells, and it also has a role in Sensing the RNA of some microorganisms, including pathogenic viruses, as TLR7 contributes to the differentiation and development of Regulatory B cells are unknown. In addition, interferon's have a regulatory role in the inflammatory response (Rosser *et al*.,2014; Chodisetti *et al*.,2020 a).

Our current data show that the concentration of TLR7 in the serum of people with gastroenteritis showed a statistical variation at (0.001), while the concentration of interferon gamma was at (0.039). The researcher Chodisetti *et al* .,(2020 b) pointed out their regulatory role for antibody-producing B cells and that increasing their gene expression has the effect of reducing interleukin-10 production. TLR7 is required for a rapid nonneutralizing IgM response that limits acute infection. The lack of neutralizing power for these antibodies in vitro suggests a mechanism involving other accessory factors or cell types, such as ADCC or complement-mediated lysis of virus particles. This IgM response is not sufficient for full recovery from infection, and clearance of infectious virus

from the plasma likely also requires the evolution of neutralizing antibodies and GC responses at later time points (Bonsignori *et al*.,2012).

TLR7 promotes early secretion of IL-10 by CD4 T cells, and this cytokine plays an important role in inhibiting acute infection. This finding is consistent with previous reports indicating that TLR7 can regulate IL-10 secretion. Importantly, IL-10 secretion is not completely abolished in the absence of TLR7 but is delayed until after the peak of proinflammatory cytokines, suggesting that additional pathways can promote IL-10 secretion during retroviral infection. Nevertheless, the timing of IL-10 secretion could be a key aspect of its ability to regulate the antiretroviral immune response (Browne *et al*.,2013). In the current study we used TLR-7 as diagnostic tool for detection the severity of norovirus and our finding shows increasing in the concentration of TLR-7 at patients group in contrast to control group , so our selection of this immunological parameter came in agreement with many previous studies on the effects of TLR against RNA virus infection, as it has an important role in increasing the immune response through increasing the immune response and increasing the production of proinflammatory cytokines. that lead to inhibition of viral replication (Lanford *et al*.,2013 ; Bam *et al*.,2017).

Activation of TLRs initiates the integration of contextual cues and signals to regulate host inflammatory and immune responses (Kumar *et al*.,2011) TLR7 recognizes viral ssRNA to induce a wide range of proinflammatory cytokines and IFNs (Walsh *et al*.,2012). Here, we noticed the role of interferon- γ (IFN-γ) and mechanism in immune response. Our results showed that there were significant differences for TLR-7 at (0.001), where performed the least significant difference (LSD) analysis to show its effect on the infection of gastroenteritis, which was caused by a virus, including *Norovirus* and *Astrovirus*, where these viruses induce multiple TLRs accompanied by excessive inflammatory factors production (Chi *et al*.,2013). While in intestinal epithelial cells, TLR7 mRNA is up regulated By

these viruses, suggesting that TLR7 mediates the inflammatory response during infection (Wang *et al*.,2016). In addition, the DNA of these viruses stimulates TLR3 signaling, which aids in the activation of macrophages and natural killer cells (Zhu *et al*.,2015). In current study, there was a significant difference in the relationship between interferon gamma and patients with gastroenteritis caused by Norovirus and Astrovirus at probability level of (0.003) and this is consistent with a study conducted in living cells on the effect of interferon gamma laboratory, where they found that infection of cells with some types of enteroviruses It triggers a response of target cells by increasing the production of IFN-γ (Arimori *et al*.,2014; Stegemann *et al*.,2018). IFN-γ exerts its direct anti-TGEV activity through a paracrine mechanism by which IFN-γ from immune cells act on TGEV-infected cells *in vivo*. The co-existence of IFN-γ and IFN-α/β in the local microenvironment of TGEV-infected. By analogy ,this is in agreement with previous studies with herpes simplex virus 1 (HSV-1) and hepatitis C virus (HCV) showing that the combination of type I IFNs and IFN-γ synergistically inhibits viral infection both *in vitro* and *in vivo* (Okuse *et al*.,2005; Shan *et al*.,2019).

In the current study we evaluated the correlation coefficient between IFN-γ & TLR-7 and gastroenteritis patients from one side and Norovirus & Astrovirus from other side, This is what a previous study indicated that an important function of toll-like receptors (TLR) and interferon's is the detection of microbes by host guard cells that direct innate immune responses and adaptive cascades. In addition to increasing the potency of antigen-presenting cells (APCs) thus providing a higher immune response (Shonyela *et al*.,2019).

Ao, Y., Xie, X., Dong, X., Jin, M., & Duan, Z. (2019). Genetic analysis of an emerging GII. P2–GII. 2 Norovirus associated with a 2016 outbreak of acute gastroenteritis in China. Virologica Sinica, 34(1), 111-114.

Abdel-Rahman, M. E., Mathew, S., Al Thani, A. A., Ansari, K. A., & Yassine, H. M. (2021). Clinical manifestations associated with acute viral gastroenteritis pathogens among pediatric patients in Qatar. Journal of Medical Virology, 93(8), 4794-4804.

Ahmed, S. M., Hall, A. J., Robinson, A. E., Verhoef, L., Premkumar, P., Parashar, U. D., ... and Lopman, B. A. (2014). Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. The Lancet Infectious Diseases. 14(8), 725-730.

Al Jassas, B., Khayat, M., Alzahrani, H., Asali, A., Alsohaimi, S., Al Harbi, H., ... & Mahbub, M. (2018). Gastroenteritis in adults. Int. J. Commun. Med. Publ. Health, 5(11), 4959-4964.

Ali, S. H. M., Al-Wadi, G. I. A., Yassin, B. A. G., Al-Janabi, M. K. W., Al-Timimi, M. F., & Hussein, H. S. (2016). Prevalence of rotavirus, adenovirus, astrovirus and enteroviruses involved in acute infantile and childhood gastroenteritis in Baghdad: a Stoolbased antigenic study. Inter. J. of Info Res and Rev, 3(6), 2467-2475.

Ali, S., Khan, S., Khan, S. N., Rauf, M., Khan, M. F., Majid, A., ... and Khan, M. Y. (2021). Molecular detection and prevalence of Rotavirus with acute gastroenteritis among the children of rural and urban areas. Brazilian Journal of Biology. 83.

Al-Sadawi, A. A., Ammar, M., & Tuwaij, N. S. (2017). Viral agent that causing diarrhoea among children in Al-Najaf province, Iraq. World J Pharma Res, 6(8), 1-11.

Al-Shuwaikh, A. M. A. (2016). Frequency of Rotavirus, Adenovirus and Astrovirus among Patients with Acute Diarrhea by Chromatographic

Immunoassay and Enzyme. Journal of Biotechnology Research Center, 10(2), 58-64.

Alspach, E., Lussier, D. M., & Schreiber, R. D. (2019). Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. Cold Spring Harbor perspectives in biology, 11(3), a028480.

Alwin, A., and Karst, S. M. (2021). The influence of microbiota-derived metabolites on viral infections. Current Opinion in Virology. 49, 151-156.

Amaral, M. S. C., Estevam, G. K., Penatti, M., Lafontaine, R., Lima, I. C. G., Spada, P. K. P., ... & Matos, N. B. (2015). The prevalence of norovirus, astrovirus and adenovirus infections among hospitalised children with acute gastroenteritis in Porto Velho, state of Rondônia, western Brazilian Amazon. Memórias do Instituto Oswaldo Cruz, 110, 215-221.

Anfruns-Estrada, E., Sabrià, A., Fuentes, C., Sabaté, S., Razquin, E., Cornejo, T., ... & Guix, S. (2020). Detection of norovirus in saliva samples from acute gastroenteritis cases and asymptomatic subjects: association with age and higher shedding in stool. Viruses, 12(12), 1369.

Ao, Y., Xie, X., Dong, X., Jin, M., & Duan, Z. (2019). Genetic analysis of an emerging GII. P2–GII. 2 Norovirus associated with a 2016 outbreak of acute gastroenteritis in China. Virologica Sinica, 34(1), 111-114.

Areeshi, M. Y., Mandal, R. K., Dar, S. A., Jawed, A., Wahid, M., Lohani, M., ... & Haque, S. (2021). IFN-γ+ 874 A> T (rs2430561) gene polymorphism and risk of pulmonary tuberculosis: a meta-analysis. Archives of medical science: AMS, 17(1), 177.

Arias, C. F., & DuBois, R. M. (2017). The astrovirus capsid: a review. *Viruses*, *9*(1), 15.

Arimori, Y., Nakamura, R., Yamada, H., Shibata, K., Maeda, N., Kase, T., and Yoshikai, Y. (2014). Type I interferon plays opposing roles in

cytotoxicity and interferon-γ production by natural killer and CD8+ T cells after influenza a virus infection in mice. Journal of Innate Immunity. 6(4), 456-466.

Arowolo, K. O., Ayolabi, C. I., Adeleye, I. A., Lapinski, B., Santos, J. S., & Raboni, S. M. (2020). Molecular epidemiology of astrovirus in children with gastroenteritis in southwestern Nigeria. Archives of virology, 165(11), 2461-2469.

Atmar, R. L., Ramani, S., & Estes, M. K. (2018). Human noroviruses: recent advances in a 50-year history. Current opinion in infectious diseases, 31(5), 422-432.

Ayolabi, C. I., Ojo, D. A., and Akpan, I. (2012). Astrovirus infection in children in Lagos, Nigeria. African Journal of Infectious Diseases. 6(1), 1-4.

Bam, R. A., Hansen, D., Irrinki, A., Mulato, A., Jones, G. S., Hesselgesser, J., ... and Yant, S. R. (2017). TLR7 agonist GS-9620 is a potent inhibitor of acute HIV-1 infection in human peripheral blood mononuclear cells. Antimicrobial Agents and Chemotherapy. 61(1), e01369-16.

Bao, M., and Liu, Y. J. (2013). Regulation of TLR7/9 signaling in plasmacytoid dendritic cells. Protein and Cell. 4(1), 40-52.

Barclay, L., Cannon, J. L., Wikswo, M. E., Phillips, A. R., Browne, H., Montmayeur, A. M., ... & Vinjé, J. (2019). Emerging novel GII. P16 noroviruses associated with multiple capsid genotypes. *Viruses*, *11*(6), 535.

Barlow, Gavin; Irving, William L.; Moss, Peter J. (2020). "20. Infectious disease". In Feather, Adam; Randall, David; Waterhouse, Mona (eds.)

Beier, R., Pahlke, C., Quenzel, P., Henseleit, A., Boschke, E., Cuniberti, G., and Labudde, D. (2014). Selection of a DNA aptamer against norovirus capsid protein VP1. FEMS Microbiology Letters. 351(2), 162-169.

Bender AT, Tzvetkov E, Pereira A, Wu Y, Kasar S, Przetak MM, Vlach J, Niewold TB, Jensen MA, and Okitsu SL (2020) TLR7 and TLR8 differentially activate the IRF and NF-kB pathways in specific cell types to promote inflammation. Immunohorizons 4:93–107.

Benninga, M. A., Nurko, S., Faure, C., Hyman, P. E., Roberts, I. S. J., and Schechter, N. L. (2016). Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology. 150(6), 1443-1455.

Bergallo, M., Galliano, I., Daprà, V., Rassu, M., Montanari, P., and Tovo, P. A. (2018). Molecular detection of human astrovirus in children with gastroenteritis, Northern Italy. The Pediatric Infectious Disease Journal. 37(8), 738-742.

Billiau, A., & Matthys, P. (2009). Interferon-γ: a historical perspective. *Cytokine & growth factor reviews*, *20*(2), 97-113.Biscaro, V., Piccinelli, G., Gargiulo, F., Ianiro, G., Caruso, A., Caccuri, F., and De Francesco, M. A. (2018). Detection and molecular characterization of enteric viruses in children with acute gastroenteritis in Northern Italy. Infection, Genetics and Evolution. 60, 35-41.

Biscaro, V., Piccinelli, G., Gargiulo, F., Ianiro, G., Caruso, A., Caccuri, F., & De Francesco, M. A. (2018). Detection and molecular characterization of enteric viruses in children with acute gastroenteritis in Northern Italy. Infection, Genetics and Evolution, 60, 35-41.

Bitencurt, E. L. R., Siqueira, J. A. M., Medeiros, T. B., Bandeira, R. D. S., de Souza Oliveira, D., de Paula Souza e Guimaraes, R. J., ... & Gabbay, Y. B. (2019). Epidemiological and molecular investigation of norovirus and astrovirus infections in Rio Branco, Acre, Northern Brazil: a retrospective study. Journal of Medical Virology, 91(6), 997-1007.

Bogdanoff, W. A., Campos, J., Perez, E. I., Yin, L., Alexander, D. L., & DuBois, R. M. (2017). Structure of a human astrovirus capsid-antibody

complex and mechanistic insights into virus neutralization. *Journal of virology*, *91*(2), e01859-16.

Bonsignori, M., Pollara, J., Moody, M. A., Alpert, M. D., Chen, X., Hwang, K. K., ... & Haynes, B. F. (2012). Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. *Journal of virology*, *86*(21), 11521-11532.

Boonchan, M., Guntapong, R., Sripirom, N., Ruchusatsawat, K., Singchai, P., Rungnobhakhun, P., ... & Motomura, K. (2018). The dynamics of norovirus genotypes and genetic analysis of a novel recombinant GII. P12-GII. 3 among infants and children in Bangkok, Thailand between 2014 and 2016. Infection, Genetics and Evolution, 60, 133-139.

Bosch, A., Pintó, R. M., & Guix, S. (2014). Human astroviruses. Clinical microbiology reviews, 27(4), 1048-1074.

Browne, E. P. (2013). Toll-like receptor 7 inhibits early acute retroviral infection through rapid lymphocyte responses. Journal of virology, 87(13), 7357-7366.

Bucardo, F., Reyes, Y., Svensson, L., and Nordgren, J. (2014). Predominance of norovirus and sapovirus in Nicaragua after implementation of universal rotavirus vaccination. PloS One. 9(5), e98201.

Bunyan, I. A., & Al-Salem, H. L. (2022). Gene Sequencing of ENTB Gene Among Klebsiella Pneumonia Isolates from Different Site of Infection. Journal of Complementary Medicine Research, 13(1), 107-107.

Campillay-Véliz, C.P., Carvajal, J.J., Avellaneda, A.M., Escobar, D., Covián, C., Kalergis, A.M. and Lay, M.K. (2020) . Human Norovirus Proteins: Implications in the Replicative Cycle, Pathogenesis, and the Host Immune Response. Front. Immunol. 11:961. doi: 10.3389/fimmu.2020.00961.

Cannon, J. L., Barclay, L., Collins, N. R., Wikswo, M. E., Castro, C. J., Magaña, L. C., ... & Vinjé, J. (2017). Genetic and epidemiologic trends of norovirus outbreaks in the United States from 2013 to 2016 demonstrated emergence of novel GII. 4 recombinant viruses. Journal of clinical microbiology, 55(7), 2208-2221.

Cheung, S. K., Kwok, K., Zhang, L. Y., Mohammad, K. N., Lui, G. C., Lee, N., ... & Chan, M. C. W. (2019). Higher viral load of emerging norovirus GII. P16-GII. 2 than pandemic GII. 4 and epidemic GII. 17, Hong Kong, China. Emerging infectious diseases, 25(1), 119.

Cannon, J. L., Bonifacio, J., Bucardo, F., Buesa, J., Bruggink, L., Chan, M. C. W., ... & Vinjé, J. (2021). Global trends in norovirus genotype distribution among children with acute gastroenteritis. Emerging infectious diseases, 27(5), 1438.

Carsetti, R., Quintarelli, C., Quinti, I., Mortari, E. P., Zumla, A., Ippolito, G., and Locatelli, F. (2020). The immune system of children: the key to understanding SARS-CoV-2 susceptibility. The Lancet Child & Adolescent Health. 4(6), 414-416.

Chan, M. C., Hu, Y., Cheng, H., Podkolzin, A. T., Zaytseva, E. V., Komano, J., ... & Chan, P. K. (2019). Global spread of norovirus GII. 17 Kawasaki 308, 2014-2016.

Chaudhary, R., & Maurya, G. K. (2019). Restriction fragment length polymorphism.

Chen, J., Yang, H., & Sheng, Z. (2020). Ellagic acid activated PPAR signaling pathway to protect ileums against castor oil-induced diarrhea in mice: application of transcriptome analysis in drug screening. *Frontiers in Pharmacology*, *10*, 1681.

Chhabra, P., de Graaf, M., Parra, G. I., Chan, M. C. W., Green, K., Martella, V., ... and Vinjé, J. (2019). Updated classification of norovirus

genogroups and genotypes. The Journal of General Virology. 100(10), 1393.

Chi, C., Sun, Q., Wang, S., Zhang, Z., Li, X., Cardona, C. J., ... and Xing, Z. (2013). Robust antiviral responses to enterovirus 71 infection in human intestinal epithelial cells. Virus Research. 176(1-2), 53-60.

Chodisetti SB, Fike AJ, Domeier PP, Singh H, Choi NM, Corradetti C, et al. Type II but not Type I IFN signaling is indispensable for TLR7-promoted development of autoreactive B cells and systemic autoimmunity. J Immunol. (2020-b) 48:1–8. doi: 10.4049/jimmunol.1901175.

Chu, D. K. W., Poon, L. L. M., Guan, Y., and Peiris, J. S. M. (2008). Novel astroviruses in insectivorous bats. Journal of Virology. 82(18), 9107-9114.

Corcoran, M. S., Van Well, G. T. J., and Van Loo, I. H. M. (2014). Diagnosis of viral gastroenteritis in children: interpretation of real-time PCR results and relation to clinical symptoms. European Journal of Clinical Microbiology and Infectious Diseases. 33(10), 1663-1673.

Cortez, V., Meliopoulos, V. A., Karlsson, E. A., Hargest, V., Johnson, C., and Schultz-Cherry, S. (2017). Astrovirus biology and pathogenesis. Annual Review of Virology. 4, 327-348.

Cotten, M., Petrova, V., Phan, M. V., Rabaa, M. A., Watson, S. J., Ong, S. H., ... and Baker, S. (2014). Deep sequencing of norovirus genomes defines evolutionary patterns in an urban tropical setting. Journal of Virology. 88(19), 11056-11069.

Dai, Y. C., Xia, M., Huang, Q., Tan, M., Qin, L., Zhuang, Y. L., ... and Zhang, X. F. (2017). Characterization of antigenic relatedness between GII. 4 and GII. 17 noroviruses by use of serum samples from norovirus-infected patients. Journal of Clinical Microbiology. 55(12), 3366-3373.

Das, M., Angeli, F., Krumeich, A. J., and van Schayck, O. C. (2018). The gendered experience with respect to health-seeking behaviour in an urban

slum of Kolkata, India. International Journal for Equity in Health. 17(1), 1-14.

Day, P. M., Thompson, C. D., Lowy, D. R., & Schiller, J. T. (2017). Interferon gamma prevents infectious entry of human papillomavirus 16 via an L2-dependent mechanism. *Journal of Virology*, *91*(10), e00168-17.

Deshpande, A., Putcha, B. D. K., Kuruganti, S., & Walter, M. R. (2013). Kinetic analysis of cytokine-mediated receptor assembly using engineered FC heterodimers. *Protein Science*, *22*(8), 1100-1108.

Dey, S. K., Nahar, S., Akter, T., Sultana, H., Akter, A., Sarkar, O. S., ... & Ahmed, F. (2014). A retrospective analysis of viral gastroenteritis in Asia. Journal of Pediatric Infectious Diseases, 9(02), 053-065.

Doll, M. K., Quach, C., and Buckeridge, D. L. (2018). Evaluation of the impact of a rotavirus vaccine program on pediatric acute gastroenteritis hospitalizations: estimating the overall effect attributable to the program as a whole and as a per-unit change in rotavirus vaccine coverage. American Journal of Epidemiology. 187(9), 2029-2037.

Donato, C., and Vijaykrishna, D. (2017). The broad host range and genetic diversity of mammalian and avian astroviruses. Viruses. 9(5), 102.

Dong, J., Dong, L., Méndez, E., & Tao, Y. (2011). Crystal structure of the human astrovirus capsid spike. *Proceedings of the National Academy of Sciences*, *108*(31), 12681-12686.

Dotson, D., Woodruff, E. A., Villalta, F., & Dong, X. (2016). Filamin A is involved in HIV-1 Vpu-mediated evasion of host restriction by modulating tetherin expression. *Journal of Biological Chemistry*, *291*(8), 4236-4246.

Dowling, J. K., and Mansell, A. (2016). Toll-like receptors: the swiss army knife of immunity and vaccine development. Clinical and translational immunology. 5(5), e85.

DuBois, R. M., Freiden, P., Marvin, S., Reddivari, M., Heath, R. J., White, S. W., & Schultz-Cherry, S. (2013). Crystal structure of the avian astrovirus capsid spike. *Journal of virology*, *87*(14), 7853-7863.

Dutta, S. K., & Tripathi, A. (2017). Association of toll-like receptor polymorphisms with susceptibility to chikungunya virus infection. *Virology*, *511*, 207-213.

El-Shabrawi, M., Salem, M., Abou-Zekri, M., El-Naghi, S., Hassanin, F., El-Adly, T., & El-Shamy, A. (2015). The burden of different pathogens in acute diarrhoeal episodes among a cohort of Egyptian children less than five years old. Gastroenterology Review/Przegląd Gastroenterologiczny, 10(3), 173-180.

El-Zayat, S. R., Sibaii, H., & Mannaa, F. A. (2019). Toll-like receptors activation, signaling, and targeting: an overview. Bulletin of the National Research Centre, 43(1), 1-12.

Enosi Tuipulotu, D., Netzler, N. E., Lun, J. H., Mackenzie, J. M., and White, P. A. (2018). TLR7 agonists display potent antiviral effects against norovirus infection via innate stimulation. Antimicrobial Agents and Chemotherapy. 62(5), e02417-17.

Enweronu-Laryea, C. C., Armah, G., Sagoe, K. W., Ansong, D., Addo-Yobo, E., Diamenu, S. K., ... and Tate, J. E. (2018). Sustained impact of rotavirus vaccine introduction on rotavirus gastroenteritis hospitalizations in children< 5 years of age, Ghana, 2009–2016. Vaccine. 36(47), 7131-7134.

Ettayebi, K., Crawford, S. E., Murakami, K., Broughman, J. R., Karandikar, U., Tenge, V. R., ... and Estes, M. K. (2016). Replication of human noroviruses in stem cell–derived human enteroids. Science. 353(6306), 1387-1393.

Ezeonwu, B. U., Ibeneme, C. A., Aneke, F., and Oguonu, T. (2013). Clinical Features of Acute Gastroenteritis in Children at University of Nigeria

Teaching Hospital, Ituku-Ozalla, Enugu. Annals of Medical and Health Sciences Research. 3(3), 361-364.

Fadhil, H. Y., Al-Kareim, M. A., Aufi, I. M., & Alhamdani, F. G. (2020). Molecular detection of astrovirus using reverse transcriptase quantitative polymerase chain reaction technique. The Iraqi Journal of Agricultural Science, 51, 191-200.

Fensterl, V., Chattopadhyay, S., and Sen, G. C. (2015). No love lost between viruses and interferons. Annual Review of Virology. 2, 549-572.

Fraenkel, a.b. B. Bo¨ttiger c , A. So¨derlund-Strand c , M. Inghammar **.** Risk of environmental transmission of norovirus infection from prior room occupants. Journal of Hospital Infection. 117 (2021) 74e80.

Gao, Z., Liu, B., Yan, H., Li, W., Jia, L., Tian, Y., ... & Pang, X. (2019). Norovirus outbreaks in Beijing, China, from 2014 to 2017. Journal of Infection, 79(2), 159-166.

Gelaw, A., & Liebert, U. G. (2022). Molecular Detection of Enteric Viruses in Under-Five Children with Diarrhea in Debre Tabor, Northwest Ethiopia. Infection and Drug Resistance, 15, 1981.

Gholam, A. I., Alosaimi, E. A., Aldhafeeri, M. D., Alahmari, A. S., Alharbi, A. S., Bohassan, R. H., ... & Albaiji, F. M. (2019). Gastroenteritis Diagnosis and Management in Children: A simple Literature Review. Arch Pharm Pract, 10(3), 43-6.

Gong, X. H., Wu, H. Y., Li, J., Xiao, W. J., Zhang, X., Chen, M., et al. (2018). Epidemiology, aetiology and seasonality of infectious diarrhoea in adult outpatients through active surveillance in Shanghai, China, 2012-2016: acrosssectional study. BMJ Open 8:e019699. doi:10.1136/bmjopen-2017-019699.

Gordon-Alonso, M., Hirsch, T., Wildmann, C., & van der Bruggen, P. (2017). Galectin-3 captures interferon-gamma in the tumor matrix reducing

chemokine gradient production and T-cell tumor infiltration. *Nature communications*, *8*(1), 1-15.

Gosu, V., Basith, S., Kwon, O. P., and Choi, S. (2012). Therapeutic applications of nucleic acids and their analogues in Toll-like receptor signaling. Molecules. 17(11), 13503-13529.

Graziano, V. R., Wei, J., and Wilen, C. B. (2019). Norovirus attachment and entry. Viruses. 11(6), 495.

Grigsby, A., Herron, J., & Hunter, B. R. (2019). Does the addition of dextrose to IV crystalloid therapy provide clinical benefit in acute dehydration? A systematic review and meta-analysis. Canadian Journal of Emergency Medicine, 21(5), 638-645

Guix, S., Caballero, S., Villena, C., Bartolomé, R., Latorre, C., Rabella, N., ... & Pintó, R. M. (2002). Molecular epidemiology of astrovirus infection in Barcelona, Spain. Journal of clinical microbiology, 40(1), 133-139.

Guix, S., Pérez-Bosque, A., Miró, L., Moretó, M., Bosch, A., and Pintó, R. M. (2015). Type I interferon response is delayed in human astrovirus infections. PLoS One. 10(4), e0123087.

Haidong, Z. (2005). Single-strand conformational polymorphism analysis. In Hypertension (pp. 149-158). Humana Press.

Hall, A.J., et al., Norovirus disease in the United States. Emerg Infect Dis, 2013. 19(8): p. 1198 205.

Hamad, A. Y., Mohammed, N. S., & Hasan, A. S. (2022). Molecular detection and phylogenetic analysis of Astrovirus and Noroviruses from sewage water in Diyala-Iraq. Journal of Pharmaceutical Negative Results, 339-347.

Hartman, S., Brown, E., Loomis, E., & Russell, H. A. (2019). Gastroenteritis in children. American family physician, 99(3), 159-165.

Hasan, H., Nasirudeen, N. A., Ruzlan, M. A. F., Mohd Jamil, M. A., Ismail, N. A. S., Wahab, A. A., & Ali, A. (2021). Acute Infectious Gastroenteritis:

The Causative Agents, Omics-Based Detection of Antigens and Novel Biomarkers. Children, 8(12), 1112.

Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. Genomics, 107(1), 1-8.

Hosmillo, M., Lu, J., McAllaster, M. R., Eaglesham, J. B., Wang, X., Emmott, E., ... and Goodfellow, I. G. (2019). Noroviruses subvert the core stress granule component G3BP1 to promote viral VPg-dependent translation. Elife, 8, e46681.

Hungerford, D., Vivancos, R., Read, J. M., Bonnett, L. J., Bar-Zeev, N., Iturriza-Gómara, M., ... and French, N. (2018). Mitigating bias in observational vaccine effectiveness studies using simulated comparator populations: application to rotavirus vaccination in the UK. Vaccine. 36(45), 6674-6682.

Hussein, A. A., Hussein, R. A., & Shaker, M. J. (2018). Enteric viruses co-infection with giardiasis among diarrheal children in Diyala Province-Iraq. Journal of PurE and aPPliEd Microbiology, 12(2), 793-799.Johnson, C., Hargest, V., Cortez, V., Meliopoulos, V. A., & Schultz-Cherry, S. (2017). Astrovirus pathogenesis. Viruses, 9(1), 22.

ICTV (2020).International Committee on Taxonomy of Viruses.

Indrio, F., Neu, J., Pettoello-Mantovani, M., Marchese, F., Martini, S., Salatto, A., and Aceti, A. (2022). Development of the Gastrointestinal Tract in Newborns as a Challenge for an Appropriate Nutrition: A Narrative Review. Nutrients. 14(7), 1405.

Iturriza-Gómara, M., Jere, K. C., Hungerford, D., Bar-Zeev, N., Shioda, K., Kanjerwa, O., ... & Cunliffe, N. A. (2019). Etiology of diarrhea among hospitalized children in Blantyre, Malawi, following rotavirus vaccine introduction: a case-control study. The Journal of infectious diseases, 220(2), 213-218.

Jacobsen, S., Höhne, M., Marques, A. M., Beslmüller, K., Bock, C. T., & Niendorf, S. (2018). Co-circulation of classic and novel astrovirus strains in patients with acute gastroenteritis in Germany. Journal of Infection, 76(5), 457-464.

Janowski, A. B. (2021). Beyond the gastrointestinal tract: the emerging and diverse tissue tropisms of astroviruses. *Viruses*, *13*(5), 732.

Januarie, K. C., Uhuo, O. V., Iwuoha, E., & Feleni, U. (2021). Recent advances in the detection of interferon-gamma as a TB biomarker. *Analytical and Bioanalytical Chemistry*, 1-15.

Jeffs, E., Williman, J., Martin, N., Brunton, C., & Walls, T. (2019). The epidemiology of non-viral gastroenteritis in New Zealand children from 1997 to 2015: an observational study. *BMC Public Health*, *19*(1), 1-9.

Jenkins, K. A., Vaughan Jr, G. H., Rodriguez, L. O., & Freeland, A. (2021). Acute Gastroenteritis on Cruise Ships—Maritime Illness Database and Reporting System, United States, 2006–2019. MMWR Surveillance Summaries, 70(6), 1.

Jensen, S., and Thomsen, A. R. (2012). Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion. Journal of Virology. 86(6), 2900-2910.

Jin, M., Wu, S., Kong, X., Xie, H., Fu, J., He, Y., ... & Duan, Z. (2020). Norovirus outbreak surveillance, China, 2016–2018. Emerging infectious diseases, 26(3), 437.

Johnson, C., Hargest, V., Cortez, V., Meliopoulos, V. A., & Schultz-Cherry, S. (2017). Astrovirus pathogenesis. *Viruses*, *9*(1), 22..

Jones, M. K., Grau, K. R., Costantini, V., Kolawole, A. O., De Graaf, M., Freiden, P., ... and Karst, S. M. (2015). Human norovirus culture in B cells. Nature Protocols. 10(12), 1939-1947.

Kahn, G., Fitzwater, S., Tate, J., Kang, G., Ganguly, N., Nair, G., ... and Santosham, M. (2012). Epidemiology and prospects for prevention of rotavirus disease in India. Indian Pediatrics. 49(6), 467-474.

Kak, G., Raza, M., and Tiwari, B. K. (2018). Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomolecular Concepts. 9(1), 64-79.

Kauer, R. V., Koch, M. C., Hierweger, M. M., Werder, S., Boujon, C. L., & Seuberlich, T. (2019). Discovery of novel astrovirus genotype species in small ruminants. *PeerJ*, *7*, e7338.

Kawasaki, T., and Kawai, T. (2014). Toll-like receptor signaling pathways. Front Immunol. 5: 461.

Kazemi, A., Tabatabaie, F., Agha-Ghazvini, M. R., and Kelishadi, R. (2006). The role of rotavirus in acute pediatric diarrhea in Isfahan, Iran. Pak J Med Sci. 22(3), 282-5.

Khalil, M., Azhar, E., Kao, M., Al-Kaiedi, N., Alhani, H., Al Olayan, I., ... & DeAntonio, R. (2015). Gastroenteritis attributable to rotavirus in hospitalized Saudi Arabian children in the period 2007–2008. Clinical epidemiology, 7, 129.

Khan, M. A. (2021). Epidemiological studies on gastroenteritis in children in the Bannu district, Khyber Pakhtunkhwa, Pakistan. Journal of Public Health. 1-8.

Khumela, R., Kabue, J. P., Traore, A. N., and Potgieter, N. (2021). Human Astrovirus in Symptomatic and Asymptomatic Children: A Cross-Sectional Study on Hospitalized and Outpatients from Rural Communities of South Africa between 2017–2021. Pathogens. 10(11), 1398.

Kim, H., Won, Y. J., Kang, L. H., Lee, A. R., Han, J. I., Suh, C. I., and Paik, S. Y. (2019). Complete sequence analysis of human norovirus GII. 17 detected in South Korea. Epidemiology and Infection, 147.

Kim, J. S., Lee, W. J., Lee, S. K., Lee, E. J., Hyun, J., Kim, H. S., ... & Kim, H. S. (2019). Molecular epidemiology of human astrovirus in stool samples from patients with acute gastroenteritis in Korea, 2013–2017. Annals of Laboratory Medicine, 39(4), 367.

Kim, M., and Ko, G. (2013). Quantitative characterization of the inhibitory effects of salt, humic acid, and heavy metals on the recovery of waterborne norovirus by electropositive filters. Journal of Water and Health. 11(4), 613-622.

Kirby, A., Dove, W., Ashton, L., Hopkins, M., & Cunliffe, N. A. (2010). Detection of norovirus in mouthwash samples from patients with acute gastroenteritis. Journal of Clinical Virology, 48(4), 285-287.

Koukou, G., Niendorf, S., Hornei, B., Schlump, J. U., Jenke, A. C., & Jacobsen, S. (2019). Human astrovirus infection associated with encephalitis in an immunocompetent child: a case report. Journal of Medical Case Reports, 13(1), 1-5.

Kumar H, Kawai T, Akira S (2011) Pathogen recognition by the innate immune system. Int Rev Immunol 30(1): 16±34. https://doi.org/10.3109/08830185.2010.529976 PMID: 21235323.

Kumthip, K., Khamrin, P., Ushijima, H., & Maneekarn, N. (2018). Molecular epidemiology of classic, MLB and VA astroviruses isolated from< 5 year-old children with gastroenteritis in Thailand, 2011–2016. Infection, Genetics and Evolution, 65, 373-379.

Lanford, R. E., Guerra, B., Chavez, D., Giavedoni, L., Hodara, V. L., Brasky, K. M., ... and Tumas, D. B. (2013). GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology. 144(7), 1508-1517.

Lazear HM et al (2019) Shared and distinct functions of type I and type III interferons. Immunity 50(4):907–923.

Lazear, H. M., Schoggins, J. W., and Diamond, M. S. (2019). Shared and distinct functions of type I and type III interferons. Immunity. 50(4), 907-923.

Leaker, B. R., Singh, D., Lindgren, S., Almqvist, G., Eriksson, L., Young, B., & O'Connor, B. (2019). Effects of the Toll-like receptor 7 (TLR7) agonist, AZD8848, on allergen-induced responses in patients with mild asthma: a double-blind, randomised, parallel-group study. *Respiratory research*, *20*(1), 1-11.

Lee, C. C., Chiu, C. H., Lee, H. Y., Tsai, C. N., Chen, C. L., and Chen, S. Y. (2021). Clinical and virological characteristics of viral shedding in children with norovirus gastroenteritis. Journal of Microbiology, Immunology and Infection.

Lee, S., and Baldridge, M. T. (2017). Interferon-lambda: a potent regulator of intestinal viral infections. Frontiers in Immunology. 8, 749.

Lee, S., Liu, H., Wilen, C. B., Sychev, Z. E., Desai, C., Hykes Jr, B. L., ... & Virgin, H. W. (2019). A secreted viral nonstructural protein determines intestinal norovirus pathogenesis. Cell host & microbe, 25(6), 845-857.

Lester, S. N., and Li, K. (2014). Toll-like receptors in antiviral innate immunity. Journal of Molecular Biology. 426(6), 1246-1264.

Letunic, I., & Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic acids research*, *47*(W1), W256-W259.

Li, T. F., Hosmillo, M., Schwanke, H., Shu, T., Wang, Z., Yin, L., ... and Zhou, X. (2018). Human norovirus NS3 has RNA helicase and chaperoning activities. Journal of Virology. 92(5), e01606-17.

Li, W., Yan, H., Liu, B., Tian, Y., Chen, Y., Jia, L., ... & Wang, Q. (2021). Epidemiological characteristics and genetic diversity of norovirus infections among outpatient children with diarrhea under 5 years of age in Beijing, China, 2011–2018. Gut Pathogens, 13(1), 1-11.

Li, W., Yan, H., Liu, B., Tian, Y., Chen, Y., Jia, L., ... & Wang, Q. (2021). Epidemiological characteristics and genetic diversity of norovirus infections among outpatient children with diarrhea under 5 years of age in Beijing, China, 2011–2018. Gut Pathogens, 13(1), 1-11.

Lilkova, E., Petkov, P., Ilieva, N., Krachmarova, E., Nacheva, G., & Litov, L. (2019). Molecular modeling of the effects of glycosylation on the structure and dynamics of human interferon-gamma. Journal of molecular modeling, 25(5), 1-13.

Liu, W., Li, M., Wang, Z., and Wang, J. (2020). Ifn-γ mediates the development of systemic lupus erythematosus. BioMed Research International.

Liu, W., Zhang, S., & Wang, J. (2022). IFN-γ, should not be ignored in SLE. *Frontiers in Immunology*, *13*, 954706.

Logan, C., O'Leary, J. J., & O'Sullivan, N. (2007). Real-time reverse transcription PCR detection of norovirus, sapovirus and astrovirus as causative agents of acute viral gastroenteritis. Journal of virological methods, 146(1-2), 36-44.

Lopman, B. A., and Grassly, N. C. (2016). Editorial commentary: pediatric norovirus in developing countries: a picture slowly comes into focus. Clinical Infectious Diseases. 62(10), 1218-1220.

Lu, L., Zhong, H., Xu, M., Su, L., Cao, L., Jia, R., & Xu, J. (2019). Genetic diversity and epidemiology of Genogroup II noroviruses in children with acute sporadic gastroenteritis in Shanghai, China, 2012–2017. BMC Infectious Diseases, 19(1), 1-10.

Lu, L., Zhong, H., Xu, M., Su, L., Cao, L., Jia, R., & Xu, J. (2021). Molecular and epidemiological characterization of human adenovirus and classic human astrovirus in children with acute diarrhea in Shanghai, 2017–2018. BMC Infectious Diseases, 21(1), 1-10.

Lu, Y., Ma, M., Wang, H., Wang, D., Chen, C., Jing, Q., ... & Yang, Z. (2020). An outbreak of norovirus-related acute gastroenteritis associated with delivery food in Guangzhou, southern China. BMC Public Health, 20(1), 1-7.

Lum, S. H., Turner, A., Guiver, M., Bonney, D., Martland, T., Davies, E., ... & Wynn, R. (2016). An emerging opportunistic infection: fatal astrovirus (VA 1/HMO-C) encephalitis in a pediatric stem cell transplant recipient. *Transplant Infectious Disease*, *18*(6), 960-964.

Luo, X., Deng, J. K., Mu, X. P., Yu, N., and Che, X. (2021). Detection and characterization of human astrovirus and sapovirus in outpatients with acute gastroenteritis in Guangzhou, China. BMC gastroenterology. 21(1), 1-8.

Maclachlan NJ, Dubovi EJ, Barthold SW, Swayne DE, Winton JR (2017). *Fenner's Veterinary Virology* (Fifth ed.). Amsterdam: Elsevier/Academic Press. ISBN 978-0-12-800946-8.

Mans, J. (2019). Norovirus infections and disease in lower-middle-and low-income countries, 1997–2018. Viruses. 11(4), 341.

Marvin, S. A. (2016). The immune response to Astrovirus infection. *Viruses*, *9*(1), 1.

Marvin, S. A., Huerta, C. T., Sharp, B., Freiden, P., Cline, T. D., and Schultz-Cherry, S. (2016). Type I interferon response limits astrovirus replication and protects against increased barrier permeability in vitro and in vivo. Journal of Virology. 90(4), 1988-1996.

McCartney, S. A., Thackray, L. B., Gitlin, L., Gilfillan, S., Virgin IV, H. W., and Colonna, M. (2008). MDA-5 recognition of a murine norovirus. PLoS Pathogens. 4(7), e1000108.

Mendes da Silva, L. D., Gatto, M., Miziara de Abreu Teodoro, M., de Assis Golim, M., Pelisson Nunes da Costa, E. A., Capel Tavares Carvalho, F., ... & Calvi, S. A. (2017). Participation of TLR 2 and TLR 4 in Cytokines

Production by Patients with Symptomatic and Asymptomatic Chronic Chagas Disease. *Scandinavian Journal of Immunology*, *85*(1), 58-65.

Mitab, H. H. (2013). Comparison of multiplex real time PCR with ELISA for diagnosis of viral Gastroenteritis in children of Al-Muthana province..

Mo, Q. H., Wang, H. B., Dai, H. R., Lin, J. C., Tan, H., Wang, Q., & Yang, Z. (2015). Rapid and simultaneous detection of three major diarrhea-causing viruses by multiplex real-time nucleic acid sequence-based amplification. Archives of virology, 160(3), 719-725.

Mohammad, H. A., Madi, N. M., & Al-Nakib, W. (2020). Analysis of viral diversity in stool samples from infants and children with acute gastroenteritis in Kuwait using Metagenomics approach. Virology journal, 17(1), 1-12.

Moser, L. A., Carter, M., and Schultz-Cherry, S. (2007). Astrovirus increases epithelial barrier permeability independently of viral replication. Journal of Virology. 81(21), 11937-11945.

Mukherjee, S., & Tripathi, A. (2019). Contribution of Toll like receptor polymorphisms to dengue susceptibility and clinical outcome among eastern Indian patients. *Immunobiology*, *224*(6), 774-785.

Nadan, S., Taylor, M. B., & Page, N. A. (2021). Circulation of classic and recombinant human astroviruses detected in South Africa: 2009 to 2014. Journal of Clinical Virology, 135, 104719.

Nadan, S., Taylor, M. B., Groome, M. J., Cohen, C., Madhi, S. A., & Page, N. A. (2019). Epidemiology of human astroviruses among children younger than 5 years: Prospective hospital-based sentinel surveillance in South Africa, 2009-2014. Journal of Medical Virology, 91(2), 225-234.

Naglak, E. K., Morrison, S. G., & Morrison, R. P. (2016). Gamma interferon is required for optimal antibody-mediated immunity against genital Chlamydia infection. *Infection and immunity*, *84*(11), 3232-3242.

Nicholas Wohlgemutha, Rebekah Honcea,b, Stacey Schultz-Cherry. Astrovirus evolution and emergence, Infection, Genetics and Evolution 69 (2019) 30–37.

Obili, R. C. R., Das, S., Mangla, V., and Nundy, S. (2020). Gender Differences in Gastrointestinal, Hepatobiliary and Pancreatic Surgery and Perceived Relevance on Outcomes—A Single Center 22-Year Observational Study in India (1996-2018). Surgical Science. 11(11), 365.

Offersen, R., Nissen, S. K., Rasmussen, T. A., Østergaard, L., Denton, P. W., Søgaard, O. S., and Tolstrup, M. (2016). A novel Toll-like receptor 9 agonist, MGN1703, enhances HIV-1 transcription and NK cell-mediated inhibition of HIV-1-infected autologous CD4+ T cells. Journal of Virology. 90(9), 4441-4453.

Okuse, C., Rinaudo, J. A., Farrar, K., Wells, F., & Korba, B. E. (2005). Enhancement of antiviral activity against hepatitis C virus in vitro by interferon combination therapy. *Antiviral research*, *65*(1), 23-34.

Omosigho, P., Izevbuwa, O. E., Rotimi, E. O., Olalekan, J., and Othoigbe, M. O. (2022). Prevalence and risk factors of Astrovirus gastroenteritis in children in Offa, Kwara State, North Central Nigeria. Microbes and Infectious Diseases. 3(3), 596-605.

Oude Munnink, B. B., & Van der Hoek, L. (2016). Viruses causing gastroenteritis: the known, the new and those beyond. Viruses, 8(2), 42.

Ouyang, S., Gong, B., Li, J. Z., Zhao, L. X., Wu, W., Zhang, F. S., ... & Liang, M. (2012). Structural insights into a human anti-IFN antibody exerting therapeutic potential for systemic lupus erythematosus. *Journal of molecular medicine*, *90*(7), 837-846.

Parrón-Bernabé, I., Plasencia, E., Cornejo Sánchez, T., Jané-Checa, M., Pérez, C., & Izquierdo, C. (2021). Human Astrovirus Outbreak in a Daycare Center and Propagation among Household Contacts.

Patinote, C., Karroum, N. B., Moarbess, G., Cirnat, N., Kassab, I., Bonnet, P. A., and Deleuze-Masquéfa, C. (2020). Agonist and antagonist ligands of toll-like receptors 7 and 8: Ingenious tools for therapeutic purposes. European Journal of Medicinal Chemistry. 193, 112238.

Pawlotsky, J. M. (2011). Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus. *Hepatology*, *53*(5), 1742-1751.

Pérot, P., Lecuit, M., & Eliot, M. (2017). Astrovirus diagnostics. Viruses 9: 10.

Platts-Mills, J. A., Amour, C., Gratz, J., Nshama, R., Walongo, T., Mujaga, B., ... & Houpt, E. R. (2017). Impact of rotavirus vaccine introduction and postintroduction etiology of diarrhea requiring hospital admission in Haydom, Tanzania, a rural African setting. Clinical Infectious Diseases, 65(7), 1144-1151.

Qi, L., Xiang, X., Xiong, Y., Ling, H., Shen, H., Deng, W., ... and Li, Q. (2018). Outbreak of acute gastroenteritis caused by norovirus genogroup II attributed to contaminated cold dishes on a cruise ship in Chongqing, China, 2017. International Journal of Environmental Research and Public Health. 15(12), 2823.

Raafat, I. I., El Guindy, N., Shahin, R. M. H., Samy, L. A., & El Refai, R. M. (2018). Toll-like receptor 7 gene single nucleotide polymorphisms and the risk for systemic lupus erythematosus: a case-control study. *Zeitschrift für Rheumatologie*, *77*(5), 416-420.

Rani, M., Rajyalakshmi, S., Pakalapaty, S., and Kammilli, N. (2021). Norovirus Structure and Classification. Norovirus. 31.

Rivera-Dominguez G, Ward R. Pediatric Gastroenteritis. Treasure Island (FL): StatPearlsPublishing;2022Jan.Availablefromhttps://www.ncbi.nlm.nih.gov/books/NBK499939.

Roach, S. N., and Langlois, R. A. (2021). Intra-and cross-species transmission of astroviruses. Viruses. 13(6), 1127.

Romo-Saenz, C. I., Medina-Soltero, M. R., Delgado-Gardea, M. C. E., Tamez-Guerra, P., Contreras-Palma, M., del Carmen Gonzalez-Horta, M., ... & Infante-Ramirez, R. (2020). Human enteric circulating viruses and co-infections among hospitalized children with severe acute gastroenteritis in Chihuahua, Mexico, during 2010-2011. Jundishapur Journal of Microbiology, 13(2).

Rönnelid, Y., Bonkoungou, I. J. O., Ouedraogo, N., Barro, N., Svensson, L., & Nordgren, J. (2020). Norovirus and rotavirus in children hospitalised with diarrhoea after rotavirus vaccine introduction in Burkina Faso. Epidemiology & Infection, 148.

Rosser EC, Oleinika K, Tonon S, Doyle R, Bosma A, Carter NA, et al. Regulatory B cells are induced by gut microbiota-driven interleukin-1b and interleukin-6 production. Nat Med. (2014) 20:1334–9. doi: 10.1038/ nm.3680.

Roth, A. N., and Karst, S. M. (2016). Norovirus mechanisms of immune antagonism. Current Opinion in virology. 16, 24-30.

Sabatier, M., Bal, A., Destras, G., Regue, H., Quéromès, G., Cheynet, V., ... & Josset, L. (2020). Comparison of nucleic acid extraction methods for a viral metagenomics analysis of respiratory viruses. Microorganisms, 8(10), 1539.

Saied, M., Neda, M., Fatemeh, F., Raheleh, S. S. N., & Fatemeh, A. (2013). Epidemiology of astrovirus infection in young children hospitalized with gastroenteritis in Iran, over a period of seven years, using reverse transcriptase-polymerase chain reaction (RT-PCR). Journal of Public Health and Epidemiology, 5(1), 37-42.

Sambrook, J., & Russell, D. W. (2006). Detection of Mutations by Single-strand Conformational Polymorphism. Cold Spring Harbor Protocols, 2006(1), pdb-prot3815.

Schoggins, J. W. (2018). Recent advances in antiviral interferon-stimulated gene biology. F1000Research. 7.

Schrader, C., Schielke, A., Ellerbroek, L., and Johne, R. (2012). PCR inhibitors–occurrence, properties and removal. Journal of Applied Microbiology. 113(5), 1014-1026.

Steyer, A., Jevšnik, M., Petrovec, M., Pokorn, M., Grosek, Š., Steyer, A. F., ... & Strle, F. (2016). Narrowing of the diagnostic gap of acute gastroenteritis in children 0-6 years of age using a combination of classical and molecular techniques, delivers challenges in syndromic approach diagnostics. The Pediatric infectious disease journal, 35(9), e262.

Shan, L., Fu, F., Xue, M., Zhu, X., Li, L., Feng, L., and Liu, P. (2019). Interferon gamma inhibits transmissible gastroenteritis virus infection mediated by an IRF1 signaling pathway. Archives of Virology. 164(11), 2659-2669.

Shen, N., Fu, Q., Deng, Y., Qian, X., Zhao, J., Kaufman, K. M., ... & Tsao, B. P. (2010). Sex-specific association of X-linked Toll-like receptor 7 (TLR7) with male systemic lupus erythematosus. *Proceedings of the National Academy of Sciences*, *107*(36), 15838-15843.

Shonyela, S. M., Yang, W., Yang, G., and Wang, C. (2019). Innate Cytokine Responses and Toll-Like Receptor Induced by Recombinant Porcine Rotavirus VP6 and VP7 Proteins Expressing in Lactobacillus plantarum NC8 Strain Colonization in Mice. World Journal of Vaccines. 10(1), 17-31.

Siah, S. P., Merif, J., Kaur, K., Nair, J., Huntington, P. G., Karagiannis, T., ... and Millar, D. S. (2014). Improved detection of gastrointestinal pathogens using generalised sample processing and amplification panels. Pathology. 46(1), 53-59.

Siqueira, J. A. M., de Souza Oliveira, D., de Carvalho, T. C. N., Portal, T. M., Justino, M. C. A., da Silva, L. D., ... and Gabbay, Y. B. (2017). Astrovirus infection in hospitalized children: molecular, clinical and epidemiological features. Journal of Clinical Virology. 94, 79-85.

Snowden, J. S., Hurdiss, D. L., Adeyemi, O. O., Ranson, N. A., Herod, M. R., and Stonehouse, N. J. (2020). Dynamics in the murine norovirus capsid revealed by high-resolution cryo-EM. PLoS Biology. 18(3), e3000649.

Stegemann-Koniszewski, S., Behrens, S., Boehme, J. D., Hochnadel, I., Riese, P., Guzmán, C. A., ... and Bruder, D. (2018). Respiratory influenza A virus infection triggers local and systemic natural killer cell activation via toll-like receptor 7. Frontiers in Immunology. 9, 245.

Sun, Z., Xu, J., Niu, P., Jin, M., Gao, Q., Zhang, R., ... & Ma, X. (2022). Aetiological Characteristics of Infectious Diarrhea in Yantai City, Shandong Province, China in 2017. Viruses, 14(2), 216.

Sunkara, T., Rawla, P., Yarlagadda, K. S., & Gaduputi, V. Eosinophilic gastroenteritis: diagnosis and clinical perspectives. Clin Exp Gastroenterol. 2019; 12: 239–53.

Tam, C. C., O'Brien, S. J., Tompkins, D. S., Bolton, F. J., Berry, L., Dodds, J., ... & IID2 Study Executive Committeea. (2012). Changes in causes of acute gastroenteritis in the United Kingdom over 15 years: microbiologic findings from 2 prospective, population-based studies of infectious intestinal disease. *Clinical Infectious Diseases*, *54*(9), 1275-1286.

Teunis, P. F., Le Guyader, F. S., Liu, P., Ollivier, J., and Moe, C. L. (2020). Noroviruses are highly infectious but there is strong variation in host susceptibility and virus pathogenicity. Epidemics. 32, 100401.

Thorne, L. G., and Goodfellow, I. G. (2014). Norovirus gene expression and replication. Journal of General Virology. 95(2), 278-291.

Thwiny, H. (2015). Detection of Norovirus in stool samples by RT-PCR from under five years age children hospitalized for acute gastroenteritis in Basrah, Iraq. University of Thi-Qar Journal of Science. 5(2), 47-54.

Timurkan, M. Ö., Aydin, H., & Aktaş, O. (2017). Frequency and molecular characterization of human norovirus in Erzurum, Turkey. Turkish Journal of Medical Sciences, 47(3), 960-966.

Toh, Y., Harper, J., Dryden, K. A., Yeager, M., Arias, C. F., Méndez, E., and Tao, Y. J. (2016). Crystal structure of the human astrovirus capsid protein. Journal of Virology. 90(20), 9008-9017.

Tohma, K., Lepore, C. J., Martinez, M., Degiuseppe, J. I., Khamrin, P., Saito, M., ... and Parra, G. I. (2021). Genome-wide analyses of human noroviruses provide insights on evolutionary dynamics and evidence of coexisting viral populations evolving under recombination constraints. PLoS Pathogens. 17(7), e1009744.

Tsague, B. N., Louya, V. M., Ntoumi, F., Adedoja, A., Vouvoungui, C. J., Peko, S. M., & Abena, A. A. (2020). Occurrence of human astrovirus associated with gastroenteritis among Congolese children in Brazzaville, Republic of Congo. International Journal of Infectious Diseases, 95, 142-147.

Tsai, A., Irrinki, A., Kaur, J., Cihlar, T., Kukolj, G., Sloan, D. D., and Murry, J. P. (2017). Toll-like receptor 7 agonist GS-9620 induces HIV expression and HIV-specific immunity in cells from HIV-infected individuals on suppressive antiretroviral therapy. Journal of Virology. 91(8), e02166-16.

Varela, M. F., Rivadulla, E., Lema, A., and Romalde, J. L. (2019). Human sapovirus among outpatients with acute gastroenteritis in Spain: A one-year study. Viruses. 11(2), 144.

van Dongen, J. A., Rouers, E. D., Schuurman, R., Bonten, M. J., & Bruijning-Verhagen, P. (2021). Acute gastroenteritis disease burden in infants with medical risk conditions in the Netherlands. The Pediatric infectious disease journal, 40(4), 300.

Verhoef, L., Hewitt, J., Barclay, L., Ahmed, S., Lake, R., Hall, A. J., ... & Koopmans, M. (2015). Norovirus Genotype Profiles Associated with Foodborne Transmission, 1999 ?" 2012. Emerging infectious diseases, 21(4), 592.

Verhoef, L., Kouyos, R. D., Vennema, H., Kroneman, A., Siebenga, J., van Pelt, W., ... and Foodborne Viruses in Europe Network. (2011). An integrated approach to identifying international foodborne norovirus outbreaks. Emerging Infectious Diseases. 17(3), 412.

Villabruna, N., Izquierdo Lara, R. W., Szarvas, J., Koopmans, M. P., and Graaf, M. D. (2020). Phylogenetic investigation of norovirus transmission between humans and animals. Viruses. 12(11), 1287.

Viskovska, M. A., Zhao, B., Shanker, S., Choi, J. M., Deng, L., Song, Y., ... and Venkataram Prasad, B. V. (2019). GII. 4 norovirus protease shows pH-sensitive proteolysis with a unique Arg-His pairing in the catalytic site. Journal of Virology. 93(6), e01479-18.

Vu, D. L., Bosch, A., Pintó, R. M., & Guix, S. (2017). Epidemiology of classic and novel human astrovirus: gastroenteritis and beyond. Viruses, 9(2), 33.

Vu, D. L., Cordey, S., Brito, F., and Kaiser, L. (2016). Novel human astroviruses: Novel human diseases. Journal of Clinical Virology. 82, 56-63.

Vu, D. L., Sabrià, A., Aregall, N., Michl, K., Sabrià, J., Rodriguez Garrido, V., ... & Guix, S. (2020). A Spanish case-control study in< 5 year-old children reveals the lack of association between MLB and VA astrovirus and diarrhea. Scientific reports, 10(1), 1-9.

Walker, F. C., and Baldridge, M. T. (2019). Interactions between noroviruses, the host, and the microbiota. Current Opinion in Virology. 37, 1-9.

Walsh, E. R., Pisitkun, P., Voynova, E., Deane, J. A., Scott, B. L., Caspi, R. R., and Bolland, S. (2012). Dual signaling by innate and adaptive immune receptors is required for TLR7-induced B-cell–mediated autoimmunity. Proceedings of the National Academy of Sciences. 109(40), 16276-16281.

Walter, M. R. (2020). The role of structure in the biology of interferon signaling. Frontiers in immunology, 11, 606489..

Wang C, Ji L, Yuan X, Jin Y, Cardona CJ, Xing Z (2016) Differential regulation of TLR signaling on the induction of antiviral interferons in human intestinal epithelial cells infected with enterovirus 71. PLoS One 11(3):e0152177. https://doi.org/10.1371/journal.pone.0152177 PMID: 27007979.

Wang, J., Li, L., Xu, Y., Mao, T., Ma, Y., Sun, X., ... & Duan, Z. (2022). Identification of a novel norovirus species in fox. Infection, Genetics and Evolution, 98, 105214.

Wei, H., Khamrin, P., Kumthip, K., Yodmeeklin, A., & Maneekarn, N. (2021). High divergence of human astrovirus genotypes circulating in pediatric patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand, 2017–2020. Scientific Reports, 11(1), 1-8.

Wicherska-Pawłowska, K., Wróbel, T., & Rybka, J. (2021). Toll-Like Receptors (TLRs), NOD-Like Receptors (NLRs), and RIG-I-Like Receptors (RLRs) in Innate Immunity. TLRs, NLRs, and RLRs Ligands as Immunotherapeutic Agents for Hematopoietic Diseases. *International Journal of Molecular Sciences*, *22*(24), 13397.

Wohlgemuth, N., Honce, R., & Schultz-Cherry, S. (2019). Astrovirus evolution and emergence. Infection, genetics and Evolution, 69, 30-37.

Wu, W., Qiu, S., Huang, H., Xu, R., Bao, E., & Lv, Y. (2021). Immune-related gene expression in the kidneys and spleens of goslings infected with goose nephritic astrovirus. *Poultry Science*, *100*(4), 100990.

Yang, L., Ijaz, I., Cheng, J., Wei, C., Tan, X., Khan, M. A., ... & Fu, J. (2018). Evaluation of amplification refractory mutation system (ARMS) technique for quick and accurate prenatal gene diagnosis of CHM variant in choroideremia. The application of clinical genetics, 11, 1.

Yang, Y., Xia, M., Wang, L., Arumugam, S., Wang, Y., Ou, X., ... and Li, X. (2019). Structural basis of host ligand specificity change of GII porcine noroviruses from their closely related GII human noroviruses. Emerging Microbes and Infections. 8(1), 1642-1657.

Yao, P., & Fox, P. L. (2020). Aminoacyl-tRNA synthetases in cell signaling. In *The Enzymes* (Vol. 48, pp. 243-275). Academic Press.

Yin, D., Tian, J., Yang, J., Tang, Y., and Diao, Y. (2021). Pathogenicity of novel goose-origin astrovirus causing gout in goslings. BMC Veterinary Research. 17(1), 1-8.

York, R. L., Yousefi, P. A., Bogdanoff, W., Haile, S., Tripathi, S., & DuBois, R. M. (2016). Structural, mechanistic, and antigenic characterization of the human astrovirus capsid. *Journal of virology*, *90*(5), 2254-2263.

Yue, M., Feng, L., Wang, J. J., Xue, X. X., Ding, W. L., Zhang, Y., & Deng, X. Z. (2014). Sex-specific association between X-linked Toll-like receptor 7 with the outcomes of hepatitis C virus infection. *Gene*, *548*(2), 244-250.

Zaki, M. E. S., Mashaly, G. E. S., Alsayed, M. A. L., & Nomir, M. M. (2020). Molecular study of human astrovirus in Egyptian children with acute gastroenteritis. Germs, 10(3), 167.

Zaraket, H., Abou-El-Hassan, H., Kreidieh, K., Soudani, N., Ali, Z., Hammadi, M., ... and Dbaibo, G. (2017). Characterization of astrovirus-

associated gastroenteritis in hospitalized children under five years of age. Infection, Genetics and Evolution. 53, 94-99.

Zaraket, H., Abou-El-Hassan, H., Kreidieh, K., Soudani, N., Ali, Z., Hammadi, M., ... & Dbaibo, G. (2017). Characterization of astrovirus-associated gastroenteritis in hospitalized children under five years of age. Infection, Genetics and Evolution, 53, 94-99.

Zhang P., Su H., Peng R., Chan JF-W., Bai S., Wang G., Huang Y., Hu X., Luo J., Liu S., Li Y., Xue L., Yang F., Zhao M., Zhang Y., Tang C., Shen S., Cui X., Niu L., Lu G., Yuen K-Y., Deng F., Zhang W., Yin F and Du J (2022) Identification of a Novel Astrovirus in Pinnipeds. Front. Microbiol. 13:845601. doi: 10.3389/fmicb.2022.845601.

Zhang, C., Niu, P., Hong, Y., Wang, J., Zhang, J., and Ma, X. (2015). A probe-free four-tube real-time PCR assay for simultaneous detection of twelve enteric viruses and bacteria. Journal of Microbiological Methods. 118, 93-98.

Zhang, D., Lou, X., Yan, H., Pan, J., Mao, H., Tang, H., ... & Ma, X. (2018). Metagenomic analysis of viral nucleic acid extraction methods in respiratory clinical samples. BMC genomics, 19(1), 1-8.

Zhang, T., Zhu, J., Su, B., Cao, L., Li, Z., Wei, H., ... & He, Q. (2020). Effects of TLR7 polymorphisms on the susceptibility and progression of HIV-1 infection in Chinese MSM population. *Frontiers in immunology*, *11*, 589010.

Zhang, Z., Ohto, U., Shibata, T., Taoka, M., Yamauchi, Y., Sato, R., ... & Shimizu, T. (2018). Structural analyses of toll-like receptor 7 reveal detailed RNA sequence specificity and recognition mechanism of agonistic ligands. *Cell reports*, *25*(12), 3371-3381.

Zhao, W., Wu, Z., Yao, Y., Qin, A., & Qian, K. (2020). The isolation and molecular characterization of an astrovirus from “Yellow” Chickens, China. Frontiers in Veterinary Science, 7, 581862.

Zhirakovskaia, E., Tikunov, A., Tymentsev, A., Sokolov, S., Sedelnikova, D., & Tikunova, N. (2019). Changing pattern of prevalence and genetic diversity of rotavirus, norovirus, astrovirus, and bocavirus associated with childhood diarrhea in Asian Russia, 2009–2012. Infection, Genetics and Evolution, 67, 167-182.

Zhu, K., Yang, J., Luo, K., Yang, C., Zhang, N., Xu, R., ... and Leng, Q. (2015). TLR3 signaling in macrophages is indispensable for the protective immunity of invariant natural killer T cells against enterovirus 71 infection. PLoS Pathogens. 11(1), e1004613.

Zhuo, R., Parsons, B. D., Lee, B. E., Drews, S. J., Chui, L., Louie, M., ... & Pang, X. (2018). Identification of enteric viruses in oral swabs from children with acute gastroenteritis. The Journal of Molecular Diagnostics, 20(1), 56-62.