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Research Article

SEQUENCING AND MOLECULAR PHYLOGENETIC ANALYSES OF VP4 GENE FROM ROTAVIRUS IN IRAQI CHILDREN

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Abstract- Group A rotavirus leftovers a main reason of diarrhea in infants and young children particularly in developing countries. Nursing changes in rotavirus strains is essential to measure the possible effectiveness of vaccines in exact geographic sites. Stool samples were collected from children below 2 years suffering after severe diarrhea. Positive samples were amplified to examine and characterized VP4 gene. The greatest predominant genotype was G1P[8] (6/13)(46.15) followed by G3P[8] (2/13)(15.38), G2P[6] (1/13)(7.69), G4P[8] (1/13)(7.69), G9P[8] (1/13)(7,69). A phylogenetic analysis and sequencing identity matrix to VP4 gene of isolates detected in the present study exposed that G1P8 (Iq4-f2,Iq4-f5, Iq4-f7, Iq4-f6, Iq4-f4) in lineage 1 of phylogenetic tree, similarity between those isolates 100%. G1P8 (Iq4-s3) and G4P8 (Iq4-f1) of lineage 1 similarity between these isolates (94%), and isolates Iq4-s3 and Iq4-f1 identity (93-94%) to next isolates (Iq4-f2, Iq4-f5, Iq4-f7, Iq4-f6, Iq4-f4). Two isolates (Ig4-f8, Ig4-f9) in lineage 2 was similar (99%). Vaccine isolate of vp4 gene (lineage 1) similarity (89%) to G4P8 (Iq4-f1), (88%) to G1P8 (Iq4-f2, Iq4-f4, Iq4-f5, Iq4-f6, Iq4-f7). The distribution of genotypes that own neither VP4 specificity through the obtainable rotavirus vaccine presently in use may embody a challenge to the consequence and accomplishment of vaccination.

Keywords- Rotavirus, Sequencing, Phylogenetic Analyses, Viral Genes

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Introduction

Diarrhea is a worldwide community health problem, and despite an important reduction in diarrhea-related mortality in developed and certain developing countries, diarrhea is motionless a significant reason of illness in these countries [1]. Rotavirus is between the key viral agents that source human diarrhea [2]. Approximately all children have knowledgeable at least one rotavirus infection by age 5 [3]. The virus encodes six structural (VP1, VP2, VP3, VP4, VP6, VP7) and five to six nonstructural proteins (NSP1–NSP5/6) [4]. Rotaviruses be in eight dissimilar species (A–H), with the types A–C recognized to affect humans, through the A group existence the maximum dominant worldwide [5]. The classification of group A rotavirus depends on the difference in its superficial viral proteins: VP4 and VP7 [6]. Two approved rotavirus vaccines have been commercially accessible worldwide since 2006 including Rotarix, human G1P[8] monovalent rotavirus vaccine, and pentavalent RotaTeq vaccine (bovine reassortant chimeric virus strains stating human G1–G4, and P[8]) [7]. While equally vaccines elicit protective immunity to homotypic and heterotypic rotavirus strains [8], there is a unceasing need for a improved understanding of the changing molecular features of rotavirus strains and their grade of homology to the vaccine strains. Rotarix vaccine has lately been introduced in the compulsory nationwide Iraq vaccination program in January 2013. Although rotavirus is the utmost common source of diarrhea in children in Iraq, one insufficient study have screened the molecular description of rotavirus strains circulating in diverse areas of Iraq [9]. The present study aimed to determine the circulation of P genotype through direct gene sequencing and to compare their relatedness to different rotavirus isolates and vaccine.

Materials and Methods

Samples collection

Rotavirus observation was showed at the Pediatric national central public health laboratory (CPHL) in Bagdad from September to January 2017. Children <2 years of age who were known by acute gastroenteritis, with episodes of diarrhea watery, mucus in stools, and vomiting were included in the study. Rectal swabs from 77 affected children were collected from the national central public health laboratory (CPHL) in Baghdad. The children's ages reached from 2 months to 2 years. Clinical appearances and case histories were noted. Separate swabs were reserved in 1-mL sterile saline comprising gentamicin sulfate. Swabs were routinely treated and kept at -80°C pending further analysis.

Antigen detection: Fecal samples were verified for the attendance of rotavirus antigen using commercial immunochromatographic test (ABON Biopharm, London, U. K.) in concordance to manufacturer's procedure. Rotavirus antigen-positive fecal samples were kept at –70°C until the instant of genotyping testing.

RNA extraction and (RT-PCR): Viral RNA was removed from 10% (wt/vol) rectal swab supernatants by the viral RNA extraction kit (Qiagen, Germany), according to the manufacturer's orders. Viral RNA was extracted from samples that rejoined positively to a rapid chromatographic antigen discovery test. The extracted RNA was used as a template for conventional RT-PCR using one-step RT-PCR kit (Qiagen, Germany). Intensification of the partial VP4 gene (663 bp) was carried out via the oligonucleotide primers: VP4F 5`-TATGCTCCAGTNAATTGG-3` and VP4R 5`-ATTGCATTTCTTTCCATAATG-3` [10].

The RT-PCR amplicons were exposed to gel electrophoresis (1.5%).

RT-PCR amplicons were directed sequencing

Dissimilar gene sequences were examined using MEGA 6. Homology (BLASTn) searches for separate gene sequences were completed using highly similar sequences (megablast) against existing distributed rotavirus sequences in the GenBank databases. CLUSTAL W program was used to perform the multiple sequence alignment and phylogenetic trees built using the Neighbor-Joining method [11]. The sequence identity percentage was performed using the Bioedit programe (v 7.2) by Sequence identity matrix.

Results and Discussion

Isolation and amplification of Vp4 gene

In the present study, antigen detection was used to screen the rotavirus and positively detect rotavirus in 30/77 samples. Upon analysis positive samples using RT-PCR for P type, only 13/30 indicated positive reactions for P genotype and bands for Vp4 gene (663 bp) were detected on agarose gel electrophoresis [Fig-1]. The detail that specific samples that were positive on the antigen revealing test were negative on RT-PCR does not specify the relative sensitivity of the two tests. The negative response in RT-PCR strength be related to either the presence of point mutations not documented by the primers used for the discovery of P genotyping, or as yet nameless P genotype. Also, earlier studies have established a reduced sensitivity of the PCR for the detection of P genotypes due to primer mismatch, since 52.5 % and 7% were not positively genotyped for G and P genotypes, respectively [12].

RT-PCR is a extremely sensitive and specific examine used for rotavirus genotyping [13]; though, continuous gene mutations in the virus may decrease the sensitivity of the assay over time. So, the primers used for genotyping should be altered regularly [10].

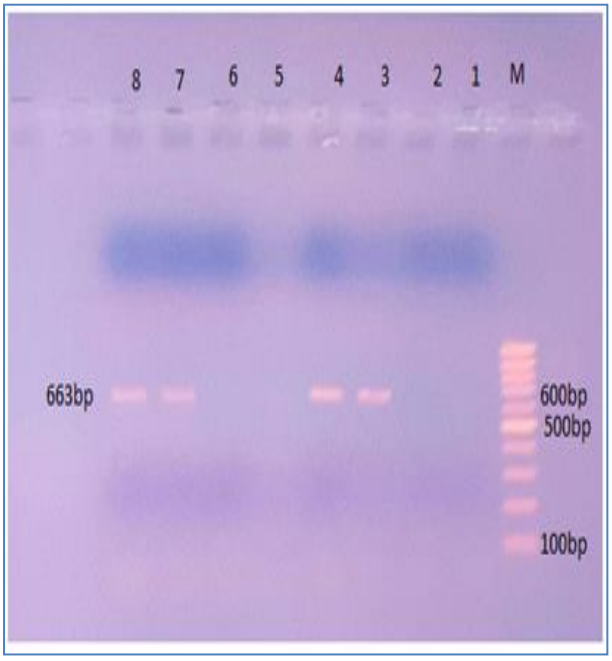

Fig-1 Amplification of a complete vp4 gene of rotavirus by using RT-PCR. Amplicons size was with 663 bp. Run on agarose gel 1% and visualized with trans illuminator, ladder ranged 1000-100 bp.

BLAST analysis of the different RVA isolates

The study results showed that six strains belong to G1P8, followed by one G4P8, tow G3P8, G2P6, one G9P8, and tow isolates untypeable, [Table-1]. Sequence similarity values were calculated for sequencing nucleotides using Bio Edit program, [Table-2]. The greatest sequence similarity for VP4 gene among local isolates were seen between isolates (Iq4-f2, Iq4-f4, Iq4-f5, Iq4-f6, Iq4-f7), those isolates showed 100% similarity between themselves and their genes are closely related (99%) to G1P8 previously identified in Thailand , these isolates founded in lineage 1 of phylogenetic tree, it is possible that these genotypes were amalgamation with the immigration of the foreign workers pending from southeast Asia as the phylogenetic analysis has revealed. G1P8 strains circulate internationally through numerous studies have exposed that in the 2000s the main circulation [14] in the present study the G1P8 was founded in 6/13 (46.15) isolates. Thus representative the circulation of this sub lineage in Iraq, this is consistent with the result from Saudi Arabia [15]. Lineage 1 also containing on vaccine isolate(G1P8) these isolate similarity (89-88%) to other isolates that founded in lineage 1, it is contains isolates (Iq4-f1, Iq4-s3) those isolates presented more than 94% resemblance between themselves and their genes are carefully related (98-99%) to G4P8, G1P8 previously identified in Slovenia, India. The nucleotide sequence identity 94% to isolates (Iq4-f2, Iq4-f4, Iq4-f5, Iq4-f6, Iq4-f7), of lineage 1. Although the similarity observed in isolates above, but the distances of evolutionary relationships among different viral types showed divergence as shown as in the phylogenetic tree [Fig-2]. The result is similarity to a previous study performed in Paraguay [16]. Lineage 2 consist of two isolates untypeable (Iq4-f8, Iq4-f9), the nucleotide sequence identity between these isolates were 99% and their genes are closely related (100-99%) to untypeable strains previously identified in Lebanon, re-assortment is facilities by a high degree of co-infection, which is characteristically much advanced in developing countries. They do seem to have experienced genetic drift since their introduction [17]. Lineage 3 contains on isolates (Iq4-s4, Iq4-f3) those isolates showed more than 98% similarity between themselves, and (91-93%) identity to isolate (Iq4-s6). Isolates (Iq4-s4, Iq4-f3) closely related (99%) to G3P8 Previously identified in Italy. Isolate (Iq4-s6) similarity 98% to G9P8 previously identified in Pakistan, G3P8, G9P8 have been the most common types causing severe disease in children in most countries during the last 20 years G3P8, G9P8 strains generally represent minor causes of disease, however outbreaks can occur at intervals of 2-5 years [18]. Present findings are consistent with other previous studies in china root branch of phylogenetic tree contained on lineage 4 that contain isolate (Iq4-s5), it is closely related 99% to G12P6, G2P6 previously in Pakistan G12P6 uncommon genotype might be due to whichever the expression of the natural variation of different human RV-A [19]. genotypes ended time in rotavirus infected children or they were circulating without discovery. Our result agreement with previous study in Saudi Arabia [15].

Table-1 *sequence characteristic of vp4 gene of 13 Rotavirus isolates and Rotarix vaccine.*

strain	genotype	Sequence id	score	expect	identities	No.nucleotide
Iq4-f1	G4P[8]	KJ432853.1	1040	0.0	98%	30 to 625
(Iq4-f2,Iq4-f4,Iq4-f5,Iq4-f6,Iq4-f7)	G1P[8]	KU754144.1	1086	0.0	99%	147 to 740
Iq4-f3	G3P[8]	KT988164.1	1018	0.0	99%	214 to 785
Iq4-f8	untypeable	KR181922.1	1090	0.0	99%	152 to 744
Iq4-f9	untypeable	KR181927.1	1077	0.0	99%	152 to 737
vaccine	G1P[8]	JQ926466.1	1085	0.0	99%	202 to 783
Iq4-s3	G1P[8]	KT387262.1	1072	0.0	99%	151 to733
Iq4-s4	G3P[8]	KT988164.1	1014	0.0	99%	220 to 783
Iq4-s5	G2P6	JN001881.1	1005	0.0	99%	72 to 624
Iq4-s6	G9P[8]	JX273740.1	957	0.0	98%	52 to 596

Iq, indicate to Iraq, number 4 indicate to vp4 gene, f indicate to first run, s indicate to second run. f1, f2, f3,.....f9 indicate to number isolate), (s3, s4,.....s6 indicate to number isolate.

Table-2 *Sequence Similarity matrix of VP4 gene of Rotavirus local isolates*

Seq->	Iq4-f1	Iq4-f2	Iq4-f4	Iq4-f5	Iq4-f6	Iq4-f7	Iq4-f3	Iq4-f8	Iq4-f9	Iq4-s3	Iq4-s4	Iq4-s5	Iq4-s6	vaccine
Iq4-f1	id													
Iq4-f2	0.93	id												
Iq4-f4	0.93	100%	id											
Iq4-f5	0.93	100%	100%	id										
Iq4-f6	0.93	100%	100%	100%	id									
Iq4-f7	0.93	100%	100%	100%	100%	id								
Iq4-f3	0.82	0.82	0.82	0.82	0.82	0.82	id							
Iq4-f8	0.83	0.83	0.83	0.83	0.83	0.83	0.78	id						
Iq4-f9	0.83	0.82	0.82	0.82	0.82	0.82	0.79	0.99	id					
Iq4-s3	0.94	0.94	0.94	0.94	0.94	0.94	0.85	0.84	0.84	id				
Iq4-s4	0.82	0.81	0.81	0.81	0.81	0.81	0.98	0.78	0.77	0.84	id			
Iq4-s5	0.62	0.63	0.63	0.63	0.63	0.63	0.65	0.63	0.63	0.64	0.65	id		
Iq4-s6	0.79	0.79	0.79	0.79	0.79	0.79	0.91	0.75	0.74	0.81	0.93	0.66	id	
vaccine	0.89	0.88	0.88	0.88	0.88	0.88	0.85	0.84	0.84	0.90	0.84	0.63	0.80	id

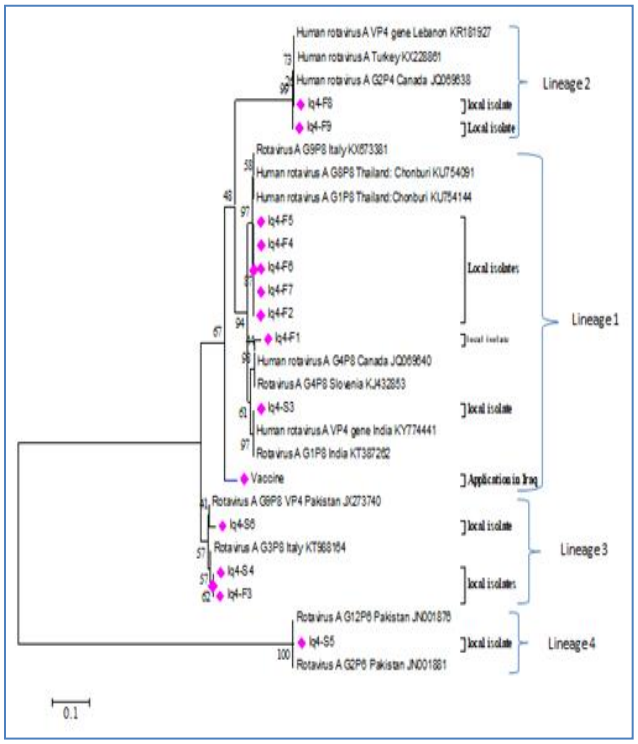

Fig-2 Evolutionary relationships as demonstrated as a based on complete vp4 gene of local strain of RVA. The pink color represents the local isolates. The evolutionary history was inferred using the Neighbor-Joining method. The phylogenetic tree was conducted in MEGA6.

Conclusion

The current study has established the local variation of genotype prevalence that often happens over time. The most predominant genotype in current study is G1P [8] followed by G4P [8], G3P [8], G2P [6], G9P [8] and tow isolates untypeable. These were emphasized the significance of incessant investigation of rotavirus genotypes circulating in human and additional animal species in instruction for improvement and understanding the epidemiology of rotavirus A infection inside the region. More wide epidemiological studies are similarly obligatory to assess the potential effectiveness of present vaccines in our geographic sites.

Application of research: The application of research is to finding evolutionary relationships of the most important viral gene (vp4). This will help to focusing on important functionally part of this gene that may be useful for vaccines design.

Research Category: Sequencing and Molecular Phylogenetic Analysis

Abbreviations:
RT-PCR: Reverse transcription polymerase chain reaction

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