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Detection of Rotavirus Infection in Iraq's Children and its Correlation with Rotarix Vaccine.

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Abstract

This study was aimed to determine the prevalence of Rotaviruses in Karbala and Basrah provinces in Iraq, the genetic recombination of Rotavirus strains in human with another hosts and release a novel strains may lead limited activity of Rotarix vaccine. Rotaviruses were detected by Immunochromatography Test (ICT) then the positive samples were tested by reverse transcription PCR (RT-PCR) using specific primers to vp4gene, this gene is responsible for stimulation the immune system to produce neutralizing antibodies about of 200 diarrheal stool samples, 100 samples from pediatrics hospital of Karbala province and another 100 samples from Maternity and children hospital in Basrah province . The vp4 gene was implemented in rotavirus vaccine of Iraqi immunization program. The RT- PCR results showed that 56.3% (27/48) of samples positive in children under five years of age in Karbala province while Basrah province revealed 58.5% (31/53) positive samples in children. The results of sequencing of human and Rotarix vaccine revealed that there was relation between them and animal strains according to genetic reassortment between human and another species while in comparing with international strains there were closely related with Lebanon and Turkey human strains. Necessarily for further studies are needed to study the genetic alterations with viral recombination of rotavirus to detect the Rotarix vaccine activity against novel Rotaviral strains.

Keywords: Rotaviruses, Rotarix, vaccine, RT- PCR

1. Introduction

Reoviridae family included Rotaviruses which are naked viruses with double stranded RNA (dsRNA). And about eight species from Rotavirus A to Rotavirus H were detected in this family [1] .The rotavirus genome consists of six non-structural (NSP1-NSP6) proteins and 11 dsRNA segments which code for the six structural proteins (VP1-VP4, VP6, VP7), VP4 with VP7 responsible for attached of virus with host receptors [2]. VP4 cleavage into two fragmentsVP8 and VP5 when a activated by proteolytic.at the tip of the VP5* attached in circular form with VP8 [3]. Binary system was classified RotavirusesA into 37 P and 27G kinds depend on vp4 (Protease sensitive) and VP7

(Glycoprotein) [4]. The infectious rotavirus particle and complete is about 100 nm in diameter, virion is genomefully infective particle surrounding by three-layered icosahedral protein capsid (TLP) similar to wheels (lat. rota) and this form gave the Rotavirus term [5]. Next icosahedral arrangement has been identified the single layered particle (SLP = core shell) consist of 120 particles of the viral protein2 (VP2) [6]. Newly, The three-dimensional (3D) structure of virion RV was detected by cryomicroscopy (cryoEM) with 4.3˚A determination of tomography [3]. The mechanism of RV entry was explained by communications of VP4 with other proteins of RV [7] Meanwhile VP4 spikes developed either in presence or lack of trypsin [8]. The faecal–oral route main transmition way of The virus, chiefly by direct contact of persons with other and less changes are wanted to induce disease in predisposed [9]. Outer capsid protein VP4 during its VP8 domain causes Rotavirus connection to host cells [10] and attachment partners on the host cell surface, containing by sialoglycans and histo-blood group antigens (HBGAs) [11]. Rotavirus–HBGA connections are influenced by protease sensitive P genotype, and studying these connections has induced new visions into variation of host species and transmission between divers species of different rotavirus strains [12]. In fact, alterations in rotavirus epidemiology among populations had been affected by genetic differences in HBGA expression [13].The function of HBGAs in the related effect on vaccine efficacy and susceptibility to rotavirus vaccine strains are important developing areas of research attended with public health [12]. Rotavirus attached and neutralized their antigen through the structural protein in outercapsid which are vp4 and vp7 genes Estes and [14]. TM (Wyeth-Lederle Vaccines) was pullout according to its side effect with intussusception in 1999. Then in 2006 another vaccine was improved with pentavalent reassortant human –bovine and finally RotatixTM [15]. Immunoglobulin A (IgA) give a good protection against infection with rotavirus , this suggestion was conducted by studies achieved on hygienic children infected with usual rotavirus [16].The total serum anti rotavirus IgA level, determined shortly after infection, generally reflects intestinal IgA levels and seems to be the best marker of protection [17]. The first immunity induced in human after birth for 6 months was obtained from mothers then the role of vaccination have been achieved for active immunization through the first years of children may expos to dangerous infection. In 2019 this vaccine induced in many country like Swedish national immunization program [18].The extra intestinal influence of the rotavirus vaccine, which is called the “rotavolution”, has recently become a growing field of debate and research. It has been proposed that the effect of rotavirus infections depend on host susceptibility, host genetics and the interaction with the intestinal microbiota. The effects has been proposed going far beside the classic gastrointestinal symptoms which are just the top of an iceberg. An evaluation from 2019 stated that the currently known rotavirus infection pathology is just the tip of an iceberg and that new questions about the influence of the rotavirus vaccine influence have

emerged. For example, it has been proposed that rotavirus infections can activate autoimmune diseases. Therefore, rotavirus vaccines might lead to reduced incidences of autoimmune related conditions such as diabetes mellitus and coeliac disease, due to immunological susceptibility and mechanisms. These hypotheses need to be explored further [19].

2. Materials and Methods

2.1 Materials

Rotavirus Detection
Immuno Chromatography (IC)Kit
RNA purification kit
FAVROGEN FavroPrep™Viral Nuclic Acid Extraction Kit
Reverse Transcription Kit
Bioneer Accupower® RocketScript™RT PreMix

Table1. Amplification Primers

Target gene	Primer Sequence 5´- 3	Product Size (bp)	Reference
Vp4	Forward, TATGCTCCAGTNAATTGG Reverse, ATTGCATTTCTTTCCATAATG	663	Durmaz *et al.*, 2014

2.2 Methods

A total of 200 diarrheal stool samples, 100 samples from pediatrics hospital of Karbala province and another 100 samples from Maternity and children hospital in Basrah province all children were under 5 years of age and suffering from diarrhea. were collected from hospitalized patients during the period from November 2018 to August 2019.

3. Detection of Rotavirus Antigen

3.1 LumiQuick, Adeno-Rota Virus Antigen Comb Test Card

Immuno Chromatography (IC)

The specimens were screened for Rotavirus antigen through Immuno Chromatography Test (ICT) according manuscripting company.

3.2 RNA Extraction Kit

FavroPrep™ for isolation viral nuclic acid from supernatant stool samples were used . It based on mini spin column (silica matrix) for the isolation of viral RNA according manuscripting company.

3.3 Reverse Transcription Kit

according manuscripting company.

3.3.1 cDNA Synthesis

Reagents and samples were brought to room temperature before starting with the reaction, 10 ul of RNA were added to the reaction tube and the final volume is completed up to 20 ul with RNase free water and mixed gently and spun-down by microcentrifuge at 3000 rpm. The reaction tube then placed in the thermal cycler programmed at 37 ,42, 95 respectively as manuscripting company. During cDNA generation random priming gives random coverage to all regions of the RNA to generate a cDNA pool containing various lengths of cDNA. Synthesized cDNA is immediately used as template for PCR, or stored at -20°C for farther use.

3.3.2 PCR Amplification of vp4 gene

PCR reaction tube containing Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers from Bioneer company (Korea), adding 2.5 µl from forward primer TATGCTCCAGTNAATTGG (10 pmols/µl) and 2.5 µl from reverse primer, ATTGCATTTCTTTCCATAATG (10 pmols/µl) for vp4 gene. Seven microliter of cDNA template was added for each reaction tube, and the volume was made up to 20 µl with Deionized Nuclease –Free water and tubes were then spun down with a micro centrifuge for the adequate mixing of the components of the reaction. The thermo-cycling conditions were done with initial denaturation stage at 92°C for 5 min then denaturation stage also at 92°C but for 30 sec while annealing stage done at 45°C for 30 sec finally elongation stage done with72°C for 1 min.

3.3.3 DNA Sequencing

Sequencing of samples was done by Macrogen company (Korea). And sequencing result were viewed and compared with reference DNA sequence using Blast software.

3.3.4 Statistical Analysis

The Molecular Evolutionary Genetics Analysis version 7 (MEGA7) Software is used for both DNA and protein phylogenetic analysis using neighbor joining algorithm (20).

3.3.5 Phylogenetic Tree Analysis

Sequencing of samples was done by Macrogen company (Korea). And sequencing result were viewed and compared with reference DNA sequence using Blast software.

4. Results

4.1 Prevalence of Rotavirus in Human Stool Samples

The characteristic features of study population are summarized in table 2. All samples were tested from diarrheic children with acute gastroenteritis by imunochromotography test (ICT). In this study, stool samples of all children were less than 5year old with acute gastroenteritis were screened for Rotavirus antigen. The result showed 48 samples out of 100 were positive Rotavirus infection from pediatric teaching hospital in Karbala province. While in Basrah province 53 out of 100 samples were positive in diarrheic children, this difference was not significant (P>0.05) between two provinces .

Table 2. The result of Human Rotavirus Detected in Karbala Province and Basrah Province by Imunochromotography test (ICT).

Province	Total samples	Positive	Negative
		no.(%)	no.(%)
Kerbala	100	48 (48)	52 (52)
Basrah	100	53 (53)	47 (47)
Total	200	101 (50.5)	99 (49.5)

X2 = 0.5 df= 1 P=0.479

4.2 Prevalence of Rotavirus in Human Stool Samples

4.2.1 Diarrheic Human Samples

In order to Immuno Chromatography assay results, conventional PCR was carried out to detection the two genes of outer layer protein's VP4 663bp. After RNA was extracted then transcribed to cDNA these samples were amplified with primers for the 3' and 5' ends of VP4 gene. The study enrolled 27 samples from 48 Rotavirus positive samples (by an Immuno Chromatography assay) in Kerbala province shown in table 3, while in Basra province the result of RT-PCR results demonstrated 31 samples from 53 Rotavirus positive samples (by an Immuno Chromatography assay) as shown table 3 this difference was not significant (P>0.05) between two provinces. The positive results for VP4 gene were shown in figure 1. Also Rotarix vaccine were checked for gene of VP4 663 bp that demonstrated in this study as shown in figure 2.

Table 3. The results of human Rotavirus detected in Kerbala province and Basrah province by Polymerase Chain Reaction (PCR).

Province	Total samples	PCR (VP4) Positive no.(%)	PCR (VP4) Negative no.(%)
Kerrbala	48	27(56.3)	21(43.7)
Basrah	53	31(58.5)	22(41.5)
Total	101	58(57.4)	43(42.6)

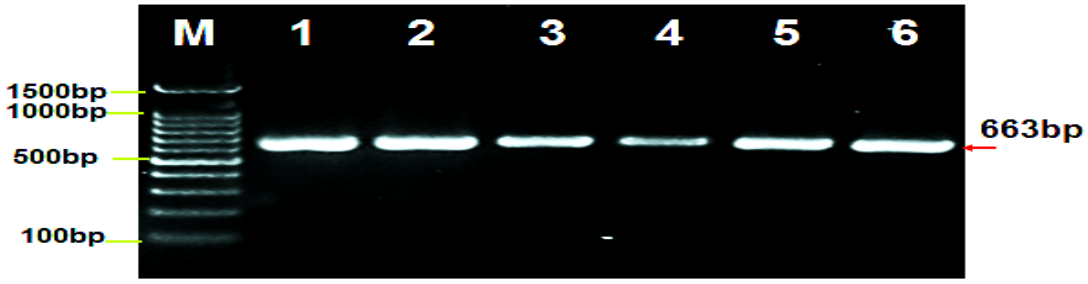

Figure (1): Agarose gel electrophoresis image showed RT-PCR product analysis for VP4 gene M (Marker ladder 100bp). Lane (1-10) some positive VP4 gene in Rotavirus at 663bp product size.

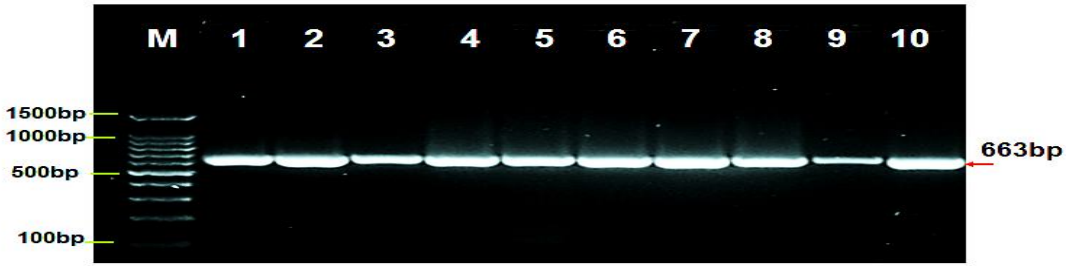

Figure (2): Agarose gel electrophoresis image showed RT-PCR product analysis for VP4 gene M (Marker ladder 100bp). Lane (1-6) positive VP4 gene in Rotavirus at 663bp product size.

4.2.2 Sequencing of DNA detected

The accession number of local Iraq's strain in present study demonstrated in (Table 4).

Table 4.Clinical sample from human amplified using Rotavirus vp4 gene specific primers

No	Type of gene	Current study strain (GeneBank accession No.)	Source
1	Vp4	MT074691	human
2	Vp4	MT074692	human
3	Vp4	MN995336	vaccine
4	Vp4	MN995337	vaccine
5	Vp4	MT074687	vaccine

4.2.3 Sequencing of DNA detected

The amplified vp4 gene with 663bp from human and vaccine. Using Blastn software for all local samples of vp4 gene derived from diarrheic human samples derived nucleotide sequences were aligned for matching with the reference database (GenBank) revealing the local samples from human and vaccine having slightly mismatch (99% identity) with reference human vp4 gene genbank sequence accession ID: MH59138 in human sample while with ID: Kx228855 in Rotarix vaccine as shown in figure 3 and figure 4 respectively.

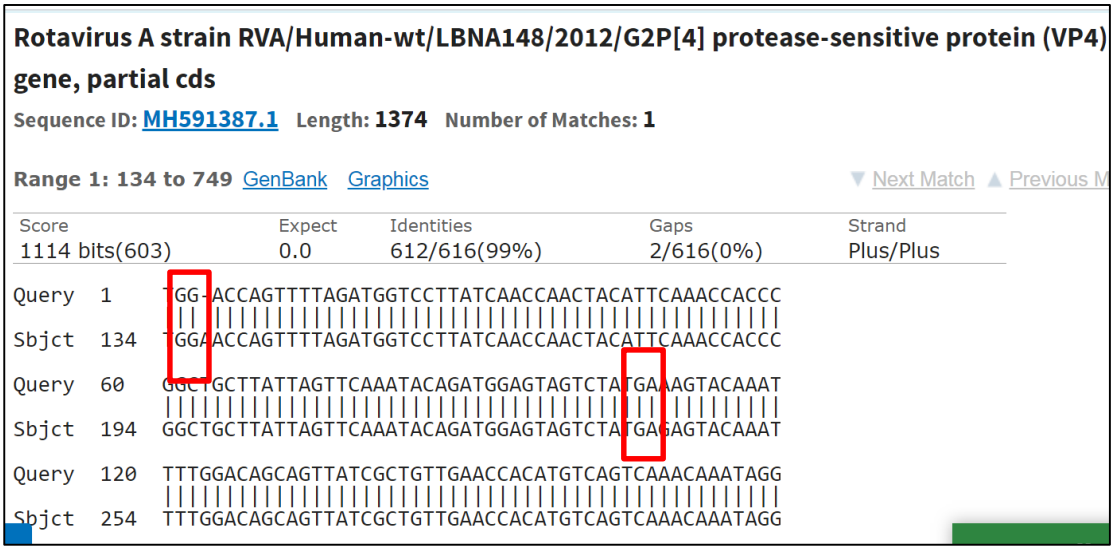

Figure (3): GenBank database alignment of VP4 gene DNA sequence from human sample showing 99% match with match with reference human Rotavirus, Mismatch sites indicated with red boxes, using Blastn.

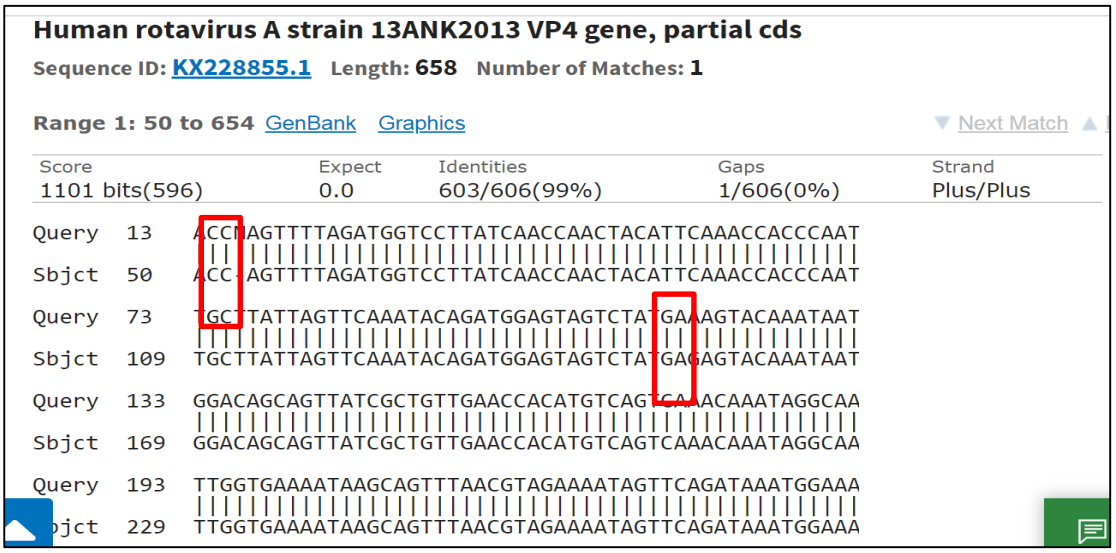

Figure (4): GenBank database alignment of VP4 gene DNA sequence from vaccine sample showing 99% match with match with reference human Rotavirus, Mismatch sites indicated with red boxes, using Blastn.

4.2.4 Phylogenetic Tree Analysis

Phylogenetic analysis where done using MEGA7 softwere , analysis done for vp4, the tree is drown to scale, with branch lengths measured in the number of sub situation per site. the analysis involved 30 nucleotide sequences for VP4 gene, figure 5.

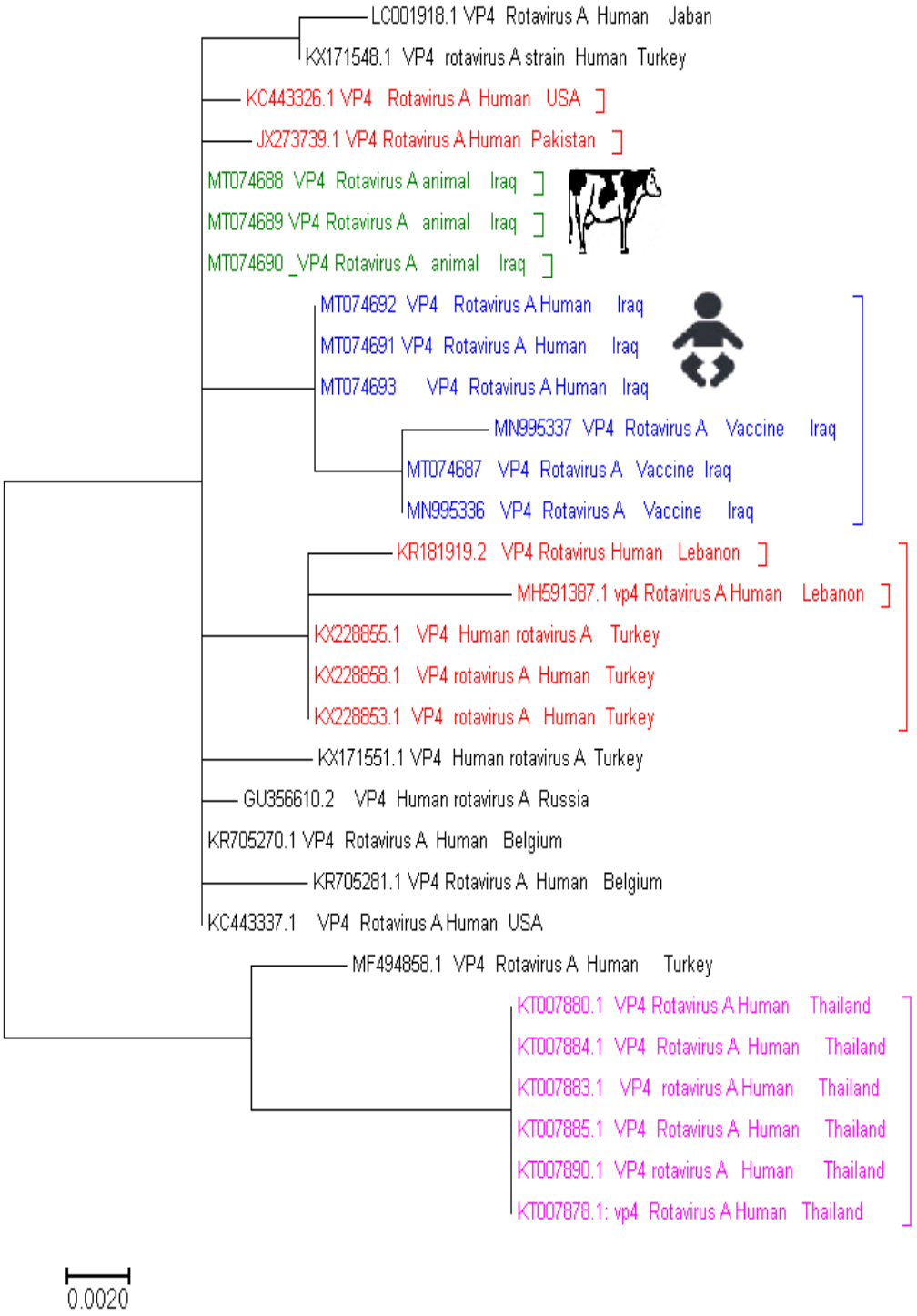

Figure (5): Rotavirus A *VP4* gene segment phylogeny for both local and international sequences molecular phylogenetic analysis was done by maximum likelihood method. The boot strap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the sequences

analyzed. blue color = current human and vaccine strains, green color = related animal strains, red color = closely related strains, pink color = less related strains, the rest samples are international sequences.

5. Discussion

Young children under 5 yeas more susceptible for gastroenteritis symptoms with Rotaviruses infection (2) in another hand diarrheal disease record about 90% in developing countries although the improvement in medical treatment (21). Rotavirus infection in children were confirmed with infection by rapid Immunochromatographic test (ICT) that showed 48% and 53% in Kerbala and Basrah provinces respectively (table 2) this difference was not significant , our results agree with (22) who collected the samples from five Iraqi provinces (Babil, Kerbala, Missan, Qadissiya, and Wasit) they found a similar prevalence (47.2%), by using (IC) test. The international scenario of rotavirus prevalence exhibited by (23) who used conventional methods (eg, IC test and ELISA test) for detection of Rotavirus also recognizing the varying manner after vaccination with common serotypes in children about 24%. Truly the picture is highly complex in human and animal may haven a diversity of genotypes with current period or in prospectively in a far time appear the dramatic of related host species that may release a unique strain rotaviruses patterns according to credited genomic diversity in numerous hosts species (24). There is an profusion of molecular epidemiological statistics on the opportunity out of both usual and unusual VP7 and VP4 proteins determined by Reverse transcription PCR amplification in world, so the present study by using RT- PCR technique for intensification of vp4 and vp7 genes (by rotavirus specific primer) from human ,animal with desired amplicon size is designed to detect the occurrence of Rotavirus and its molecular properties to detect the specific genotype which are distributed between the human and animal populations, Rotavirus was detected in (56.3%) and (58.5%) of human samples in Kerbala and Basrah provinces respectively (table 3), therefore, the results of present study were somewhat consistent with many Iraq's studies, (51.98%) in Mid Iraq was conducted by .(25)

While neighbored countries RT-PCR conducted in Turkey found (78.2%) of Rotavirus in tested samples while in children age range from thirteenth to twenty four months (38.7%) infective rate whereas higher than (28.3%) monitoring in children with twenty five to thirty six menthes (26). On other hand, Arab countries disagree with the incidence of Rotavirus accomplished in present study resembling by (27) who revealed (44%) in Kuwait while (28) reveled (61%) in Syria whereas (29) showed (50%) in Oman .These varying in incidence statistics may be due to season of specimen taken and approaches of specimen detection.

Rotaviral VP4 DNA Sequence Analysis

The present study by Blastn significant the similarity of genetic features between human and Rotarix vaccine that applied to advance studies are desired to understand the undercurrents of Rotavirus relationship between circulating strain and Rotarix strain .

Blastn showed related of vp4 gen in human, animal samples and vaccine sample related with MH559138.1 for both human and Rotarix vaccine and KX228855.1 for animal sample from GenBank (NCBI) as shown in figure (3) and figure (4) respectively. Since 2016 year Iraq has implemented the final Rotavirus vaccine Rotarix vaccine but continuous circulating novel strains according re-assortment habitat of Rotavirus encourage the observation of variations in Rotavirus genotype circulation and detection of vigorous vaccine compatible with this unique variants strains become necessary to develop vaccination protocol and induce of modern Rotavirus vaccines (30).

However, it is essentially check methods periodically to develop vaccines efficacy against Rotavirus infection in order obtain the perfect benefits from immunization with vaccine implemented (31).

Rotaviral VP4 DNA Sequence Analysis

Phylogenetic tree and correspondence analyses were achieved to conclude the association between most recurrently identified rotavirus, The close relation of local samples sequences to each other as it is clustered in one branch away from other international derived sequences. The figure (5) demonstrate the genetic diversity of local strains from different sources human, animal and vaccine strains for vp4 gene, human an vaccine strains are closely related with Lebanon at KR181919.2 and KX22885 from Turkey and with animal strains which are closely related with Pakistani strain have ID JX273739.1 and KC443326.1 (NCBI) from USA whereas less related with strains from Thailand have ID KT007880 . The differences of human strains identity with variant strains give indication about Rotavirus modification (32).The genotypic diffusion of Rotaviruses with different strains according to production of novel strain with affected conditions as changing of temperatures and geographical terrain of infected region all these variations corresponding with vaccine improvement and approach of diagnostic vaccine efficacy (33). However, in present study analysis of both vp4 gene in middle and south Iraq was detected in partial genome while in future analysis for complete genome will need to understand the molecular characterization of Rotavirus.(35 , 34)

Full complete genome analysis could be made in the upcoming to improved describe strains socializing in the pre- and post-vaccination dated. Though, to the greatest of our information, no revision from nations or areas with high vaccination attention have described in unimmunized or immunized children. No suggestion of vaccine derivative variations in vp4 were detected while slight

genetic difference in human and animal sequences throughout the study dated (36). Therefor current study, also realized about extremely probable that a choosy effect of vaccination occasioned in a different molecular outline of common G1P[8] Rotarix vaccine strains and the correlation of human strains with animal strains show the origin of human strain may be from animal sources according to recombination property of rotavirus while Rotarix vaccine prepared from human strain only this analysis lead to indicate the limited protection of Rotarix vaccine (37).

6. CONCLUSIONS

In present study the local strains are different from other local Iraqi strains while data of Rotaviruses prevalence with international strains showing closely related and continuous observing of reassortment of Rotavirus genotypes is needed in the upcoming to evaluate the Rotarix vaccine efficacy with variant Rotavirus strain. Also, full molecular analysis of genome fragments should be done frequently to track strain variety and improved outlines of vaccine preparation and insurance with vaccine update periodically in this types of recombinant viruses.

References:

[1] Matthijnssens, J. and Ranst M. V 2012. Genotype constellation and evolution of group A rotaviruses infecting humans. Curr. Opin. Virol. 2 (4):426–433.

[2] Desselberger, U. Rotaviruses. *Virus Res.* 190, 75–96 (2014).

[3] Settembre EC, Chen JZ, Dormitzer PR, Grigorieff N, Harrison SC. Atomic modelof an infectious rotavirus particle. EMBO J. 2011;30:408–16.

[4] Trojnar E, Sachsenröder J, Twardziok S, Reetz J, Otto PH, Johne R. Identification of an avian group A rotavirus containing a novel VP4 gene with a close relationshipto those of mammalian rotaviruses. J Gen Virol 2013;94(Pt 1):136–42.

[5] Flewett, T.H., Bryden, A.S., Davies, H., Woode, G.N., Bridger, J.C., Derrick, J.M.,.Relation between viruses from acute gastroenteritis of children and newborncalves. Lancet 2 (7872), 1974. 61–63.

[6] McClain, B., Settembre, E., Temple, B.R., Bellamy, A.R., Harrison, S.C. X-raycrystal structure of the rotavirus inner capsid particle at 3.8˚A resolution. J. Mol.Biol. ;2010397 (March (2)), 587–599.

[7] Crawford, S.E., Mukherjee, S.K., Estes, M.K., Lawton, J.A., Shaw, A.L., Ramig, R.F.,Prasad, B.V., 2001. Trypsin cleavage stabilizes the rotavirus VP4 spike. J. Virol.75 (July (13)), 6052–6061.

[8] Rodríguez J.Chichón F.Martín-Forero E et al. New Insights into Rotavirus Entry Machinery: Stabilization of Rotavirus Spike Conformation Is Independent of Trypsin Cleavage. PLoS Pathogens 2014; 10(5).DOI: 10.1371/journal.ppat.1004157.

[9] Estes, M.K., Greenberg, H.B. Rotaviruses. In: Knipe, D.M., Howley, P.M., et al.(Eds.), Fields Virology. , 6th ed. Wolters Kluwer Health/Lippincott Williams &Wilkins, Philadelphia, PA, pp. 2013.1347–1401.

[10] Lopez S. and Arias C.F. Multistep entry of rotavirus into cells: a Versaillesque dance. Trends Microbiol. 2004; 12:271–278.

[11] Arias CF, et al. Rotavirus entry: a deep journey into the cell with several exits. J Virol. 2015; 89:890–893.

[12] Ramani S, et al. Diversity in rotavirus-host glycan interactions: a “sweet” spectrum. Cell Mol Gastroenterol Hepatol. 2016; 2:263–273.

[13] Nordgren J, et al. Host genetic factors affect susceptibility to norovirus infections in Burkina Faso. PLoS ONE. 2013; 8:e69557.

[14] Estes MK, and Kapikian AZ. Rotaviruses. In: Knipe DM, Howley PM, editors. FieldsVirology. 5th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2007. p.1918–74.

[15] Glass, R. I., and U. D. Parashar. 2006. The promise of new rotavirus vaccines. The New England journal of medicine 354:75-77.

[16] Velázquez F.R., Matson D.O., Guerrero M. L., et al. Serum Antibody as a Marker of Protection Against Natural Rotavirus Infection and Disease. J Infect Dis. 2000;182(6):1602-9. doi: 10.1086/317619.

[17] .Franco, M.A., Angel, J., Greenberg, H.B. Immunity and correlates of protection for rotavirus vaccines. Vaccine. 2006; 24:15, 2718–2731.

[18] Ask L.S.2019. The introduction of rotavirus vaccine in sweden - setting the scene and short term outcomes. Phd,Thesis.

[19] Gomez-Rial J, Sanchez-Batan S, Rivero-Calle I et al. Rotavirus infection beyond the gut. Infect Drug Resist. 2019;12:55-64.

[20] Kumar S., Stecher G., Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016;33(7):1870-4. doi: 10.1093/molbev/msw054.

[21] .Parry J.New vaccines to Tohoolr Child Care in developing countries. BullWorld Health Organisation.2017;85(6) 426-7.

[22] Abdulazeez A.A. and AbedM.N. Genotyping of Rotavirus in Neonatal Calves with Acute Gastroenteritis in Iraq. Advances in Microbiology.2018;7(12): 863-870.

[23] Senthilkumar,(2017). A study on Rotavirus gastroenteritis in children under five years in Coimbatore. Chennai.ph.D.thesis in Microbiology- branch IV. Medical University.

[24] Bwogi J., Jere K.C., Karamagi C. et al. Whole genome analysis of selected human and animal rotaviruses identified in Uganda from 2012 to 2014 reveals complex genome reassortment events between human, bovine, caprine and porcine strains. PLoS One.2017. doi.org/10.1371/journal.pone.0178855.

[25] Bwogi J., Jere K.C., Karamagi C. et al. Whole genome analysis of selected human and animal rotaviruses identified in Uganda from 2012 to 2014 reveals complex genome reassortment events between human, bovine, caprine and porcine strains. PLoS One.2017. doi.org/10.1371/journal.pone.0178855.

[26] Durmaz R, Kalaycioglu AT, Acar S, Bakkaloglu Z, Karagoz A, et al. Prevalence of Rotavirus Genotypes in Children Younger than 5 Years of Age before the Introduction of a Universal Rotavirus Vaccination Program: Report of Rotavirus Surveillance in Turkey. PLoS ONE .2014;9(12): e113674.

[27] Marmash RW, Dalwai AK, Szucs G, Molla AM, Pacsa AS, Al Nakib W, Albert MJ: Genotypic characterization of rotaviruses and prevalence of serotype-specific serum antibodies in children in Kuwait. Epidemiol Infect 2007, 135(8):1331-1337.

[28] Teleb N: Rotavirus Surveillance Network in the Eastern Mediterranean regional. Presented at the 8th International Rotavirus Symposium 2008, June 3-4 Istanbul; 2008.

[29] Al Awaidy SA, Bawikar S, Al Busaidy S, Baqiani S, Al Abedani I, Varghesem R: Considerations for introduction of a rotavirus vaccine in Oman: rotavirus disease and economic burden. J Infect Dis 2009, 200(Suppl 1):S248-S253.

[30] Almalki S.S.R. Circulating rotavirus G and P strains post rotavirus vaccination in Eastern Mediterranean Region. Saudi Med J. 2018; 39(8): 755–756.doi: 10.15537/smj.2018.6.21394.

[31] Burnett E., Tate J.E., Kirkwood C.D. et al . Estimated Impact of Rotavirus Vaccine on Hospitalizations and Deaths from Rotavirus Diarrhea among Children <5 in Asia. HHS Public Access. Expert Rev Vaccines 2018 ; 17(5): 453–460. doi:10.1080/14760584.2018.1443008.

[32] Esposito S., Camilloni B., Bianchini S., Ianiro G. et al . First detection of a reassortant G3P[8] rotavirus A strain in Italy: a case report in an 8-year-old child . Virology Journal volume.2019; 16: 64.

[33] Lee S.K. Choi S. Shin S. H. and et al . Emergence of G8P[6] rotavirus strains in Korean neonates. Gut Pathog. 2018;10:27. doi: 10.1186/s13099-018-0255-8.

[34] Malakalinga J., Misinzo G. , Msalya G. and Kazwala R. Rotavirus Burden, Genetic Diversity and Impact of Vaccine in Children under Five in Tanzania. Pathogens. 2019; 8: 210. doi:10.3390/pathogens8040210.

[35] Carvalhoa M.F. and Gill D. Rotavirus vaccine efficacy: current status and areas for improvement. Hum Vaccin Immunother. 2019; 15(6): 1237–1250.doi: 10.1080/21645515.2018.1520583.

[36] Luchs A., Cilli A., Morillo SG., Carmona Rde C. Rotavirus Genotypes Circulating in Brazil, 2007-2012: Implications For The Vaccine Program. 2015; 57(4):305-313 DOI: 10.1590/s0036-46652015000400006.

[37] Jiang X., Liu Y. and Tan M. Histo-blood group antigens as receptors for rotavirus, new understanding on rotavirus epidemiology and vaccine strategy Rotavirus host receptor and vaccine strategy. Journal Emerging Microbes & Infections.2017; 6(1). https://doi.org/10.1038/emi.2017.30.

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