Determination of Human Sapovirus Genotypes Causing Gastroenteritis in Children under Five Years in Baghdad

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Abstract

Sapoviruses (SaVs) may cause acute gastroenteritis (AGE) in young children. To prove the presences of Sapovirus infection in Iraq AGE patients, a total of 200 stool samples from children patients under five years with AGE were collected and screened by reverse transcription real time PCR using specific primers was performed for partial sequencing SaVs capsid and probes cover all Sapovirus genogroups, this was followed by revers transcription PCR and nested PCR using the specific primers. The SaVs partial capsid gene amplicons were sequenced. Partial capsid sequences were blasted with National Center of Information Technology (NCBI) and phylogenetic analysis was conducted using MEGA 6.0 program. Out of the 200 samples tested, 18 (9%) were found SaVs positive. Amplicons were sequenced and only 7 samples were genotyped, 5(5/7) were belonged to the genotype GI.1, 1(1/7) were belonged to the GI.4, and 1(1/7) were belong to the GII.8 .The GI.1 was the dominant strain in Baghdad and the more infected age stage was the children 4 ≤5 years. SaVs was diagnosed in Baghdad in the current study for the first time. These results need more extensive surveillance study to determine the distribution and burden of virus in Iraq community.
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Keywords: Human Sapovirus; Real time PCR; Genogroups; Genotypes.

Introduction

The Caliciviridae family contains four RNA virus genera, *Norovirus, Lagovirus, Sapovirus, and Verisvirus* [1]. Norovirus genogroups (GI, GII and GIV), and Sapovirus (SaVs) classified into 15 genogroups (G) based on available virus Identification Pipeline major capsid protein (VP1) sequences [2] The (GI, GII, GIV, and GV) infect humans and causes gastroenteritis outbreaks [3;4]. SaV may cause mild gastroenteritis in young children [5] Sapovirus is a non-enveloped virus, with icosahedral geometries, and T=3 symmetry it is around 27-40 nm, non-segmented linear genome sized 8.3kb in length [6] and is slightly different from that of the Norovirus (NoV) genome. The polymerase and capsid junction is highly conserved region among the NoV and SaVs. NoVs are responsible for 47–96% of outbreaks of acute gastroenteritis (AGE), and 5–36% of sporadic cases of AGE worldwide [7]. There are some clinical differences between NoV and SaV. Vomiting and low grade fever is the most frequent symptom in NoV outbreaks where in SV diarrhea is the most frequent symptoms. "Norovirus being considered the first causative agent of viral gastroenteritis in children under five years old in Baghdad the city of Iraq and in Mosul Province "[8, 9, and 10]. There is no information available about the SaVs circulating in Iraq in compared to NoV. this study was aimed to describe epidemiological features of AGE outbreak caused by SaVs in Baghdad city.

Materials and Methods

Sample collection: Two hundred stool samples were collected from two Pediatric hospitals were chosen in Baghdad City:-Ebn – Albalady and Central Hospital from September to December 2016 these samples were collecting from children under five years old suffering from AGE symptoms, diarrhea more than three stool lose/day, and vomiting in sterile plastic wide mouth container, after bacterial culture of the tested samples and parasite examination to exclude the bacterial and parasite infected samples and stored at +4ºC until processing.

RNA extraction: Viral RNA was extracted from stool according to [8] and according to the manufacturer's instructions of

"QIAamp1Viral RNA Mini kit" provided from (Qiagen, Germany).

Molecular detection: Real time qPCR was performed on all viral suspected samples. Sets of primers and two probes were SaV124F, SaV5F, SaV1245R, SaV5TP and SaV124TP [2], targeting the highly conserved region to detect SaVs Genogroups. Amplification and detection of all SaVs Geno groups was performed using Go Taq one step RT-qPCR from (Qiagen, German) in 7500 Fast Real time PCR (Applied Biosystem).Program conditions were revers transcription at 42◦C for 20 min then hot start inactivation at 95◦C for 10 min followed by 45 cycles of denaturation at 94◦C for 15 sec, annealing and extension at 61◦C for 1 min then final extension at 72◦C for 10 min. The Samples considered positive when the threshold cycle (Ct) value was under 40 cycle .Revers transcription conventional PCR to amplify the partial capsid gene first round and second round nested PCR according to (8;9) using QIAGEN One Step RT-PCR from (Qiagen, German) .Primers used are shown in Table (1). The first round of PCR was performed in 25µl of reaction volume (5µl of RNA templet and 10 pmol of each primer. PCR amplification program was as followed conditions: revers transcription at 42°C for 20 min then hot start inactivation at 95°C for 15 min, followed by 35 cycles (94°C for 30 sec, 50°C for 30 sec, 72°C for 1 min), and final extension at 72°C for 10 min. Two reaction was performed in the second round nested PCR each reaction volume was 50 µl the first one containing 20 pmol SV-F22 and SV-R2 and 5 µl of the first PCR product, and the second reaction tube containing20 pmol 1245Rfwd and SV-R2. Amplification program as mention in first nested PCR. The PCR products were sequenced was performed Macro gen company, USA). The Sequence data were blasted with related prototype sequence in NCBI and aligned .The evolutionary history was inferred using the UPGMA method [11] in MEGA6 [12]. The evolutionary distances were computed using the Maximum Composite Likelihood method [13] and are in the units of the number of base substitutions per site. The analysis involved 27 nucleotide sequences. There were a total of 307 positions in the final dataset.

Table (1)

The first round revers transcription PCR primers, and nested second PCR primers to amplify the partial capsid gene in SaVs genotypes.

Rt-PCR type	Forward primer	Reverse primer	Amplicon size	Forward primer	Reverse primer	Amplicon Size	references
Nested	SV-F13 /SV-F14	SV-R13/ SV-R14	800	SV-F22	SV-R2	440 bp	14
				1245Rfwd	SV-R2	430 bp	15

Results and Discussion

In this study 200 samples were tested to detect SaVs in suspected AGE patients. The molecular detection dependent on RT-rPCR revealed that 18from 200 samples represented (9%) were positive to SaVs virus showed in the Table (2), 4% were from children <5 years Fig.(1) the infection with SaVs may be more common in young children as mention in some Australian studies finding that the prevalence of human AGE patient infected with SaV was less than 5% in samples [16;17]. All positive samples were amplified using nested PCR, In the first round only 10 samples was amplified and some of them gave faint bands sized 800 bp and after the second round 12 samples were produced sharp amplification bands sized 440bp and 430 bp these products were sequenced to determine the SaVs dominant genogroups and genotype in children. From these only seven SaVs isolated were classified into three genotypes GI.1 5/7 (71.4%) and GII.4 1/7(14.3) and GII.8 1/7(14.3%). Phylogenetic analysis was performed of the capsid region, to confirm the genotyping diversity of circulating strains within SaVs type.

Table (2)
The molecular detection results of SaVs genotypes of children under five with AGE symptoms.

Sample ID	RT-qPCR	Ct Value	Nested RT-PCR1	Nested RT_PCR2	Genotypes
S1R3	+	38.4	-	-	-
S1R11	+	36.2	+	+	-
S1R35	+	32.4	+	+	GII.4
S1R37	+	37.4	-	+	-
S1R60	+	35.1	+	+	GI.1
S1R73	+	33.7	+	+	GI.1
S1R77	+	38.4	-	-	-
S1R84	+	38.2	-	-	-
S1K101	+	36.7	+	+	-
S1R122	+	35.7	+	+	-
S1R132	+	37.5	-	-	-
S1R145	+	32.7	+	+	GI.1
S1K147	+	38.7	-	-	-
S1K165	+	36.6	+	+	GI.1
S1k169	+	35.7	+	+	GI.1
S1K187	+	33.6	+	+	GII.8
S1K188	+	33.1	+	+	-
S1K194	+	37.2	-	-	-

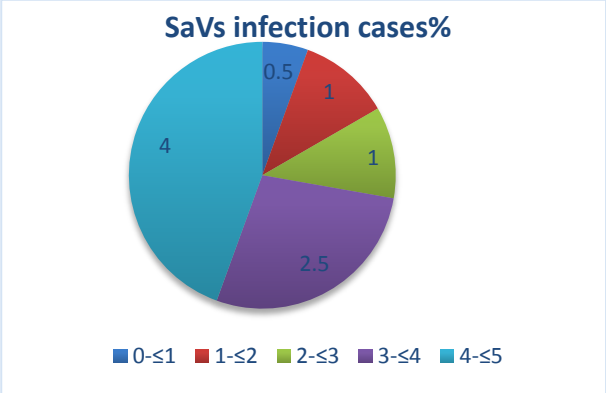

Fig.(1):The SaVs infection rate in five different age groups dependent on RT-rPCR results.

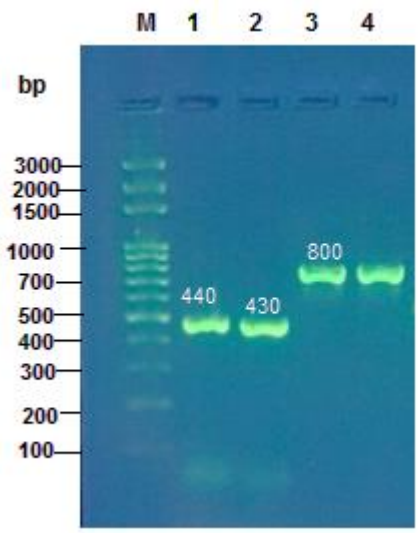

Fig.(2): Amplification SaVs, gel electrophoresis of nested PCR capsid gene product electrophoresed in (3) % agarose prepared in 1X TBE buffer and stained with red safe DNA dye using 5V/Cm, 2hr.Lane M 100bp of DNA ladder. Lane1 nested second round product 446bp lane 430 bp. lane 3 first round nested PCR product 800bp.lane2 nested second round product.

The epidemiological studies of SaVs do not acquire attention of researchers in compared with Norovirus and Rotavirus infection which top of the list in interest for being constitute a

high proportion of AGE. Many studies refers to the low rate infection of SaVs [18] refers to that SaVs was detected in 3.5 %in AGE stool samples with low virus load concentration 104- 109 genome copies / gram of stool, but the rate was rising in Tamagawa River in Japan to 20% SaV strains detected were classified into 10 genotypes: GI/1, GI/2, GI/3, GI/4, GI/5, GI/ un typed, GII/1, GII/2, GII/5, and GV/1. (19) was reported from Ethiopia 4.2% were positive for NoV and SaV RNA and this was comparable to SaV prevalence in other countries. But the prevalence of virus in river sample is higher than the human samples as a concuding of [20] and [21] referred to that the detection rate of SaVs strains worldwide circulating varied over time and the GI genotype was the predominant SaVs strain and this is agreed with our finding that the rate of SaVs was 9% and the dominant strain was GI.1 and this result are similar to the Brazilian study conducted by [22] that revealed circulation the genotypes GI.1, GI.2, GI.6, GII.1 and GV which indicated that the GI.1 is the dominant strain in Brazil. The phylogenetic tree was constructed from 7 partial sequences of capsid after aligned with NCBI database in comparing with 20 related sequences. Fig.(3) show that GI.1 strain of SaVs was 100% identity with Russia and China strain. Current study indicated that SaVs in Baghdad are responsible for only a small percentage AGE in children .This study was just a preliminary study to determine the presence of SaVs AGE children the short period of sample collection from AGE patient it was not enough to find the burden of virus, we recommended further more studies covering long period of time, different age groups and different Iraqi regions.

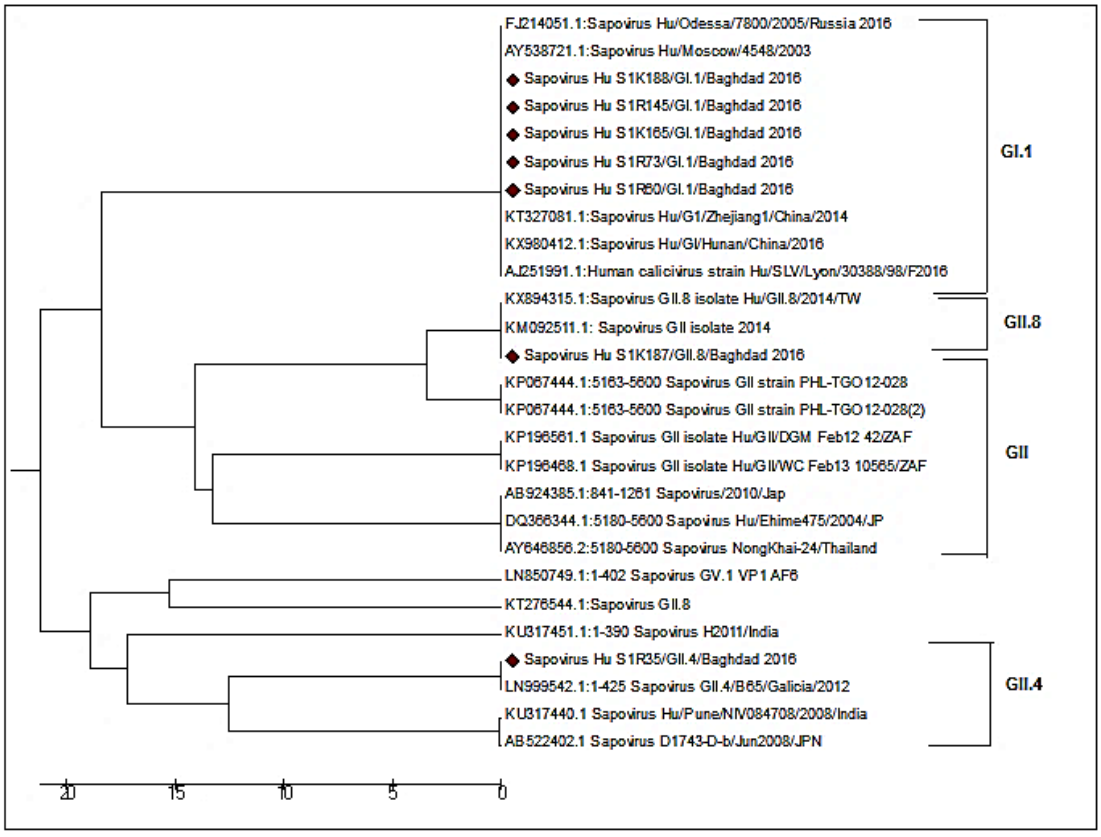

Fig.(3):The phylogenetic tree constructed from partial capsid gene of 7 Iraq SaVs nucleotide sequences pointed by red Rhomboid shape in comparing with the related Genbank reference 20 nucleotide sequences in a total of 307 positions in the final data set .The evolutionary analyses according to the UPGMA method were conducted in MEGA6 program.

Conclusion

This study indicated that SaVs in Baghdad are responsible for only a small percentage AGE in children under five years old, further analysis of SaVs in the different Iraqi regions targeting different age groups are required for better understanding of the viral distribution.

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