Tikrit Journal of Pure Science 22 (2) 2017 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

Real time-PCR, ELISA, as an identification methods for Detection respiratory syncytial virus in children

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Abstract
The study have been done to detecting HRSV infections among hospitalized children under 10 years old in Salahaldin Governorate, in addition to compare different diagnostic methods for detection of this virus.
Nasopharyngeal Swabs (NPS) and whole blood samples were taken from 400 hospitalized children between end of August 2013, to the end of May 2014, each sample was analyzed for HRSV by Real time –PCR, IgM and IgG specific to HRSV determining from blood specimens in serum using enzyme linked immune sorbent assay (ELISA) technique.
Nasopharyngeal Swab was more sensitive than the whole blood specimens for the detection of HRSV ,which is found in 1% by RT-PCR, in 81.9% by IgM –anti HRSV antibodies and in79.5% by IgG-HRSV antibodies. Autumn – winter periodwas considered as the most common time for HRSV infections with peak in September and February.

Keywords: RTIs, whole blood, Pneumonia, Cyanosis.

Introduction
Respiratory Tract infection (RTIs) is one of the important causes of deaths among children especially in developing countries [1]. It is caused by abroad spectrum of microbial agents viruses, on account for the largest number of respiratory tract infections [2]. Among these respiratory viruses are human respiratory syncytial virus (HRSV) [3]. HRSV belongs to the family paramyxoviridae, is important cause of Acute Respiratory Tract infection in children and represent a high percentage of the pediatric medical admission due to pneumonia and Bronchiolitis [4]. Human respiratory syncytial virus is a liner single stranded (SS) negative sense RNA consisting of 15,191 base pairs (bp) [5].
The laboratory diagnosis of HRSV infections is mostly done by Enzyme linked Immuno sorbent assay (ELISA) [6]. The diagnosis can also be done by real time – polymerase chain reaction assay RT-PCR [7].
HRSV has not been studied in Salahaldin Governorate of Iraq and no laboratory method have been used to detect this virus among patients in Salahaldin Governorate. The frequency of this virus as causative agent among hospitalized children with respiratory tract infections have not been clarified.
The laboratory diagnostic methods that are routinely done in Iraqi medical laboratories and hospitals, do not include the diagnosis of HRSV in their schedule investigations, and generally no regular diagnostic methods are done for respiratory viruses in Iraqi hospitals. Where as (RTIs) is so common and as RTIs in Iraq is a real problem, this study was prepared and its aim was to detect HRSV in respiratory specimens of children with (RTIs)

Materials and Methods
This study was carried out on patients attended the general hospital in Baiji in the central of Baiji city and to the teaching hospital of Tikrit city. The patients were the children less than 10 years old and they were admitted to these hospitals with respiratory tract infections. A total of 400 children who required medical treatment were included both sexes were chosen. The period of the study was started from the end of August 2013 to the end of May 2014.

Collection of Specimens
Four milliliters (mls) of venous blood were collected from each child. The blood then was divided into two parts:
The first part (3ml) was kept into a test tube without anticoagulant and centrifuged for 15 minutes at 3000 revolution per minute (RPM), the serum was then putted into one aliquot stored in one micro-centrifuged (Eppendorf) tube labeled with patient's code number and frozen upon reaching the deep freeze at -70℃ till tested. Once thawed refreezing was avoided.
The second part (1ml) was put into a test tube with anticoagulant, ethylene diamine tetramide (EDTA), and then mixed on a roller, labeled with patient's code number and frozen upon reaching the deep freeze at -70℃ till tested. Once thawed refreezing was avoided. One nasopharyngeal Swab (NPSs) was taken from each child by flocked swab applicators, thus one applicator was applied for one nostril. The nasopharyngeal Swab was placed immediately after collection in 3mls viral transport Medium (VTM) (Micro Rheologics company/ Italy).ELISA test kit for IgM anti – HRSV detection and IgG anti–HRSV detectionwere importedfrom (Cusabio company/ China) and Real Time – PCR kit for qualification of respiratory syncytial virus, was imported from (Primer design TM genesig company/ United Kingdom).

Materials
Quantification of respiratory syncytial virus (all species) Nucleocapsid (N) gene by RT-PCR kit:
This kit is designed and manufactured by primer design^{TM} genesig company/ United Kingdom.

The kit contents are:

- RSV-SPP specific primer/ probe mix (150 reactions BROWN) FAM labeled, BHQ quenched.
- RSV-SPP positive control template (for standard curve RED).
- Internal extraction control RNA (150 reactions BLUE).
- Internal extraction control primer/ probe mix (150 reactions BROWN) VIC labelled.
- Endogenous ACTB primer/ probe mix (150 reactions BROWN) FAM labeled, BHQ quenched.
- Internal extraction control/ RSV-SPP/ ACTB RT primer mix (150 reactions GREEN) required for two step protocol only.
- RNAse/ DNAse free water.

Reagents to be supplied by the user

- RNA extraction kit
- Oasig^{TM} lyophilised one step qRT-PCR mastermix kit contains complete one step qRT-PCR mastermix.

In this study, the master mix were ordered from GeNet Bio company/ Korea. The contents of the kit are:

1. Prime Q- master mix (2x), Q-9200, (1.0 ml) consists of:

1- HS prime Taq DNA polymerase.
2- Reaction buffer.
3- Enzyme stabilizer.
4- dNTPs mixture and PCR enhancer.

2. Prime Q- master mix (2x, with ROX dye), Q-9210, (1.0 ml) consists of:

1- HS prime Taq polymerase.
2- Reaction buffer.
3- Enzyme stabilizer.
4- dNTPs mixture and PCR enhancher.
5- 50X ROX dye (50µl X1).

- Optimal – Reverse transcription kit and mastermix.

Although a one step RT-PCR protocol is recommended, this kit is designed to work well with two step protocol.

Materials of two step reverse transcription/ real-time PCR kit for the detection of HRSV.

1. Synthetic Oligonucleotides (primers)

Oligonucleotids were ordered from Macrogen company/ Korea.

Table 1: The primers for target regions on nucleic acids of HRSV.

Oligo	**Oligo-dT**					
SEQ	5' – TTT TTT TTT TTT TTT TTT- 3' (18mer)					
GC%	MW		Yield		Scale (umoles)	Tm (C)
	Calculated	measured	OD	nmol		
0.0	5413.6	5426.5	4.6	27.5	0.05	33.4
Vol. for 100 pmol/ µl	purification			Modification		
275.0	MOPC					

Table 2: The primers for target regions on nucleic acids of HRSV.

Oligo	**RH examer**					
SEQ	5' – NNN NNN – 3' (6mer)					
GC%	MW		Yield		Scale (umoles)	Tm (C)
	Calculated	measured	OD	nmol		
0.0	1792.0	1793.2	2.2	33.3	0.05	12.0
Vol. for 100 pmol/ µl	purification			Modification		
333.0	MOPC					

2- Two- steps reverse transcriptase –PCR kit: These consist of the following:

1. Materials for reverse transcription (first strand cDNA synthesis): this kit (Cyclescript reverse transcriptase) was ordered from BIONEER company/ Korea. Consists of:

1- 5x rxn reaction buffer.
2- DTT.
3- 10 mM dNTPs.
4- Cycle script reverse transcriptase.

ELISA kit for IgG anti - HRSV antibodies detection (Cusabio Company/ China.Cat.NO.CSB-EQ027708HU).

This ELISA test kit is used for the qualitative determination of human anti RSV antibody (IgG) concentration in serum.

The kit compounds are the following:

1- Microplate wells: containing 96 test well coated with RSV antigen.
2- Negative control: one vial containing 0.5 ml.
3- Positive control: one vial containing 0.5 ml.
4- Sample diluent: one bottle containing 12 ml.
5- HRP-conjugate: one bottle containing 12 ml.
6- Wash buffer (20x concentrate): one bottle containing 15 ml.
7- Substrate A: one bottle containing 7 ml.
8- Substrate B: one bottle containing 7 ml.
9- Stop solution: one bottle containing 7 ml.

ELISA kit for IgM anti - HRSV antibodies detection (Cusabio Company/ China.Cat.NO.CSB-E13790h).

This ELISA test kit is used for the qualitative determination of human anti RSV antibody (IgM) concentrations in serum.

The kit compounds are the following:

1- Microplate wells: containing 96 test well coated with RSV antigen.
2- Negative control: one vial containing 0.5 ml.

3- Positive control: one vial containing 0.5 ml.
4- Sample diluent: one bottle containing 12 ml.
5- HRP-conjugate: one bottle containing 12 ml.
6- Wash buffer (20x concentrate): one bottle containing 15 ml.
7- Substrate A: one bottle containing 7 ml.
8- Substrate B: one bottle containing 7 ml.
9- Stop solution: one bottle containing 7 ml.

Methods

Quantification of respiratory syncytial virus (all species) Nucleocapsid (N) gene, alternative two step reverse transcription/ real time-PCR

1. Reverse transcription for first strand complementary DNA (cDNA) synthesis:

This is the second step in real time PCR, and also divided into two sub-steps.

1. The first substep is (called reverse transcription 1, RT1). This step includes the following:

- Two µl of the oligonucleotides primers (2 primers of HRSV) were added into a single 200 µl microcentrifuge tube; and 9µl of the eluted solution of the sample (template) were added to the same tube to get a final concentration of 11µl. The tube was then subjected to short centrifugation for few seconds at 5000 rpm before putting it in thermal-cycler which was programmed for preheat at 105 °C and heating 10 minutes at 65 °C for sample denaturation and annealing, then chilling at 4°C for 4 minutes.

2. The second substep (called reverse transcription2, RT2).

This step included the preparation of cDNA master mix by addition of 4µl of the 5x rxn reaction buffer, 2µl DTT, 2µl of 10 µl mMdNTPs, and 1µl Cycle Script reverse transcriptase were collected in to a single microcentrifuge tube to get a final concentration of 9µl. The final 9µl concentration was added to the previous 11µl to get reaction volume of 20µl which was put in the thermal cycler.

The cycler was programmed for the first strand cDNA synthesis by incubation at 50°C for 60 minutes, then incubation at 95°C for 5 minutes to step reaction. The product then was used for PCR amplification.

1. After reverse transcription, the reaction mix was prepared according to the table below for each cDNA sample.

A- RSV-SPP primer/ probe detection mixture.

Component	Volume
Prime Q- master mix	10µl
RSV-SPP primer/ probe mix (BROWN)	1µl
RNase/ DNase free water (WHITE)	3.5µl
Prime Q- master mix with ROX dye	0.5µl
Final volume	15µl

B- Endogenous ACTB detection mixture.

Component	Volume
Prime Q- master mix	10µl
Endogenous ACTB primer/ probe mix (BROWN)	1µl
RNase/ DNase free water (WHITE)	3.5µl
Prime Q- master mix with ROX dye	0.5µl
Final volume	15µl

2. Fifteen µl of this mix into each well according to real-time PCR experimental plate setup was pipetted.
3. The cDNA templates for each samples in RNase/ DNase free water was prepared (the suggested concentration was 5ng/µl), but if the concentration of RNA that was used to make the cDNA is not known, then the RT reaction mix should be diluted 1: 5 (10 µl of sample cDNA and 40 µl of water).
4. Then 5µl of cDNA template was pipetted in to each well according to the experimental plate setup. For negative control wells 5µl of RNAse/ DNAse free water was used. The final volume in each well is 20µl.
5. Real-time PCR amplification protocol.

This proposal was carried out after several experimental tests to find out the optimal concentration of reagents to be used for PCR amplification, the place was cleaned with absolute alcohol, all the solution was thawed on ice, gently vortexed, and briefly centrifuged. The protocol is mentioned in the following table:

	Step	Time	Temp
50 cycles	Enzyme activation	10 mins	95 °C
	Denaturation	10 s	95 °C
	DATA COLLECTION*	60 s	60 °C

* Fluorogenic data was collected during this step through the FAM and VIC channels.

Interpretation of results

Target	Internal control	Negative control	Positive control	Interpretation
+ve	+ve	-ve	+ve	+ve
+ve	-ve	-ve	+ve	+ve
-ve	+ve	-ve	+ve	-ve
-ve	-ve	-ve	-ve	Experiment fail
+ve	+ve	+ve	+ve	Experiment fail

ELISA test kit for IgG anti- HRSV antibodies qualitative determination concentrations in serum.

- Test procedure, according to supplied company kit:

1. All reagents and samples were prepared and brought to room temperature (18-25°C).
2. The assay layout sheet was used to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and stored unused wells at 4 °C.
3. A blank well was set without any solution.

4. One hundred µl of negative control, positive control were added, controls need test in duplicate.
5. One hundred µl of sample diluent were added to rest wells, then 10 µl of sample per well were added then covered with the adhesive strip provided and incubated for 30 minutes at 37 °C.
6. A respiratory and washing each well was done for 5 times. Washing by filling each well with wash buffer (200µl) by autowasher, and standing for 20 seconds and completed removal of liquid at each step for good performance after the last washing, any remaining wash buffer was removed by a separating or, decanting with inverted and bloted the plate against clean paper towels.
7. Then 100 µl of HRP-conjugate was added to each well (not to blank well), and covered the microtiter plate with a new adhesive strip and incubated for 20 minutes at 37 °C.
8. The aspiration/ wash process for 5 times as in step 6 was repeated.
9. Fifty µl of substrate A and 50 µl substrate B were added to each well and incubated for 10 minutes at 37 °C with protected from light.
10. Then 50 µl of stop solution was added to each well with gently tap the plate to ensure through mixing.
11. Blank well was taken as zero and the optical density (OD) of each well was determined within 10 minutes by using a microplate reader set to 450 nm.

Calculation of results

For calculation the valence of human anti - RSV antibody (IgG) the sample well was compared with control, according to following cut – off value supplied company kit:

If optical density (OD.) negative <0.05, calculate it as 0.05.

while OD. Sample/ OD. ≥ 2.1: positive.

While OD. Sample/ OD. < 2.1: negative.

ELISA test kit for IgM anti-HRSV antibodies qualitative determination concentrations in serum. - Test procedure, according to supplied company kit:

1. All reagents and samples were prepared and brought to room temperature (18-25°C).
2. The assay layout sheet was used to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and stored unused wells at 4 °C.
3. A blank well was set without any solution.
4. One hundred µl of negative control, positive control were added, controls need test in duplicate.
5. One hundred µl of sample diluent were added to rest wells, then 10 µl of sample per well were added then coverd with the adhesive strip provided and incubated for 30 minutes at 37 °C.
6. A spirating and washing by filling each well with wash buffer (200µl) by autowasher, and standing for 20 seconds and completed removal of liquid at each step for good performance after the last washed, any remaining wash buffer was removed by a spirating or, decanting with inverted and bloted the plate against clean paper towels.
7. Then 100 µl of HRP-conjugate was added to each well (not to blank well), and coverd the microtiter plate with a new adhesive strip and incubated for 20 minutes at 37 °C.
8. The aspiration/ wash process for 5 times as in step 6 was repeated.
9. Fifty µl of substrate A and 50 µl substrate B were added to each well and incubated for 10 minutes at 37 °C with protected from light.
10. Then 50 µl of stop solution was added to each well with gently tap the plate to ensure thorough mixing.
11. Blank well was taken as zero and the optical density (OD) of each well were determined within 10 minutes by using a microplate reader set to 450 nm.

Calculation of results:

For calculation the valence of human anti - RSV antibody (IgM) the sample well was compared with control, according to following to cut – off value supplied company kit:

If optical density (OD.) negative <0.05, calculate it as 0.05.

while OD. Sample/ OD. ≥ 2.1: positive.

While OD. Sample/ OD. < 2.1: negative.

Statistical Analysis:

The statistical package for social science (SPSS,U.S.A. , version 17, 2014), two approaches are used namely descriptive and analytic. Chi – square text (X2) and fisher's exact test are used to test the association between categorical variables, probabilities of 0.05 ($p \leq 0.05$) was considered as statistically significant.

Results and discussion

Detection of HRSV infection:

Detection of HRSV from nasopharyngeal swab and whole blood samples using RT-PCR technique:

The nucleic acid RNA of HRSV was detected by real time - PCR technique from NPS and whole blood samples from 23(5.75%) patients, the results showed that 4 (17.39%) out of 23 (5.75%) were positive for HRSV, while 6 (26.1%) out of 23 patients were negative for HRSV from whole blood samples. On the other hand, the results showed that 13 (56.5%) out of 23 patients were negative for HRSV from NPS. All positive whole blood cases were negative in NPS, (Figure 1).

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

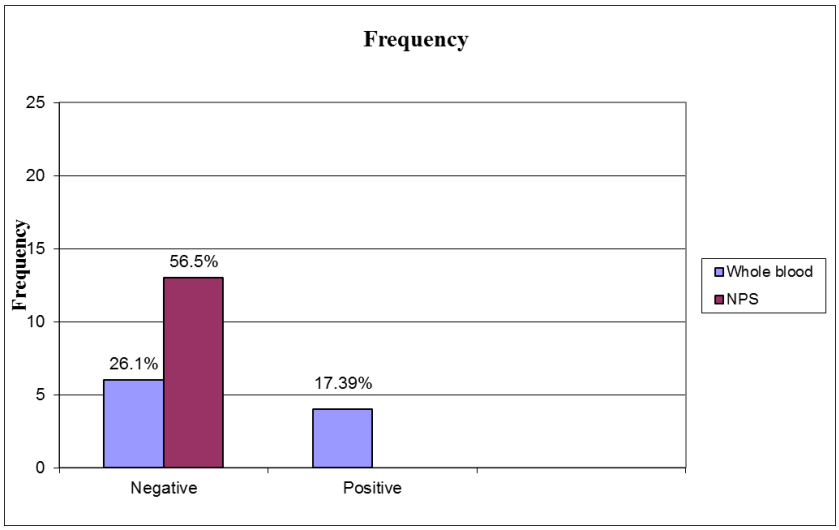

Figure 1: Detection of HRSV by the real time - PCR technique in nasopharyngeal swab (NPS) and whole blood specimens for infection children.

Flocked swab was used for the collection of NPS, also whole blood samples were the only specimens used for antigen and nucleic acid detection. Other kinds of specimens like NPA, NPW, nasal wash, tracheal aspirates were not performed in this study, thought they are the mainly specimens which were used in most of other studies for the detection of HRSV antigen or nucleic acid [8, 9, 6].

Detection of HRSV using real time-PCR:

RNA of HRSV was detected for each children from NPS and whole blood specimens by using the molecular technique real time - PCR. The results showed that 4 (1%) children were positive while the remaining 396 (99%) children were negative (Figure 2).

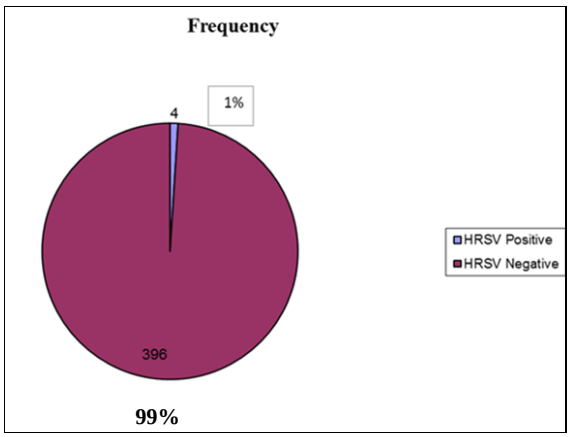

Figure 2: Frequency of HRSV infections among patients in the study using real time - PCR technique.

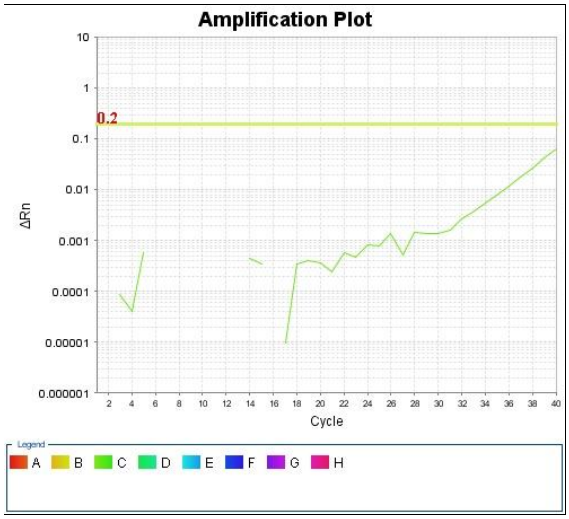

Figure 3: Detection of HRSV by RT-PCR (ACTB)

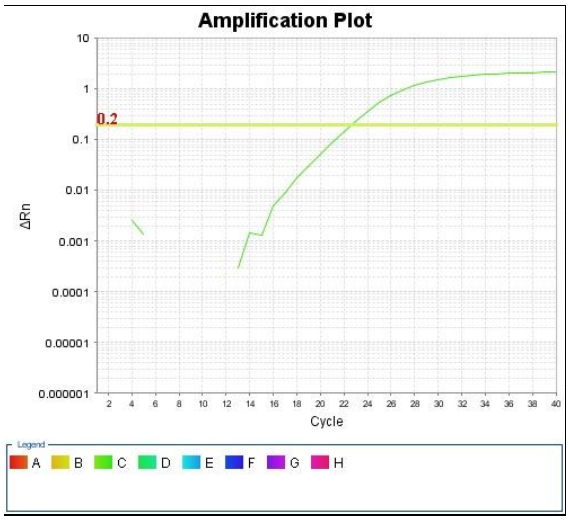

Figure 4: HRSV positive control (PC) detection by RT-PCR

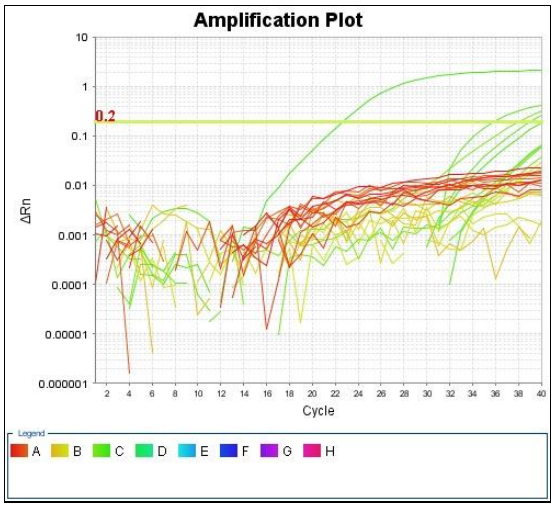

Figure 5: Results of HRSV and internal control detection by RT-PCR

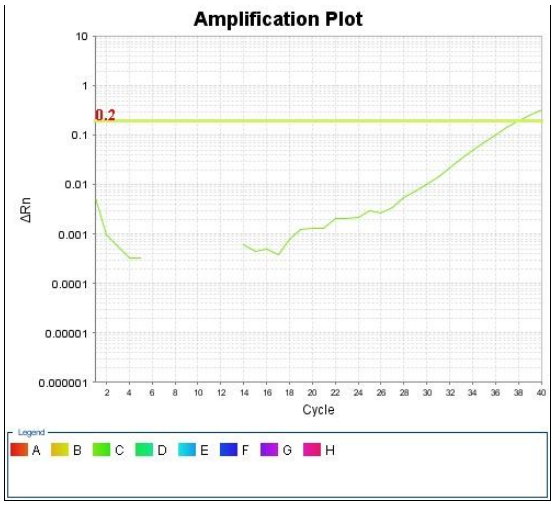

Figure 6: Detection of HRSV by RT-PCR (ACTB)

In the present study, HRSV was detected in 4(1%) out of 400 infected children using RT-PCR technique, this frequency reflected the low incidence of HRSV among children who were hospitalized suffering from RTIs. To our knowledge, it is the first record about HRSV infection in Salahaldin Governorate.

The use of RT-PCR in order to diagnosis of HRSV frequency in Iraq was not done previously, just four studies, the first previous study in Iraq studied the frequency of HRSV among children using the antigen detection methods, determined the percentage of infection as 37.6% [10]. The second study was in Baghdad/ Iraq in 2005-2006. It revealed that 45% of hospitalized children were infected with HRSV using rapid immunochromatographic test [11]. While antigen immunochromatographic test in the current study revealed a frequency of 14.6%, this low prevalence among the mentioned studies compared to this study might belong to different time of selecting specimens or different samples sizes selected, different climate and geographical factors. The third study done in Kurdistan region in Iraq in Suleimani Governorate in 2013 was detected HRSV in 81(27%) out of 300 hospitalized children using a conventional multiplex RT-PCR technique beside another diagnostic methods like ELISA, DFA, immunochromatographic strep methods [12]. And the fourth study was done in Hilla city/ Iraq also in 2013 found that 68(19.43%) from 350 of children were infected with HRSV IgM using ELISA technique [13]. While IgM anti - HRSV antibodies in the current study revealed a frequency of 68(81.9%) out of 83 patients, 68(17%) out of 400 patients, they present study is agreement with previous study by Al-Marzogi [13].

The prevalence of HRSV infections in the nearby countries revealed variations in frequency using RT-PCR, in a study done in Jordan it was 64% [14]. and in Saudi Arabia study 54.4% of children were infected by HRSV [15]. In Iran, astudy found that 45.6% of children under 5 years were infected with HRSV [16]. In USA a study found that 24% of hospitalized children were infected with HRSV [17]. And in Philippines another study found that only 8.4% of children were infected with HRSV [18].

The variation in results from different countries and even from the same country reflected the sample size as some studies were taking small sample size, or due to the different techniques were used in different studies or due to the period of study as some studies were performed during only winter season, or due to the type of sample as some studies were chose only children with bronchiolitis and pneumonia but not all respiratory infections as a whole, more over geographical variations, environmental differences and control measures used might also contribute to the differences in the percentage of HRSV infections in different countries.

Seropositivity of anti – HRSV IgM antibodies among patients with RTI.

Measurement of IgM anti-HRSV antibodies seropositive showed that 68(81.9%) out of 83 children infected with HRSV had positive antibody titers, while only 3(0.9%) out of 317 patients (False positive), who were negative for HRSV, were having positive antibody titer. The total IgM anti-HRSV antibodies was 314 (78.3%) in all hospitalized children. The results were statistically not significant (p=0.3452).

Seropositivity of IgM anti-HRSV antibodies among the 83 HRSV patients showed that positivity was found in 8 (80%) out of 10 patients aged <1 year, 10 (83.3%) out of 12 patients aged 1 to < 2 years, 9(69.2%) out of 13 patients aged 2 to <3 years, 7 (87.5%) out of 8 patients aged 3 to <4 years, 8(88.8%) out of 9 patients aged 4 to <5 years, 6(85.7%) out of 7 patients aged 5 to <6 years, 6(85.7%) out of 7 patients aged 6 to <7 years, 5(83.3%) out of 6 patients aged 7 to <8 years, 4(80%) out of 5 patients aged 8 to <9 years, and 5(83.3%) out of 6 patients aged 9 to <10 years.

The highest seropositivity was found in the age group 4 to <5 years, while the lowest rate in the age group 2 to <3 years, the results among age groups were statistically not significant (p= 0.0218) (Figure 7).

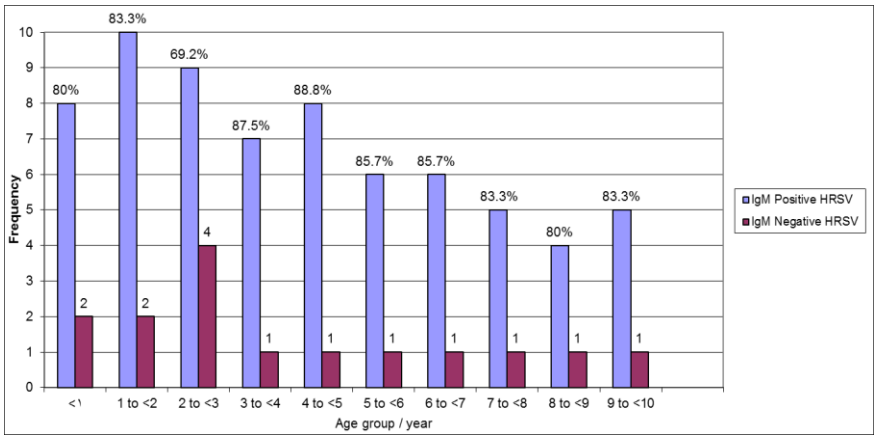

Figure 7: Seropositivity of anti – HRSV IgM antibodies among patients infected with RTI.

However, the results showed that IgM anti-HRSV antibodies as seroprevalence in children with positive HRSV antigen were significant and more frequent than in HRSV antigen negative children were important diagnostic tools [19].

Seropositivity of anti – HRSV IgG antibodies among patients with RTI.

The seropositivity of anti-HRSV IgG antibodies specific to HRSV in the study group showed that 66(79.5%) out of 83 HRSV infected patients were positive, while 251(79.2%) out of 317 patients HRSV-negative patients were IgG seropositive (Table 4-7). The difference in IgG Seropositivity between the HRSV infected patients and those who were not infected with HRSV was not statistically significant (p=0.366).

Positive IgG anti-HRSV antibodies among the 83 HRSV patients infected children were found in 8(66.7%) out of 12 in age group <1 year, 9(75%) out of 12 in age group 1 to <2 years, 8 (80%) out of 10 were aged 2 to <3 years, 7(77.8%) out of 9 were aged 3 to <4 years, 7(87.5%) out of 8 were aged 4 to <5 years, 6(75%) out of 8 were aged 5 to <6 years, 7(87.5%) out of 8 were aged 6 to <7 years, 5 (83.3%) out of 6 were aged 7 to <8 years, 4(100%) out of 4 were aged 8 to <9 years, and 5 (83.3%) out of 6 were aged 9 to <10 years.

The highest Seropositivity is found in the age group 8 to <9 years, while the lowest is in the age group <1 year, the results among age groups were not statistically significant (p= 0.3209) (Figure 8).

Positive IgG anti HRSV antibodies percentage among HRSV-negative children was (66.7%), (75%), (80%), (77.8%), (87.5%), (75%), (87.5%), (83.33%), (100%), (83.33%) in age groups <1 year, 1 to <2 years, 2 to <3 years, 3 to <4 years, 4 to < 5 years, 5 to <6years, 6to <7 years, 7 to <8 years, 8 to <9 years, 9to <10 years respectively, the results showed the presence of wavy increase in a percentage of children with positive IgG anti-HRSV antibodies in HRSV infected and non-HRSV infected group (Figure 9).

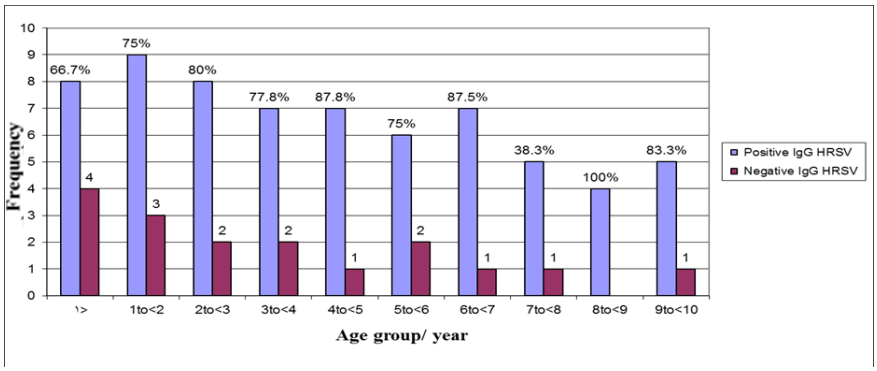

Figure 8: Seropositivity of anti – HRSV IgG antibodies among patients infected with RTI.

Table 3: Seropositivity of anti – HRSV IgG antibodies among patients infected with RTI.

Patients	Frequency of positive IgG anti-HRSV antibodies	%	Frequency of negative IgG anti-HRSV antibodies	%
HRSV infected group	66	79.5	17	20.5
HRSV no- infected group	251	79.2	66	20.8
Total	317	79.2	83	20.7

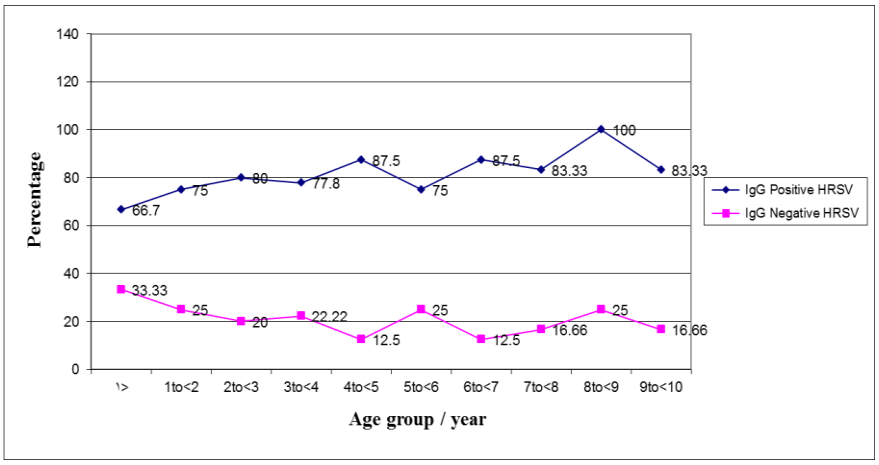

Figure 9: Percentage of anti-HRSV IgG antibodies among patients infected with RTI.

The results revealed that 79.5% of HRSV positive patients were having positive titers, while 79.2% of HRSV- negative patients were IgG seropositive. The differences in the two groups were statistically not significant, and the high rate of previous infections with HRSV among children under 10 years, or in infants under 1 year of age, may be because the transport of IgG anti - HRSV antibodies from mothers to infants during intrauterine life. A previous study by [20]. Revealed that 83% of the pregnant women as well as all the babies of these mothers were anti- HRSV IgG positive . IgG anti- HRSV antibodies were investigated among all patients in this study and the total prevalence rate was 79.2%. This result was similar to a previous study done in Baghdad/ Iraq, in which 79% of infants with ARTIs were having positive titers of antibodies [11]. Beside that the patients in the previous study were limited to one year old infants, while in this study, 66.7% of the age group< 1 year old was IgG seropositive.

The results showed the presence of wavy increase in the percentages of children with positive IgG anti - HRSV antibodies in HRSV infected group and HRSV non - infected group. This result points to the cumulative increase in HRSV infections with the increase in the age of children. A study by [21]. In Thailand found that the prevalence of IgG anti - HRSV antibodies was increasing with age: from 6 to 11 months, 11.76%; from 12 to 17 months, 41.67%; from 18 to 23 months, 60.87%; from 24 to 29 months, 88.24%; from 30 to 35 months, 78.57%; from 36 to 41 months, 94.44% and from 42 to 60 months 100%.

This figure showed increase in the frequency of infections which started in January, presented in February peaked and declined in March. In this figure also another peak, started from August, peaked in September and declined in October and November, Thus Autumn-Winter period was the most common for HRSV infections, while Summer is the least for HRSV infections.

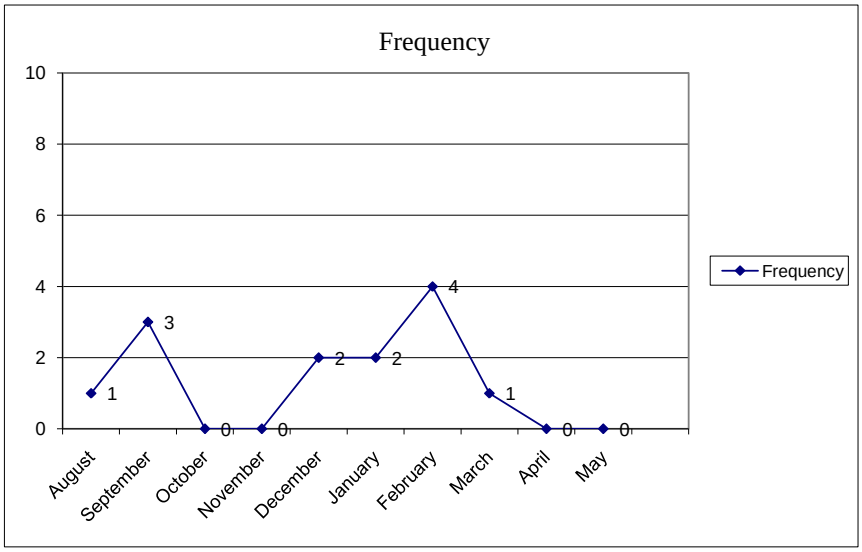

Figure 10: Seasonal distributions of HRSV infections among hospitalized children in the study

Salahaldin Governorate is located in the middle of Iraq with some mountains and hills like Hemreen and Makhool in some parts of this Governorate. The main annual temperature is high as like as Baghdad temperature and the climate is dry and windy in Winter. The HRSV infections were detected all the year in this study.

Autumn-Winter period was the most common for HRSV infections, with peak in September and another peak in February. The differences in viral distribution and seasonality might be attributed to the climate and geographical factors.

Most of other studies from different regions all over the world like Pakistan, Japan and Jordan showed Winter season predominance of HRSV infections, [22, 23, 24]. Though in Thailand and in similar climate countries, the annual peak of HRSV hospitalization among children is occurring between July and October which are the rainfall months in this country [25].

In Winter, inhalation of cold air will slow the mucociliary escalator and reduce phagocytic activity of leukocytes, thereby increasing susceptibility to infection [26]. May be because that people's stay indoors in Winter will increase the risk of HRSV transmission and especially among children who catch their infections from older people.

Conclusions

Pneumonia was the main clinical disease in HRSV in hospitalized children and the infections were higher among age group <4-5 years.

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تفاعل انزيم البلمرة المتسلسل المتعدد، الأليزا كطرق تشخيصية لفايروس الخلية العملاقة التنفسي في الأطفال

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الملخص

اجريت هذه الدراسة لتشخيص فايروس (HRSV) في الأطفال الراقدين في المستشفى تحت العشر سنوات في محافظة صلاح الدين أضافة الى مقارنة الطرق التشخيصية لانزيم البلمرة المتسلسل المتعدد الوقت الحقيقي مع طريقة الاليزا، أخذت العينات بوساطة المسحات الانفية البلعومية و عينات الدم الكلي لـ 400 طفل للفترة بين نهاية آب 2013 لغاية نهاية أيار لعام 2014 و كل عينة تم تحليلها بواسطة انزيم البلمرة المتسلسل المتعدد الوقت الحقيقي و بواسطة الاجسام المضادة نوعIgM و الاجسام المضادة نوعIgG لفايروس HRSV من عينات المصل المفصولة عن الدم بوساطة تقنية الاليزا. عينات الدم الكلي Whole blood كانت اكثر تحسساً من المسحة الانفية البلعومية NPS في تشخيص HRSV الذي تم تشخيصه في (1%) بوساطة Real time-PCR و في 81,9% بوساطة IgM anti – HRSV antibody و في 79,5% بوساطة IgG anti – HRSV antibody. فترة الخريف– الشتاء كانت أكثر الأوقات للأصابة بـHRSV مع وجود أعلى فترة للأصابة في شهري سبتمبر و شباط.