Detection of Respiratory syncytial virus infection in a sample of infants in Iraq

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Abstract

Background: Human Respiratory syncytial virus (HRSV) is one of the major causes of bronchiolitis and pneumonia in infants.

Objective: detection of Anti HRSV antibodies in infants and children by using indirect ELISA, and detection of HRSV antigen by using Respi-RSV test.

Materials and Methods: Hundred and eighty four serum samples (104 with respiratory tract infection, 54 without Respiratory tract infection, and 26 with cancer) also 100 nasal and throat swabs were collected from infants and children from Central pediatric Hospital in Baghdad in year (2005-2006). Indirect ELISA test and rapid test used for detection Human RSV antibodies and Human RSV antigen respectively

Results: Detection of anti HRSV antibodies using ELISA in children without respiratory tract infection was 26% with mean titer 494, but the antibodies was higher in patients with cancer which were 96% with mean titer 580, however anti HRSV was detected in79% in infants with respiratory tract infection (bronchiolitis and pneumonia), their mean titer (1411) was, higher than that of the previous two groups.

Human RSV viral antigen was detected by RSV-Respi kit in 45% of nasal and throat secretion collected from children with respiratory tract infection, detection of HRSV antigen samples was compatible with the detection of antiHRSV antibodies in 50%.

Conclusion: The highest percentage and titers of antiHRSV antibodies were detected in infants with respiratory tract infection than in others suffering from different cancers or without respiratory tract infection.

Human RSV antigens were detected in nasal/throat swabs in infants with bronchiolitis and pneumonia.

Keywords: Respiratory syncytial virus, bronchiolitis, Respi-RSV.

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Introduction

Human Respiratory syncytial virus (HRSV) is one of the major causes of viral respiratory tract disease in young children and infant in developed and developing, poor and rich countries [(1)]. Severe HRSV bronchiolitis and pneumonia requiring hospitalization typically occur in infant less than 9 months of age [(2)]. The HRSV is mainly associated with bronchiolitis in children suffering from underlying illnesses such as congenital heart disease and broncho-pulmonary dysphasia which are at increased risk for severe infection due to HRSV.

In our country the pediatricians noticed increasing numbers of children under 2 years suffering from lower respiratory infections and bronchiolitis which occurs mainly in winter and in some years presented as epidemic with high morbidity and mortality especially in patient underlying illnesses[(3)]. This study were decided to detection antibodies for human RSV in serum samples from infants and young children by using specific ELISA kits, and detection of viral antigen in nasal and throat swabs obtained from

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respiratory tract specimens by using Rapid test.

Materials and methods

Blood samples collected from children (54 samples from children without sings of respiratory illness, 26 with different kinds of cancer , and 104 suffering from bronchiolitis and pneumonia) during months (December-March) in the year (2005-2006), they were collected from Central Pediatric Hospital. Hundred and eighty four blood samples were collected in a disposable plastic tubes container, then the sera separated and stored at -20 C° till use. ELISA kits: Imported from DIALAB Company (Germany) used for the detection human RSV antibodies in serum.

Hundred nasal and throat swabs were collected from infants and young child, in central pediatric hospital, the inpatients with severe respiratory tract infection(bronchiolitis and pneumonia) chosen depend on clinical signs and chest x-rays , diagnosis by pediatricians and collected at time of illness; the sterile swabs were immersed into tube containing 2 ml of cooled transport media used for virus isolation- RSV-Respi-Strip test, kit were used for detection of human RSV antigen in nasopharyngeal specimens which based on the use of homogenous immunochromatographic system with colloidal gold particales. The Kits were imported from CORIS BioConcep Company, (Germany).

Statistical analysis

The data were analyzed by Chi-square test, analyses of variance (ANOVA) and least significant difference (LSD) for differentiation among the means of groups[4], P Value ≤ 0.05 was considered as significant result.

Results

A total of (54) serum samples were collected from patients with different ages ranging from 1 month Infants to 5 years children without signs of respiratory illness, only 14 serum samples had a positive titer of anti HRSV (26%) ranging from (215-875) and mean titer (494).The highest percentage of anti HRSV was (44.4%) in age 1-3 months and the lowest percentage was in 10-12 months (18%).These results showed no statistical significant differences among the age groups (P>0.05) (Table 1).

Table 1: Detection of anti HRSV antibodies in children without respiratory tract infection by indirect ELISA.

Tested sera	Age	+ve anti HRSV*			-ve antiHRSV*	
		No.of+ve	%	mean titer	No.of-ve	%
9	1-3months	4	44.4%	215.7±83.8	5	55.5%
8	4-6months	3	37%	236.6±118.6	5	62.5%
16	7-9months	3	18.8%	378.6±163.3	13	81%
11	10-12months	2	18%	875.5±512.5	9	81.8%
10	1-5 years	2	20%	763.5±650	8	80%
54		14	26%	494	40	79%

*Cut off value= 67. Over this number is +ve HRSV antibodies and below is –ve
L.S.D. = 813.
p>0.05

Twenty six (26) patients with different kinds of cancer (3 Leukemia, 2 Wilm's tumor (WT), 13 Acute lymphocytic leukemia (ALL),3 Chronic lymphocytic leukemia (CLL) and 5 Non Hodgkin's lymphoma (NHL)), at central pediatric Hospital who were under chemotherapy. Examination of sera of these patients for anti HRSV antibodies revealed that 18 of them were positive which were equal to 69% with a mean titer of 580. All cancer sera from patients aged 1-6 years were suffering from different malignancies positive for anti HRSV antibodies. Statistical analysis of the data results show highly significant differences (P<0.01) mainly in age 1-6 years group of patient with NHL than other groups (Table 2).

Table 2: Detection of anti HRSV antibodies in cancer patients according to disease type and age.

Tested sera	Type of cancer	Age / year	+ve Anti HRSV *			-ve AntiHRSV*	
			No.	%	titer	No.	%
3	Leukemia	1-2 years	3	100%	284.3±120.4	_	_
2	WL	2-3 years	2	100%	214.5±4.5	_	_
5	NHL	1-6 years	5	100%	1495±1116.5	_	_
3	CLL	6-8 years	_		_	3	100%
13	ALL	3-12 years	8	61.5%	322.6±98.7	5	38.5%
26			18	69%	580	8	31%

* Cut off value=67. Over cut off+ve, blow cut off-ve.
L.S.D. = 890.9
P <0.01

One hundred and four Infants and young children suffering from acute bronchiolitis and pneumonia from one month to one years of age. Examination of serum samples of these patients showed that 82 of them were positive (79%) for anti HRSV by indirect ELISA with mean titer of 1411, ranging (354-2867).

All pneumonia patients' sera were positive for HRSV antibodies in the age 7 months to one year, but highest percentage (76%) of broncholitis cases were in age 4-6 months and 10 month- to one year. These data showed statistical significant differences (P<0.05) among different age groups which was high in 10 months- to one year and low in 1-3 months of bronchiolitis patients, and in pneumonia patients the highest were in 10moths –to one year and lowest in 7-9 months and 1-3 months (Table 3)

Table 3: Detection of antiHRSV antibodies in Infants with respiratory tract infection by indirect ELISA.

Tested sera	Clinical disease	Age	+ve Anti HRSV *			-ve antiHRSV*	
			No.	%	Titer ± S.D.	No.	%
26	Bronchiolitis	1-3month	19	73%	1261.1±470.9	7	27%
25	=	4-6month	19	76%	2184.1±916.3	6	24%
11	=	7-9month	8	73%	1769.2±1071.1	3	27%
14	=	10-12month	11	76%	2867.1±1295.4	3	24%
11	Pneumonia	1-3month	9	82%	489.4±231.3	2	18%
7	=	4-6month	6	86%	1016.6±204.5	1	14%
2	=	7-9month	2	100%	354±80.8	0	0
8	=	10-12mon	8	100%	1373.8±352.5	0	0
104			82	79%	1411	22	21%

* Cut off value =67. Over cut off = +ve, below cut off = –ve.
L.S.D = 1570.3
P<0.05

From a total of (184) individual sera samples examined by indirect ELISA showed a high percentage of sera (79%) were positive for anti HRSV and those belong to Infants with respiratory infections followed by sera from cancer patients (69%). However, sera from patients without respiratory tract infection were (26%) positive for HRSV.These results showed highly significant statistical differences (P<0.01) among the different groups, which was higher in the group of respiratory tract infection (Table 4)

Table 4: Percentage of anti HRSV antibodies in individuals with or without respiratory tract infections and individuals with cancers.

Subjects	No. of sample	+ ve Anti HRSV %		- ve Anti HRSV%	
Infants without R.T.I	54	14	26%	40	74%
Infants with R.T.I	104	82	79%	22	21%
Individuals with cancer	26	18	69%	8	31%
	184	114	62%	70	28%

X^2=25
P<0.01

Hundred nasal and throat swabs collected from Infants and young children suffering from acute respiratory tract infection were examined by Respi test kit. Forty five (45%) tested swabs were positive for HRSV antigens. Nasal-throat swabs collected from Infant patients in December 2005 showed the highest positive percentage (70%), and the lowest was in March 2006 (25%).These data showed highly significant differences (P<0.01) among the months of the year (Table 5).

Table 5: Detection of HRSV antigen in nasal/throat swabs by using immunochromatographic assay.

Month/year	No. of patient sample	+ve HRSV antigen	-ve HRSV antigen	%
December 2005	39	27	12	70%
January 2006	26	8	18	44%
February 2006	20	6	14	30%
March 2006	15	4	11	25%
Total	100	45	55	45%

X^2=30
P<0.01

The positive result of RSV-Respi-Kits appears as two dark red lines due to presence of RSV- antigen in test samples, compared to negative result which shows only one dark red line (Figure 1).

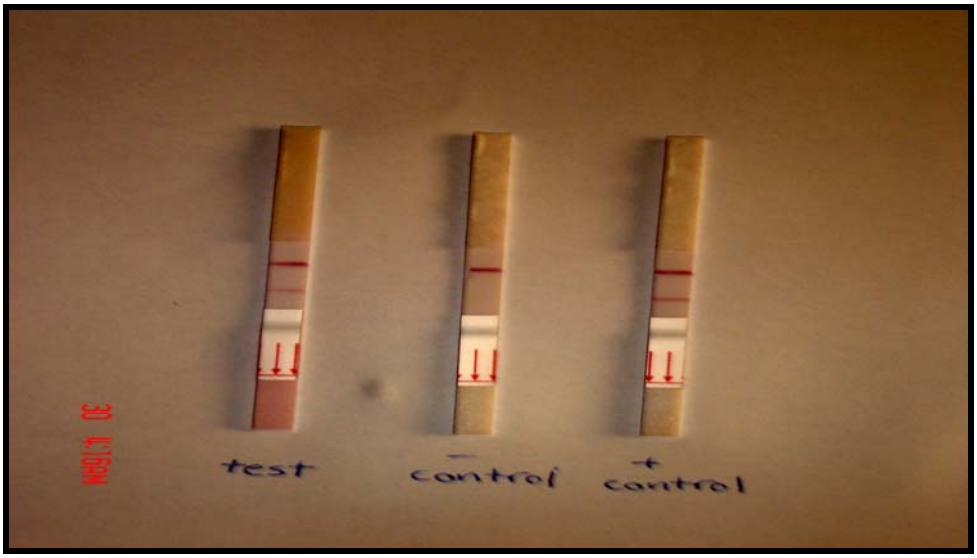

Figure 1: RSV-Respi-test appears as two dark red lines due to presence of RSV-antigen in nasal/ throat swabs, compared to negative result which shows only one dark line.

Forty one Infants who were suffering from acute bronchiolitis and pneumonia were examined for presence of both HRSV antigen and antibodies. Results revealed that (54%) of patients were positive HRSV antigen in the nasopharyngeal swabs by using Respi-test kit but 46% samples were negative for HRSV antigen which was 46%. Thirty four (34) serum samples of the same patients showed positive result for anti HRSVantibodies which make 83% of the tested sera. Twenty patients were positive for both HRSV antigen and antibodies constituted about (50%) of the tested sera. The result showed statistically high significant difference (P<0.01) among different age groups, which was high in group 10 months to one year than other groups (Table 6).

Table 6: Detection of HRSV antigen and antibodies in Infants with respiratory illness.

No. of sample	Respiratory infection	Age	+ve HRSV antigen		+ve HRSV antibody		+ve RSV antigen +ve antibody		
			No.	%	No.	%	No.	%	titer
11	Broncholitis	1-3 months	5	45%	8	73%	4	36%	263.5±125
9	=	4-6 months	5	65%	8	89%	5	56%	527±196
3	=	7-9 months	1	33%	3	100%	1	33%	67
2	=	10-12 months	1	50%	2	100%	1	50%	308
6	Pneumonia	1-3 months	3	50%	4	67%	2	33%	120±21.2
3	=	4-6 months	2	67%	2	67%	2	67%	489±379
2	=	7-9 months	2	100%	2	100%	2	60%	354±180
5	=	10-12 months	3	60%	5	100%	3	60%	1410±829
41			22	54%	34	83%	20	50%	440

L.S.D=766
P<0.01

Discussion

Human respiratory syncytial virus has been recognized as most important causative agent of serious respiratory tract infection in infants and young children [(2)]. It account for approximately 50% of all pneumonias [(5)] and about 70% of bronchiolitis, hospitalization are associated with HRSV infection in developed world and in Europe, HRSV accounts for 42 - 45% of hospital admissions for lower respiratory tract infections in children younger than 2 years of age[(6)].

In Iraq, pediatricians recognized an increasing number of infants with bronchiolitis and pneumonia as reported in hospital admission in winter months [(3)].Our study is the first in the country to records the presence of RSV in respiratory tract infection in infants by detection of RSV viral antigens and specific viral antibodies in ill Infants.

The antibodies which was found in children with other disease condition, could be due to previous RSV infection or may be a maternal type of antibody as reported in other studies[(7)] who

mentioned that the presence of specific HRSV antibodies in the sera of all full-term newborn due to transplacental transfer of maternal antibodies. The level of these antibodies decline slowly during the first few months of life and it is believed that after 7 month of age the detectable antibodies are usually due to natural HRSV infection[8]. Also breast-fed Infants have the added benefit of maternal antibodies in form of colostrom and milk [9]. However, the presence of antiHRSV in Infants without signs of respiratory tract may be due to asymptomatic infection with HRSV [10].

The detection of antiHRSV in sera of patients with different kind of cancer indicated that these patients were probably infected with HRSV; this result is higher than the result obtained in other study [11], who reported 37% in cancer patients, and agreed with other studies [12], who explained the high infection in immunocompromized patients since they are more susceptible to HRSV infection. Also these patients are able to shed the virus for a longer period of time than the normal Infants [13]. In addition, the patients are usually under immunosupressing drug therapy which causes suppression of immunity accompanied by slow recovery and causes prolonged shedding of the virus from the respiratory tract [14].

The highest percentage of antibodies was detected in infants with pneumonia. Our result agreed with other study [5] who indicated that 70% of broncholitis associated with HRSV, but our results were higher than others [6] who found 42 - 45% and this may be due to difference in the virulence of the virus and many other stress factors.

Also we noticed differences in titer of anti HRSV antibodies depending on the age of the patients tested which was low in infants from 1-3 months but the titer increased when infants becomes older, this may be due to developing antibodies which received as low titer and also to low of transfer maternal antibodies from mother to infants through the placenta[15], also the blunted antibody response may be due to the relative antibody response or due to secondary relative immunologic immaturity of infants or due to suppressive effect of maternally transmitted transplacental antibody which cause a non responder for the RSV[16].

The titer of antibodies is significantly higher in infants with bronchiolitis infection than with pneumonia, these may depends on the disease condition as in acute or active phase than in advanced phase (pneumonia), these results agree with other studies[17].

From the accumulative total samples examined (184) serum samples the incidence of HRSV infection was 62% and high percentage was in infants with respiratory tract infection (79%) followed by cancer patients (69%).

The second part of our study was to detect RSV antigen in nasopharyngeal secretion using Respi-test and indirect ELISA. The result of present study is higher than that recorded[18], who detected 31% RSV by using Respi- test and 34% by using viral culture method, but in agreement with other[19], who detected HRSV in 41% of patients samples using light cycler RT-PCR in Australia, and with [8] who found 42% to 45% of tested samples by using RT-PCR in European children, but less than that identified[6] who recorded 70% in bronchiolitis patients hospitalized in U.S.A. by using RT-PCR, studied the infection rate in Huston state showed

that 68.8% of infected infants were less than 12 months and 82.6% during 1-2 years of their life[20].

The high detection rate of HRSV was in December which is one of the cold months in Iraq; this agreed with others [21] whom found that RSV is a primary infantile RSV infection and typically represented as a winter respiratory tract infection.

Further studies on HRSV in developing countries such as Indonesia [22] and South Africa [23] and Gambia [24] indicated that RSV is a seasonal prevalent agent and has most frequent occurrence at the coldest times of year. These studies are in agreement with our results.

Results show that 50% of Infant patients (20 out of 40) were positive for both HRSV antigens in the nasal/ throat secretion and antiHRSV antibodies in their sera. These results indicate that presence of humeral antibodies doesn't appear to play a role in resolution of viral infection and clinical recovery from RSV, with virus shedding continues from the upper respiratory tract. These results agreed with others which proved that humeral antibodies did not stop viral shedding from infected mucosa [25]. Similarly studies recorded that viral shedding was blocked in presence of specific secretary immunoglobulin (IgA) at time of clinical recovery [26]. Also the frequency of lower respiratory tract infection caused by RSV is significantly low among children with RSV-neutralizing antibodies and mainly when the titer of neutralizing antibodies lower than 100[27].

We recommended to isolate the RSV in infant and identified the strain and type present in Iraq which causes illness.

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