# **Results**

In this study, fourty eight patients; 18 males and 30 females were subjected to clinical and laboratory evaluation for the diagnosis of human metapneumovirus infection.

Table 1: Distribution of the study population according to different age groups.

Age (in months)	No.	%
1st 3 months	22	45.9
4-6 m.	4	8.3
7-9 m	11	22.9
10-12 m	5	10.4
Above 1year	6	12.5
TOTAL	48	100

The patients' age ranged from 1 month to 2 years with the mean age of 6.8 months. Patients below 3 months represent 45.9% (22/48) of the study population. The other age groups represent 54.1% (26 / 48) of the study population.
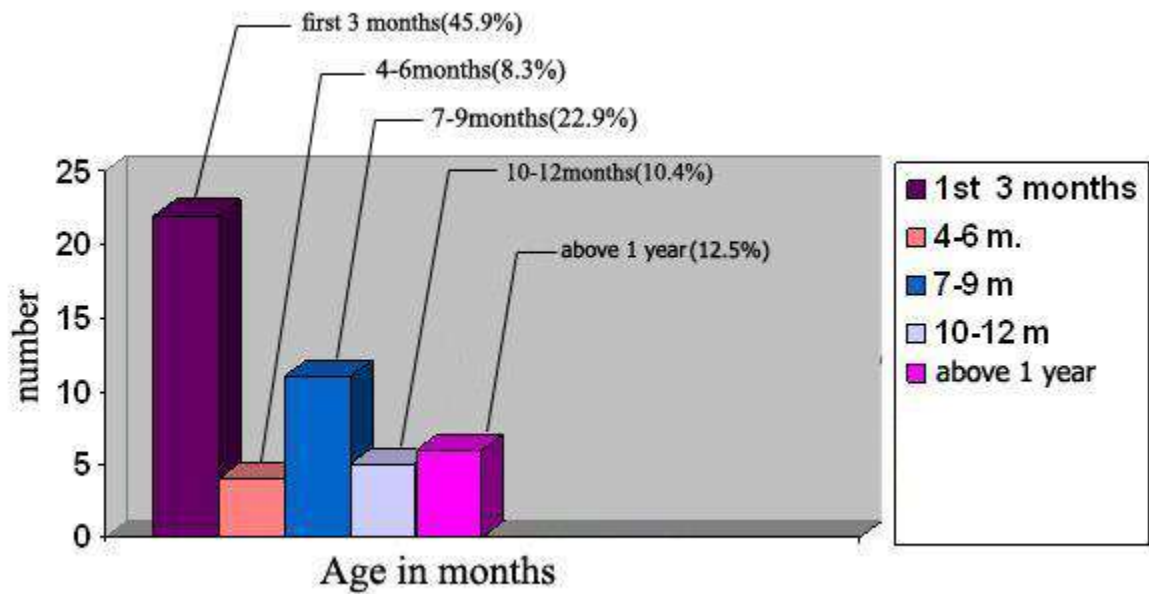
Figure (1): Distribution of patients into different age groups.

Table 2: Different clinical groups of the studied patients.

| clinical group	No.	%
Bronchiolitis	30	62.5
Bronchopneumonia	15	31.2
Acute bronchitis	2	4.2
Bronchial asthma	1	2.1
Total	48	100

Table 3: Human metapneumovirus infections in the study group as diagnosed by Duplex, real time RT-PCR.

Test	No. of examined cases					
	Positive		Negative		Total number	
	No.	%	No.	%	No.	%
Duplex, real time RT- PCR	12	25%	36	75%	48	100

Real time PCR could detect human metapneumovirus in 12 (25%) patients out of 48 pediatric patients.
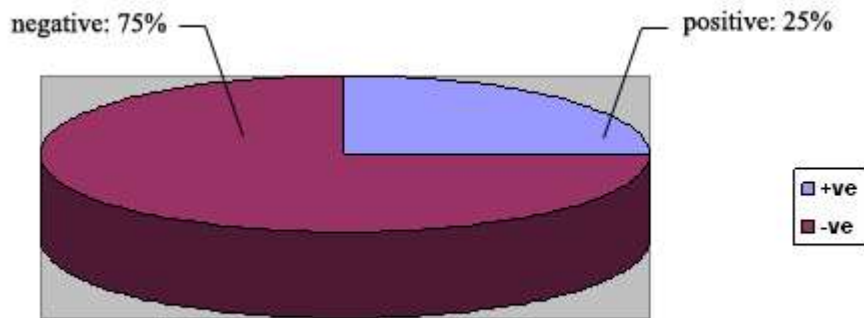
**Figure (2):** Human metapneumovirus infection in the study group as diagnosed by Duplex, real time RT- PCR.

Table 4: Results of direct immunofluorescence assay test.

Test	Positive cases		Negative cases		Total no. of patients	
DFA test	No.	%	No.	%	No.	%
	13	27.1%	35	72.9%	48	100%

From the above table it is shown that among fourty eight patients, a human metapneumovirus infection was detected in 27.1% (13/48) of patients by direct immunofluorescence assay test, whereas 72.9% (35/48) of patients were negative.
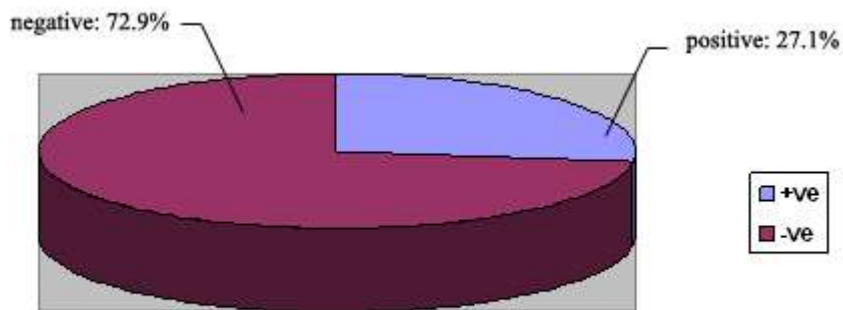
Figure (3): Results of direct immunofluorescence assay test for detection of hMPV in the study group.

table 5: Results of different methods used for diagnosis of hMPV infection.

Method of diagnosis		Duplex, real time RT- PCR		Total
		+ve	-ve	
DFA	+ve	12	1	13
	-ve	0	35	35
Total		12	36	48
Kappa		0.94		
Significance (P)		< 0.05		
Agreement		97.9		

Value of K	Strength of agreement	P>0.05 (non significant difference)
0.2	Poor	P<0.05 (significant difference)
0.21-0.4	Fair	P<0.01 (highly significant)
0.41-0.6	Moderate	
0.61-0.8	Good	
0.81-1.00	Very good	

(Campbell and Machine,1993)

Table (5) shows that in diagnosis of hMPV infection, 12 cases were positive for hMPV by both methods of identification (direct immunofluorescence assay test and Duplex, real time RT- PCR). There was a very good agreement between results of DFA and Duplex, RT-real time PCR (Kappa=0.94).

Table 6: Sensitivity, specificity, positive and negative predictive values of DFA taking Duplex real time RT- PCR as a reference method.

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value	False positive	False negative
DFA	100%	97.2%	92.3%	100%	1	0

Table (6) shows the diagnostic validity test done for DFA considering the Duplex, real time RT-PCR as a reference or standard method. As shown in the table the sensitivity of DFA was 100% while the specificity was 97.2%. There is one false positive result in the direct immunofluorescence assay test that may be due to fault technique (presence of scratch or dirt on the slide or contamination during specimen collection).
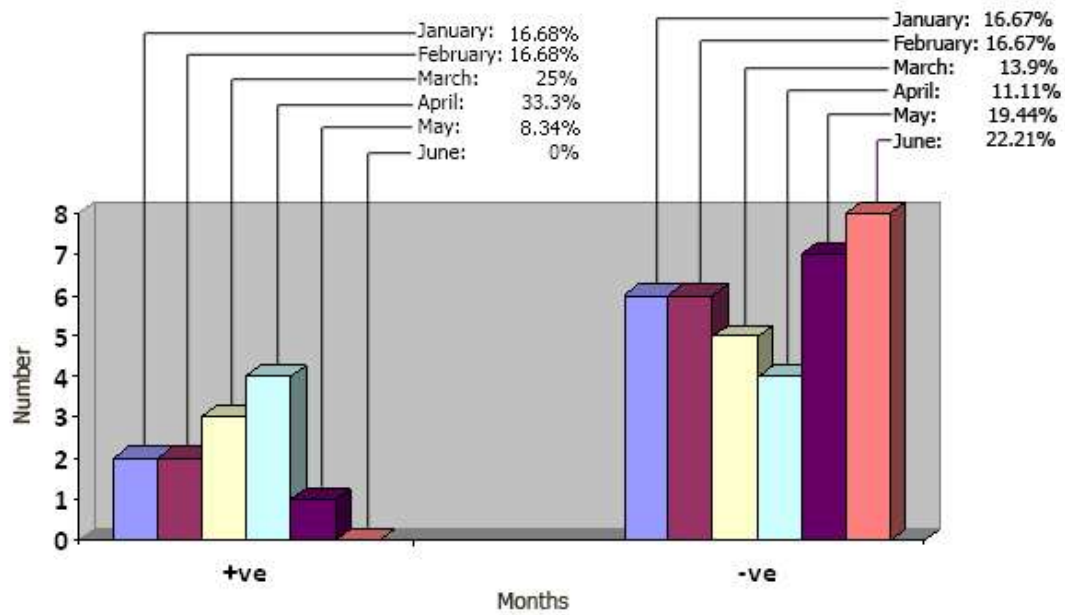
Table 7: Monthly distribution of the positive hMPV cases:

Month	No of positive cases	% of positive cases	No of negative cases	% of negative cases	Total No.
January	2	16.68%	6	16.67%	8
February	2	16.68%	6	16.67%	8
March	3	25%	5	13.9%	8
April	4	33.3%	4	11.11%	8
May	1	8.34%	7	19.44%	8
June	0	0%	8	22.21%	8
Total no. of examined cases	12	100%	36	100%	48
Chi- Square(X^2)	6.7				
Significance (P)	>0.05				

Table (7) shows the monthly distribution of positive hMPV samples. The peak of hMPV activity was during spring as 33.3% of positive cases were in April and 25% were in March but this is statistically insignificant (P>0.05).

Fig (4): Monthly distribution of hMPV infection of the studied patients.

Table 8: Time taken by each method to diagnose hMPV infection.

| Time taken by each method	Method for diagnosis of hMPV infection	
	DFA	RT-PCR
	4 hours	Less than 60 minutes after extraction procedures which took about 70 minutes.

It is noted from the above table that real time RT- PCR took less time than DFA method (2 hours including the automated extraction procedures using MagNA Pure Compact instrument).

Table 9: hMPV infection among the different studied clinical groups according to duplex real time RT-PCR results:

infection Clinical group	Positive cases		Negative cases		Total no. of cases per group		z	p
	No.	%	No.	%	No.	%		
Bronchiolitis	9	75	21	58.3	30	62.5	2.4	<0.05
Bronchopneumonia	3	25	12	33.3	15	31.2	1.04	>0.05
Acute bronchitis	0	0	2	5.7	2	4.2	0.6	>0.05
Bronchial asthma	0	0	1	2.7	1	2.1	1	>0.05
Total	12	100	36	100	48	100		

It is noted from the above table that 75% of hMPV positive cases occurred in the bronchiolitis group and this result is of significant statistical value (p <0.05). Twenty five percent of hMPV positive cases occurred in the bronchopneumonia group. No positive cases could be detected in the acute bronchitis and bronchial asthma patients groups.
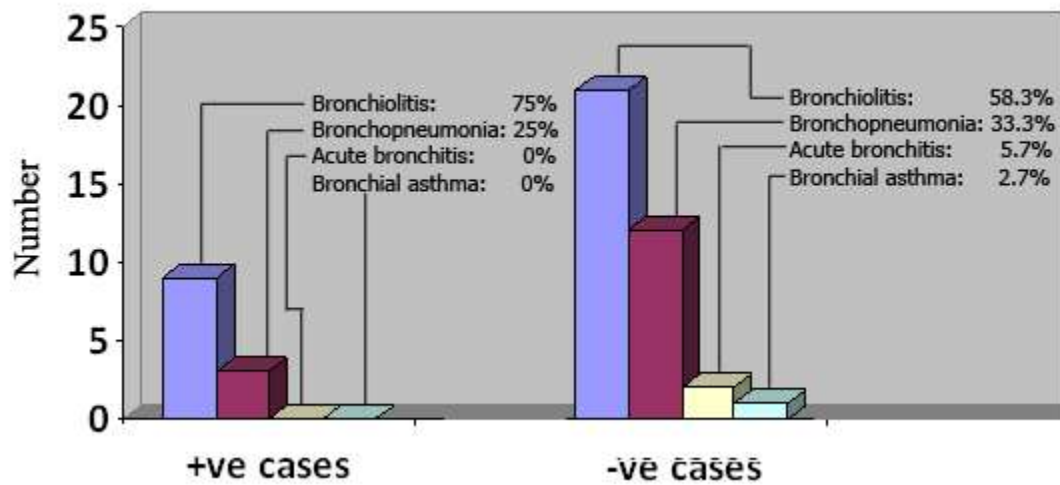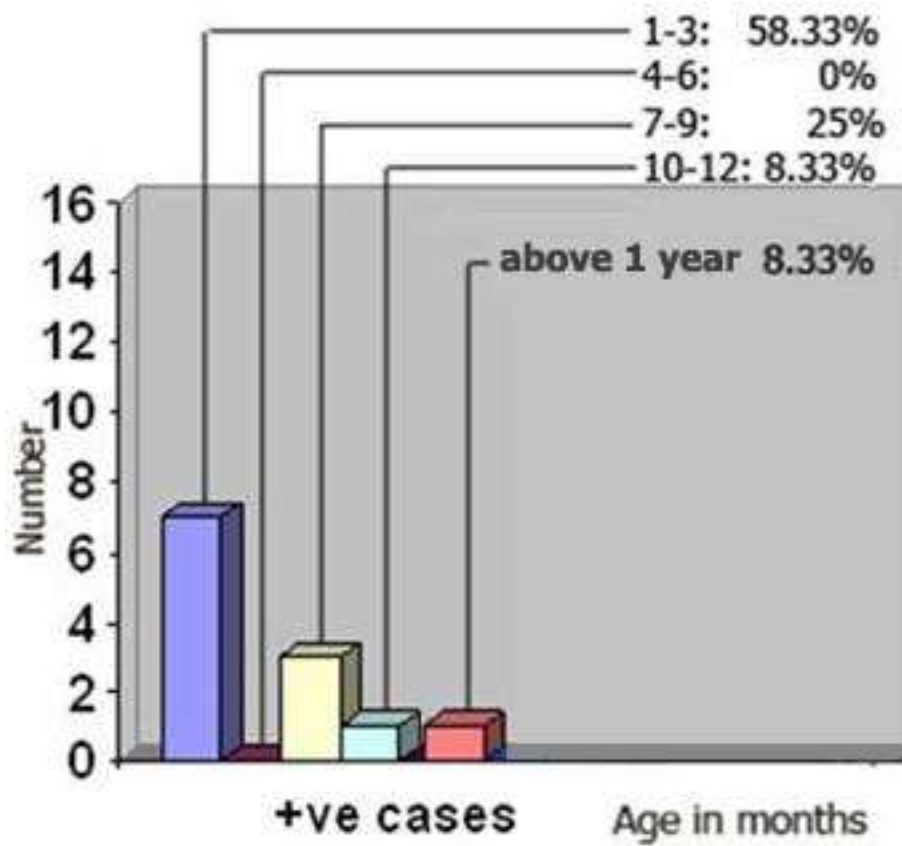
Fig (5): hMPV infection among the different studied clinical groups according to duplex, real time RT-PCR results.

Table 10: hMPV infection among the different age groups in the studied patients:

infection age in months	Positive cases		Negative cases		Total no. of cases per group		Z	P
	No.	%	No.	%	No.	%		
1-3	7	58.34	15	41.67	22	45.9	1.8	<0.05
4-6	0	0	4	11.11	4	8.3	0.7	>0.05
7-9	3	25	8	22.22	11	22.9	0.4	>0.05
10-12	1	8.33	4	11.11	5	10.4	0.5	>0.05
Above one year	1	8.33	5	13.89	6	12.5	0.5	>0.05
Total	12	100	3 6	100	48	100		

It is noted from the above table that 58.34% (7/12) of positive cases occurred in infants below three months of age and this of significant statistical value (<0.05). Twenty five percent (3/12) of positive cases occurred in infants whose ages ranged from seven to nine months. Only 8.33% (1/12) of positive cases occurred in patients aged from 10 to 12 months and in patients above one year of age also.

Fig (6): hMPV infection among the different age groups of the studied patients according to duplex, real time RT-PCR results.

Table 11: Relation between hMPV infection and sex of the patients according to RT-PCR results:

infection / sex	Positive cases		Negative cases		Total	
	No.	%	No.	%	No.	%
Male	6	50	12	33.33	18	37.5
Female	6	50	24	66.67	30	62.5
Total	12	100	36	100	48	100
Chi- Square (X^2)	0.47					
Significance (P)	>0.05					

Table (9) shows that 50% of hMPV positive cases occurred in females, 50% occur in males and there is insignificant statistical difference between hMPV infection and sex of patients (P>0.05).
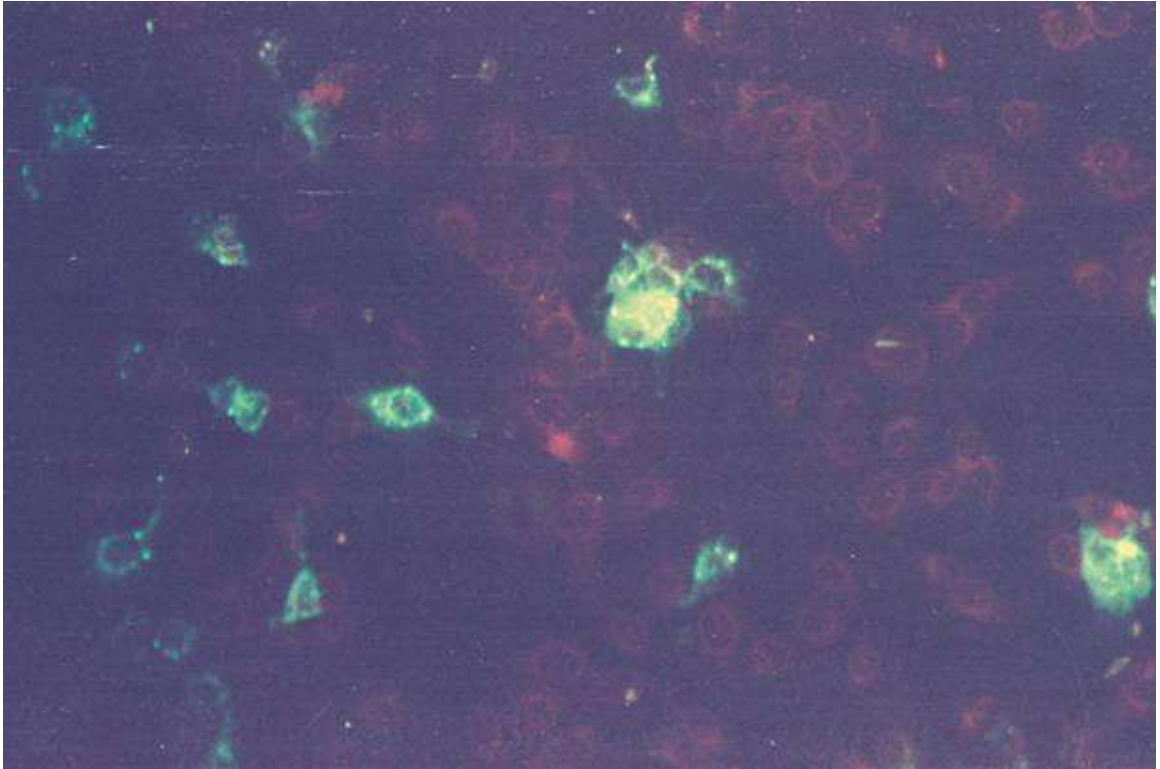
Figure (7): Direct Immunofluorescence Assay test positive for hMPV (400x) shows apple green fluorescent intracellular cytoplasmic granules and filaments seen in respiratory epithelial cells infected with hMPV. Uninfected cells stained red.
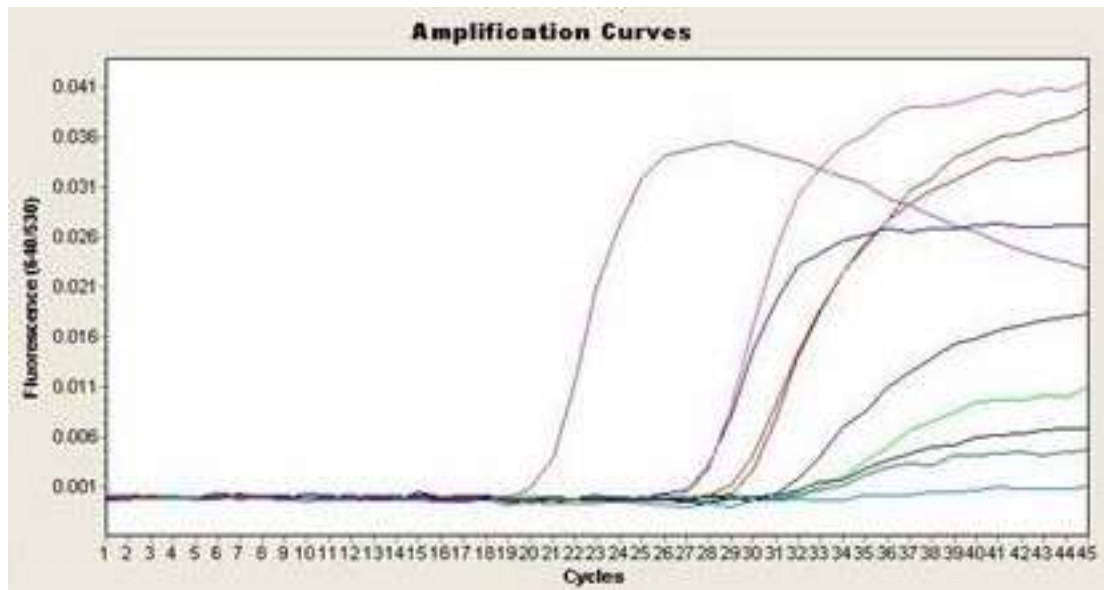
Figure (8): Direct Immunofluorescence Assay test negative for hMPV shows stained specimen did not exhibit fluorescence.
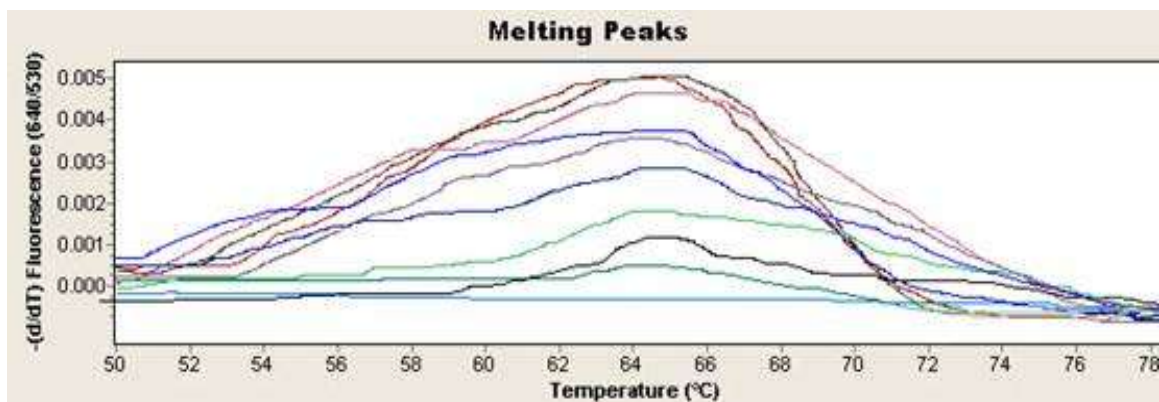
Figure (9): Melting temperature curve (Tm curve). Tm specific for hMPV was 65ºC.

Fig (10): Amplification curve of hMPV.