Detection of human *Metapneumovirus* and respiratory *Syncytial Virus* associated with asthmatic patients using direct fluorescent assay and Real time – PCR.

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الكشف عن فيروس ميتانيمو وفيروس التنفس المخلوي البشري لدى مرضى الربو بأستخدام فحص التألق المباشر وتقنية فحص الوقت الحقيقي – لتفاعل سلسلة البلمرة.

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المستخلص

هدفت الدراسة الحالية الى التحري نسبة حدوث فيروسي الجهاز التنفسي المخلوي وفيروس الميتانيمو المشخصة في مرضى الربو المتفاقم في محافظة واسط. تم اختبار عينات الدم لكل من مجموعتي المرضى والأصحاء لقياس عدد كريات الدم البيضاء الكلي والتفريقي، إذ أظهرت النتائج وجود فروق معنوية في عدد الخلايا البيضاء بين مجموعة المرضى ومجموعة الأصحاء. فضلا عن ذلك ظهرت فروق معنوية في كل من خلايا الدم البيضاء وهي العدلات واللمفاويات والحمضات والخلايا القاعدية بين مجموعتي الدراسة المختلفة.باستخدام فحص الامتزاز المناعي المرتبط بالإنزيم (الاليزا) على مصل الدم لتقدير مستوى الأجسام المضادة نوع هـ، أظهرت وجود فروق معنوية بين نتائج مجاميع المرضى مقارنتا بمجاميع الأصحاء. جمعت 80 مسحة مرضية من الأنف والحنجرة ووضعت على الفور في الوسط الناقل الخاص بالفيروسات وخزنت لحين فحصها باختبار التألق المباشر للكشف عن فيروس الجهاز التنفسي المخلوي وفيروس الميتانيمو ووجد ان نسبة الإصابة بفيروس الجهاز التنفسي المخلوي هي 16.25%، بينما كانت نسبة الإصابة بفيروس الميتانيمو هي 13.75%. عند استخدام فحص تفاعل سلسلة البلمرة بالوقت الحقيقي وجد ان الإصابة بفيروس الجهاز التنفسي المخلوي كانت 15 مريضا (18.75%).

Abstract

This study aimed to determine the incidence of *human respiratory syncytial virus* (hRSV) and *metapneumovirus* (hMPV) occurrence in asthmatic patients in Wasit province. Blood samples were collected for measure the total and differential white blood cells count (WBCs). Correlation results of total and differential WBCs count for neutrophils, lymphocytes, eosinophils and basophils count among studied groups were significant ($P < 0.001$). Enzyme linked immunosorbent assay (ELISA) technique has been applied for detection of total-IgE antibodies. Results revealed that the highest total-IgE antibodies titer in sera were significantly difference ($p < 0.01$). A total of 80 nasopharyngeal swabs

were immediately dipped in transport media and stored until using for the detection of suspected hRSV and hMPV patients group by direct fluorescent assay the results appeared 13 (16.25 %) and 11 (13.75 %) samples were given positive results for hRSV and hMPV, respectively. Results of hRSV in asthmatic patients were subjected to real time – polymerase chain reaction (RT-PCR) appeared 15 samples out of 80 samples (18.75 %) were gave positive result for this test.

Introduction

Asthma exacerbation have been shown to be a major cause of morbidity and mortality and up to 80% of the exacerbations are linked to viral infections (1). Asthma is characterized by acute episodes of airway obstruction precipitated by respiratory infection and the release of IgE depended mediators, airway inflammation resulting from an inappropriate response to either infectious or allergic antigens is a finding common to the different manifestations of asthma. Evidence for the importance of viral respiratory infections in the development of asthma comes from studies indicating that sever paramyxoviral infections early in life impart a markedly increased risk for asthma later in childhood (2).

Asthmatics with viral infection but no sensitization show lower rates of hospital admission (3). This effect is due to synergism between allergens and viruses. When RSV infects bronchial cells, the bronchial cells produce various cytokines and chemokines. These responses cause hyperresponsiveness in bronchial cells. In other words, RSV infection might create a preparatory step as the first step in the development of asthma (4). RSV is a paramyxovirus that infects nearly all children by the age of 2 years (5). Although most of these infections have no known sequelae, infants requiring hospitalization for severe RSV infection in the first 6 months of life have a nearly 8-fold increase risk of developing asthma (6).

Respiratory viruses are detected in the majority of asthma exacerbations in both children 80 – 85% and adults 75 – 80% (7). Co-infection with other virus such as *metapneumovirus* may be important (8).

hMPV a respiratory virus was first identified in Netherland, and soon after it was recognized as a new member of Metapneumovirus genus based on virological data, nucleotide sequence homology and gene constellation (9). hMPV infection results in a large number of hospitalization with substantial morbidity, resource utilization, and coast (10).

The virus had been overlooked previously because the growth of clinical isolates *In vitro* is slow, has a delayed cytopathic effect and requires added trypsin (11). The viral genome of hMPV is similar to that of *respiratory syncytial virus* (12). and children who are infected with hMPV have clinical features similar to those

infections caused by RSV ranging from acute upper respiratory tract infections to sever acute lower respiratory tract infections like bronchiolitis and pneumonia (13).
The aim of this study is to determine the incidence of *human Respiratory syncytial virus* and *human Metapneumovirus* diagnostic from nasopharyngeal in asthmatic patients in the Wasit Province, Iraq**.**

Materials and methods

Study Subjects

This study included two groups of subjects:

A- A total of 80 specimens were collected from patients suffering from exacerbation asthma who were admitted to Al-Karrama'a Teaching Hospital and Al-Zahra'a Teaching Hospital in Wasit Province / Iraq during the period from January 2013 to May 2013. The patients' age were ranged from 1 – 15 years. Two specimens were taken from each patient, as following nasopharyngeal swab and 3 – 5 ml of freshly drawn venous blood.

Nasopharyngeal swabs were taking by inserting a dry calcium alginate, aluminum-shafted swab into the nasopharyngeal area. The swab was allowed to remain in the area for 10 – 30 seconds, then rotated and withdrawn. One swab from each patient was placed in 3 ml transport medium, vircell, and the second swab put in 2 ml transport medium, vircell, then together put in an ice bag until be taken to the laboratory for real time PCR and fluorescent assay respectively, then they were stored at -80 $^{\circ}$C for other time.

Venous blood sample 3 ml, was drown from each patients. Blood samples were divided into 2 tubes; the first, EDTA tube with 1 ml were used for measuring total and differential white blood cells, the second, gel tube with 2 ml which was left to clot and separated the serum by centrifugation at 3000 r.p.m. (14) for 10 minutes, after that, sera samples were carefully transferred to eppendorff tubes and store at –20°C until use.

B- Twenty specimens (nasopharyngeal swabs and blood) were collected from apparently healthy control group, who had no history of asthma.

- **Statistical Analysis**

All results were analyzed by statistical tests. Normally, distributed data were expressed as mean ± SD. Difference between the groups examined using the t-test and a p-value of ≤ 0.05 was taken as statistically significant.

Results and discussion

Distribution of asthma according to Age and Gender

The demographical distribution of the studied groups according to the age (Table 1). The results clarified that the age was ranged between < 1 – 15 years and the mean ± SE for asthmatic patients was 4.768 ± 3.180.

Table (1): Distribution of patients according to the age.

Age (year)	Patients group No. (%)	Control group No. (%)
5≥	62 (77.5%)	14 (70%)
6 – 10	13 (16.25%)	4 (20%)
11 – 15	5 (6.25%)	2 (10%)
Total	**80**	**20**

There were differences between the numbers of patients in varying year's months showed that highest frequency were during February and January 21 and 19 out of 80 patients 26.25 and 23.75 %, respectively (Table 2). May month had less number, this because of increase in the prevalence of respiratory disorder that induce asthma exacerbation in winter months than in other months or season like July (15). The result was matched with recorded by in Iraq, (16) who mentioned that ratio was higher in January and February in Iraq and in USA (17) who mentioned at winter, the virus peak had an increased risk of bronchiolitis in infancy and of asthma during childhood.

Table (2): Monthly distribution of asthma cases and control group

Month	Patients group No. (%)	Control group No. (%)
January	19 (23.75 %)	4 (20%)
February	21 (26.25 %)	4 (20%)
March	16 (20 %)	4 (20%)
April	13 (16.25 %)	4 (20%)
May	11 (13.75 %)	4 (20%)
Total	**80**	**20**

Analysis of white blood cells total and differentials rate

The mean level of white blood cell count was 10.659 ± 2.339 and 6.915 ± 0.831 in asthmatic patients and control groups respectively (Table 3). This study showed that there was a significant difference in the total WBCs among different study groups using t-test ($P<0.001$). The present results were almost similar to those obtained by Darwesh, (18) and Bicer *et al.,* (19).While there was a significant by using t-test ($P<0.001$) correlation in neutrophils, lymphocytes, eosinophils and basophils counts among different studied groups. The role of viral infection in developing acute

exacerbation of asthma is continuing to be defined. Subjects with asthma group had significantly increased. The present results were almost similar to those obtained by Darwesh, (18) and Al-Watify and Al-Joubori, (20).The increase lymphocytes in peripheral blood of patients refer to the role of these cells in viral and allergic infection. It is well documented that lymphocytes are important part in the defense against viral infection. This had been documented by many researchers like Itazawa *et al.* (21). Eosinophils and Basophils play a major role in allergic reactions. It contains a high affinity receptor, FcεR1 and are capable of an immediate response to allergen (22). Caughey *et al.* said that the basophils increase in asthma patients (23). Monocytes there were a non - significant correlation between asthmatic patients and control groups. The present results were almost similar to those obtained by Alaa and Thanaa, (24).

Table (3): The total and differential white blood cells count of asthmatic patients and control group

Parameter mean ± SD	patients group	Control group
WBCs x 10^3 cell/mm^3	10.659 ± 2.339 **A**	6.915 ± 0.831 **B**
NEU x 10^3 cell/mm^3	5.985 ± 1.277 **A**	4.431 ± 0.735 **B**
LYM x 10^3 cell/mm^3	3.064 ± 0.556 **A**	1.877 ± 0.213 **B**
MONO x 10^3 cell/mm^3	0.511 ± 0.432 **A**	0.46 ± 0.169 **A**
EOS x 10^3 cell/mm^3	1.008 ± 0.381 **A**	0.102 ± 0.094 **B**
BAS x 10^3 cell/mm^3	0.222 ± 0.163 **A**	0.044 ± 0.013 **B**
*** The same letter in one row means that there is no significant difference between these value**		

Analysis of total IgE

The IgE concentration are significantly (P<0.001) in asthmatic patients group have an expected IgE concentration 32.113 ± 6.676, 46.733 ± 19.474 and 90.484 ± 22.162 compared with control group 8.724 ± 9.957, 15.847 ± 8.423 and 14.472 ± 5.570 respectively, the distribution of IgE concentration was according to the age group (Table 4).These results are agreed with the study of Tavakkol *et al.*, who found a high significant differences in concentrations of IgE in patients compared with healthy individuals (25). Satwani *et al.*, (26) showed eosinophilia along with raised serum IgE level which consider a significant allergic marker. Several studies have reports of elevated of total serum IgE in asthmatic patients (18, 27 and 28). Therefore, it is in accordence with the well known fact that IgE plays a central role in the pathophysiology of allergic disorder such as asthma.

Table (4): Concentration of IgE level for asthmatic patients and controls.

Age group (years)	Group	IgE level IU/ml
< 1 – 2	Asthmatic patients	32.113 ± 6.676 **A**
	Control	8.724 ± 9.957 **B**
3 – 5	Asthmatic patients	46.733 ± 19.474 **A**
	Control	15.847 ± 8.423 **B**
6 – 15	Asthmatic patients	90.484 ± 22.162 **A**
	Control	14.472 ± 5.570 **B**
*** The same letter in one age group means that there is no significant difference between these value**		

Detection of *human respiratory syncytial virus* and *Metapneumovirus* by direct fluorescent test

Virus identified by specific, fluorescent monoclonal antibodies in respiratory specimens, this was along with a diagnostic procedure that used in clinical virology laboratories (29).

A total of 80 nasopharyngeal swabs of suspected hRSV and hMPV patients group were used in direct fluorescent test, 13 (16.25 %) and 11 (13.75 %) samples appeared positive results for hRSV and hMPV respectively, on the other hand three positive cases were infected with hRSV and hMPV (Table 5).

Table (5): Diagnosis of hRSV and hMPV by direct fluorescent assay.

		HRSV		HMPV		HRSV & HMPV from positive cases
Group	**Number of cases**	**positive cases**	**Negative cases**	**positive cases**	**Negative cases**	
Asthmatic patients	80	13 (16.25%)	67 (83.75%)	11 (13.75%)	69 (86.25%)	3
Control	20	0 (0%)	20 (100%)	0 (0%)	20 (100%)	0

The results appeared under fluorescent microscope by examining different fields of each slid at a magnification of 200X interpretation of hRSV and hMPV for this test. hRSV according to D^3 FastPoint L-DFA RSV/MPV infected cells observed the golden–yellow fluorescence (R-phycoerythin (PE)) dye is cytoplasmic. While hMPV infected cells observed the apple–green

fluorescence (fluorescein isothiocyanate (FITC)) dye is cytoplasmic (Figure 1 and 2). Mix infected by RSV/MPV infected cells observed two dyes (Figure 3) and negative samples, cells did not observe two dye (Figure 4).

This study is the first report that describe hMPV in Wasit province, Iraq. The results of hMPV have agreement with a study carried out in Hilla, that refered to the rate infection 13.3% (30). Also Esper *et al.*, (31) pointed the hMPV infection about 8.1%. The results agreed with Aziz *et al.*, (32), who found 23% hospitalized children suffered from Bronchiolitis in Suleimania city. In the present study, the hRSV result refers to the highest infection rate among patients who have asthma from hMPV. This is according to the relationship between hRSV infection and formation of exacerbation asthma which induced by the virus (33). The present results were almost similar to those obtained by Al-Marzoqi *et al.*, (34) who mentioned that ratio was 19% . The prevalence of RSV in this study is relative low compared with data reported in Baghdad by Odisho *et al.* (35), who reported that the percentage is reached to 79% among the children who have respiratory tract infection. In Kuwait, Khadadah *et al.* (36) reports that the percentage of infection is 36.8% in hospitalized children who have respiratory tract infection. On the other hand, the studies indicate that hMPV may cause upper or lower respiratory tract illness in patients between age 2 months and 87 years, it may co-circulate with RSV, and HMPV infection may be associated with asthma exacerbation (37).

Mixed viral infections hRSV and hMPV were found in 3 cases from positive cases of hRSV and hMP. Since the circulation of hMPV may overlap with hRSV, simultaneous infection with both RSV and hMPV may contribute to sever disease (38). The co-infection was found by Esaa *et al.* (39) ; and Toivonen *et al.*(40) who detected the co-infection of hRSV and hMPV that 11.2% and 2.8%; and 13% and 5% , respectively.

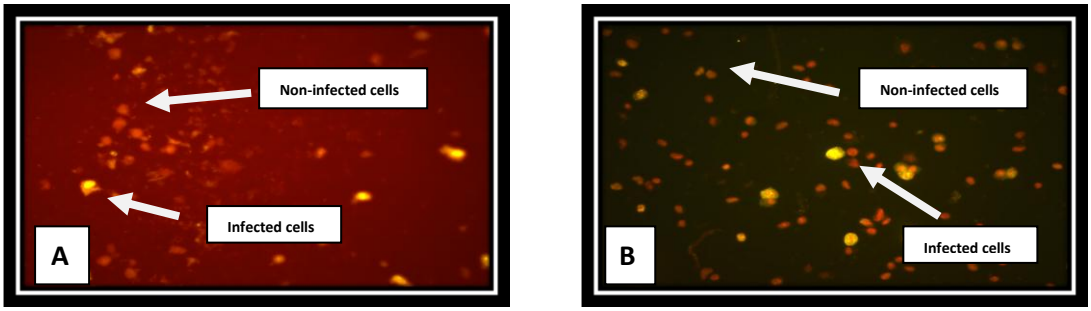
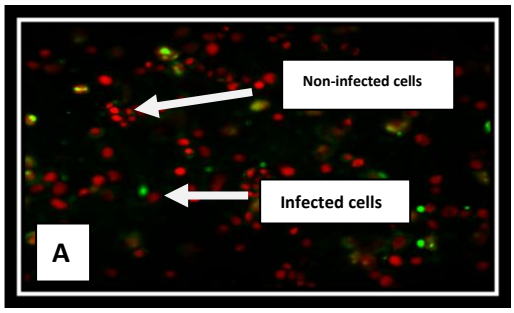


Figure (1): hRSV according to D[3] FastPoint L-DFA RSV/MPV infected cells observed golden-yellow fluorescence (R-phycoerythin (PE)) dye A. cytoplasmic positive control and B. positive sample 200X magnification.



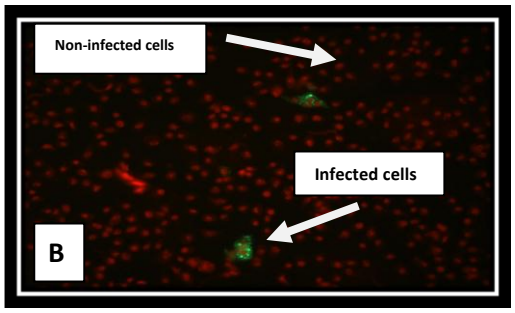


Figure (2): hMPV according to D^3 FastPoint L-DFA RSV/MPV infected cells observed apple – green fluorescence (fluorescein isothiocyanate (FITC)) dye A. cytoplasmic positive control and B. positive sample 200X magnification.

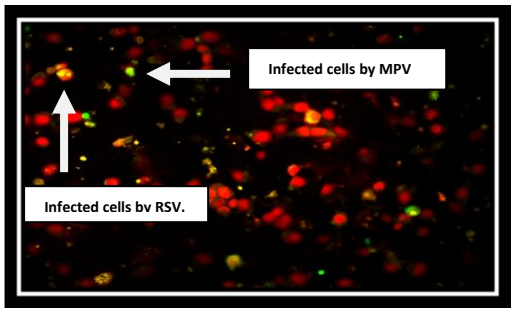


Figure (3): hRSV and hMPV according to D^3 FastPoint L-DFA RSV/MPV infected cells observed cytoplasmic mix dyes is 200X magnification.

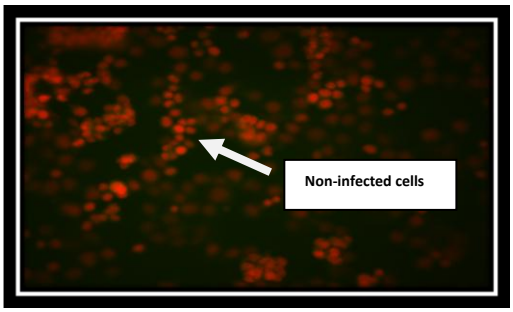


Figure (4): Negative sample appears non infected cells that did not observe cytoplasmic fluorescent dyes is 200X magnification.

Detection of *Respiratory syncytial virus* by Real Time – PCR

A total of 80 nasopharyngeal swabs of suspected *human respiratory syncytial virus* infection of asthmatic patients were tested by real time – polymerase chain reaction, (RT-PCR), 15 samples (18.75 %) give positive result (Table 6).

Table (6): Diagnosis of hRSV by Real time PCR

		HRSV	
Group	**Number of cases**	**positive cases**	**Negative cases**
Asthmatic patients	**80**	**15 (18.75%)**	**65 (81.25%)**
Control	**20**	**0 (0%)**	**20 (100%)**

Many respiratory infections caused by bacteria or viruses that often simillar clinical features and symptoms which are difficult to distinguish clinically (41). Therefore, detection of such agents require to sensitive and effective method to give correct treatment and avoiding an unnecessary use of antibiotics. RT-PCR has been shown to be a better test to diagnosis than conventional assays and real-time PCR significantly reduces time to give results and has an advantage than conventional PCR as detection is dose in closed system in real time and minimum risk of contamination (42).

In the present study, a RT-PCR assay was performed along with Direct fluorescent assay (DFA). RT-PCR assay was developed to detect hRSV in 80 patients. Using both RT-PCR and DFA, overall 19 of 80 (23.75) (Table 7). However, 6 positive cases by RT-PCR appeared negative by DFA. Most of patients were having exacerbation asthma. Only 9 positive cases in RT-PCR out of 13 positive cases in AFD. This study provides comparative sensitivity values of RT-PCR versus AFD for *respiratory syncytial virus* (Table 8).

Table (7): Conformation of hRSV by RT-PCR.

Number of examination cases by DFA			RT-PCR Positive	%
DFA	**Positive**	13	9	69.23
DFA	**Negative**	67	6	8.05
Total		80	15	18.75

Table (8): Sensitivity and specificity of RT-PCR and DFA in detection of hRSV.

Test		DFA		Total
		Positive	**Negative**	
RT-PCR	**positive**	9	6	15
	Negative	4	61	65
Total		13	67	

Sensitivity = $true\ positive \div (true\ positive + false\ negative) \times 100$.

The sensitivity of RT-PCR vs DFA is 69.23 %.

Specificity = $true\ negative \div (true\ negative + false\ positive) \times 100$.

The specificity of RT-PCR vs DFA is 60.33 %.

The sensitivity of rapid RSV antigen testing in asthmatic patients have been as high as 69.23 % that used of RT-PCR has allowed large epidemiologic studies to well-known pathogens such as RSV (43). If a nonimmunocompromised child have RSV-positive by RT-PCR, it means that the child is acutely infected with RSV, has been ill recently with RSV, or they become ill with RSV. Almost all children have negative results by RT-PCR after 12 – 21 days; but occasionally a child will remain positive for up to 4 weeks. During these longer periods of shedding that detected by RT-PCR, the change increases by another undetected viral infection which may be present, especially among young children have frequent viral infections during the respiratory disorder season. Because of the small amount of viral antigen that usually present in nasopharngeal aspirates collected from RSV-infected, current antigen detection assay may have low sufficient sensitivity to detect and diagnose RSV (44). The results of RT-PCR were agreed with Jartti *et al.* (45) and Sung *et al.* (46) who had reported that percentage of infection 18% and 8.4% respectively. Brice *et al.*, (19) concluded that the RSV infection in RT-PCR 32%. The present results almost similar to those obtained by Shameran and Al-Mola, (47); and Ali *et al.* (48), in Iraq when who found the RSV infection of 24% and 20%, respectively.

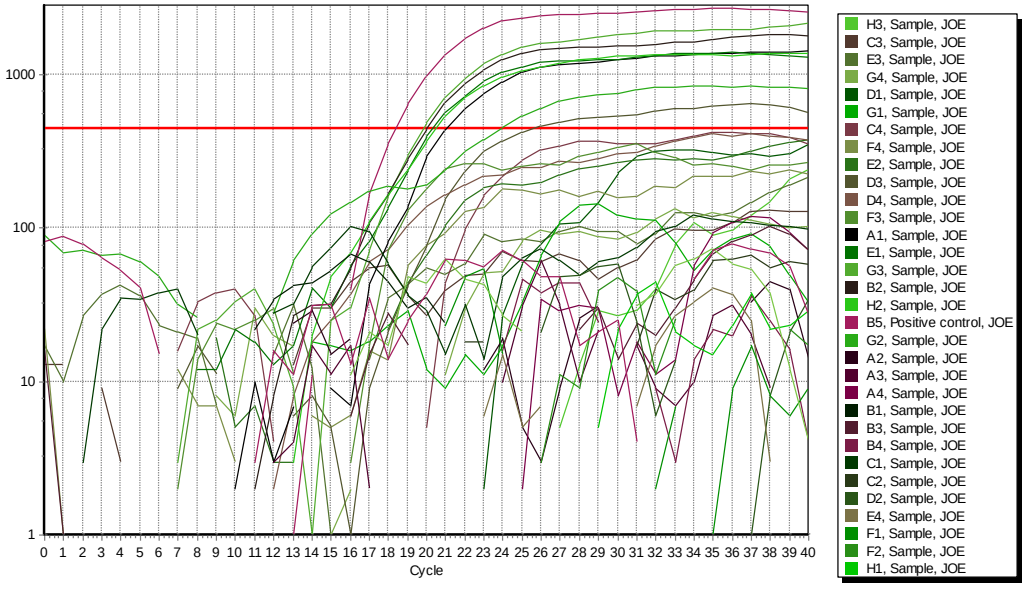


Figure (5): Real-Time PCR amplification log plot of *Human respiratory Syncytial virus* (HRSV) from nasopharyngeal swabs samples. where, the positive control was appeared at (19.08 CT: Threshold cycle number), Internal positive control which appeared at (20.84 CT: Threshold cycle number), and the tested samples was positive reaction at (21.04 to 26.95 CT: Threshold cycle number), whereas,

the negative control samples were not amplification under Threshold cycle number.

References

1- Schwantes, E. A.; Denlinger, L. C.; Evans, M. D.; Gern, J. E.; Jarjour, N. N. and Mathur, S. K. (2015). Severity of virus – induced asthma symptoms is inversely related to resolution IFN-λ expression. J. Allergy Clin. Immunol.; 135 (6): 1656-1658.

2- Murphy, D. M. and O'Byrne, P. M. (2010). Recent advances in the pathophysiology of asthma. CHEST.; 137: 1417 – 1426.

3- Green, R. H.; Brightling, C. E.; Woltmann, G.; Parker, D.; Wardlaw, A. J. and Pavord, I. D. (2002). Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum netrophilia and poor response to inhaled corticosteroids. Thorax.; 57 (10): 875 – 879.

4-Tukagoshi, H.; Ishioka, T.; Noda, M.; Kozawa, K. and Kimura, H. (2013). Molecular epidemiology of respiratory viruses in virus – induced asthma. Front. Microbiol.; 4: 278.

5-Meissner, H. C. (2005). Selected population at increase risk from *respiratory syncytial virus* infection. Pediatr. Infect. Dis. J.; 22: S40 – 44.

6-Sigurs, N.; Bjarnason, R.; Sigurbergsson, F. and Kjellman B. (2000). *Respiratory syncytial virus* bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am. J. Respir. Crit. Care. Med.; 161 (5): 1501 – 1607.

7-Grissell, T. V.; Powell, H.; Shafren, D. R.; Boyle, M. J.; Hensley, M. J.; Jones P. D.; Whitehead B. F. and Gibson, P. G. (2005). Interleukin-10 gene expression in acute virus-induced asthma. Am. J. Respir. Crit. Care. Med.; 172: 433 – 439.

8-Isaacs, D. and Joshi, P. (2002). Respiratory infections and asthma. M. J. A.; 117: S50 – S51.

9-Van den Hoogen, B. G.; Bestebroer, T. M.; Osterhaus, A. D. M. E. and Fouchier, R. A. M. (2002). Analysis of the genomic sequence of human *Metapneumovirus*. Virol.; 295: 119 – 132.

10-Davis, C. R.; Stockmann; C.; Pavia, A. T.; Byington, C. L.; Blaschkel, A. J.; Hersh, A. L.; Thorell, E. A.; Korgenskil, K.; Daly, J. and Ampofol K. (2015). Incidence, Morbidity, and Costs of *Humane Metapneumovirus* Infection in Hospitalized Children. J. Ped. Infect. Dis.; 4 (2): 27 – 35.

11- Stephane, B.; Quynh, N. P.; Mario, H. S.; Brian, R. M.; Peter L. C. and Ursula, J. B. (2006). Modification of the trypsin – dependent cleavage activation site of the *human metapneumovirus* fusion protein to be trypsin independent does not increase replication or spread in rodents or nonhuman primates. Virol. J.; 80: 5798 – 5806.

12-Boivin G.; De Serres, G.; Côtė S.; Gilca, R.; Abed Y.; Rochette L.; Bergeron, M. G. and Dėry, P. (2003). *Human metapneumovirus* infections in hospitalized children. Emerg. Infect. Dis.; 9: 634 – 640.

13-Heikkinen, T.; Osterback, R.; Peltola, V. and Vainionpää, R. (2008). *Human metapneumovirus* infections in children. Emerg. Infect. Dis.; 14: 101 – 106.

14-Bishop, M. C.; Dben – von Laufer, J. C.; Fody, E. P. and thirty three contributers. (1985). Clinical chemistry principles, procedures, and correlations. The Murray Printing Company, Philadelphia, USA: 181 – 182.

15-Malmstrom, k.; Pitkaranta, A.; Carpen, O.; Pelkonen, A.; Malmberg, L. P.; Turpeinen, M.; Kajsaari, M.; Sarna, S.; Lindahl, H.; Heahtela, T. and Makela, M. J. (2006). *Human rhinovirus* in bronchial epithelium of infants with recurrent respiratory symptoms. J. Allergy Clin. Immunol.; 118: 591 – 596.

16-Al – Shami J. A. J. and Al – obaidi, N. (2009). Review of wheezing in children in maternity and children teaching hospital in Al – Diwaniyah / Iraq. Babylon Med. J.; 6 (3 – 4): 477 – 483.

17-Wu, P.; Dupont, W. D.; Griffin, M. R.; Carroll, K. N.; Mitchel, E. F.; Gebretsadik. T. and Hartert, T. V. (2008). Evidence of a causal role of winter virus infection during infancy in early children asthma. Am. J. Respir. Crit. Care Med.; 178: 1123 – 1129.

18-Darwesh, M. F. (2011). Immunological aspects on asthmatic patients. Collage of Science, Kufa university, Iraq: 1 – 6.

19-Bicer, S.; Giray, t.; Col, D.; Erdag, G. C.; Vitrinel, A.; Gurol, Y.; Celik, G.; Kaspar, C. and Kucuk, O. (2013). Virological and clinical characterization of respiratory infections in hospitalized children. Italian J. Pediatr.; 39(22): 2 – 10.

20-Al – watify, D. G. O. and Al – Joubori, S. J. H. (2014). Inhibitory effects of synthetic glucocorticoids drugs on hypothalamus – pituitary – adrenal gonads axes and differential white blood cells in asthmatic patients. Babylon Uni. J. Pure Appl. Sci.; 6 (22): 1786 – 1793.

21-Itazawa, T.; Adachi, Y.; Imamura, H.; Okabe, Y.; Yamamoto, J.; Onoue, Y.; Adachi, Y. S.; Miyawaki, T. and Murakami, G. (2001). Increased lymphoid MxA expression in acute asthma exacerbation in children. Allergy.; 56: 895 – 898.

22-Wong, C. K.; Lp, W. and Lom, C. W. K. (2004). Biochemical assessment of intracellular signal transduction pathways in eosinophils: implication for pharmacotherapy. Clin. Labo. Scie.; 41 (1): 79 – 113.

23-Caughey, G.; Xiang, J.; Walter, N. and Paul, J. (2007). White blood cells in lung produce histamine see in allergies. Exper. Med. J.; 89: 222 – 227.

24-Alaa, J. H. and Thanaa A. M. (2013). Immunological study of patients with asthma. Babylon J. Uni. Pure Appl. Sci.; 7 (21): 2400 – 2407.

25-Tavakkol, J. A.; Hosseini, R. F.; Farahabadi, S. H.; Heydarian, F.; Boskabady, M. H.; Khoshnavaza,

R.; Razavi, A.; Karimiani, E. G. and Ghasemi, G. (2007). Association of the expression of IL-4 and IL-13 genes, IL-4 and IgE serum levels with allergic asthma. Iran J. Allergy Asth. Immunol.; 6 (2): 67 – 72.

26-Satwani, H.; Rehman A.; Ashraf, S. and Hassan, A. (2009). Is serum total IgE levels a good predictor of allergies in children. J Pak. Med. Assoc.; 59 (10).

27-Raesan, S. J. (2014). Association of HLA DQA1 and HLA DRB1 Alleles with asthma patients in Basra city. Basrah J. Res. Sci.; 40 (2): 129 – 137.

28-Almaamory, I. A. S. (2015). Study specific Immunoglobulin E, G antibodies and bacterial which induced asthmatics. J. Nat. Sci. Rec.; 5 (7): 21 – 25.

29-Manoha, C.; Bour, J. B.; Pitoiset, C.; Darniont, M.; Aho, S. and Pothier, P. (2008). Rapid and sensitive detection of *metapneumovirus* in clinical specimens by indirect fluorescence assay using a monoclonal antibody. J. Med. Virol.; 80: 154 – 158.

30-Al – Mola, G. A.; Ragheb, A. and Abass, I. R. (2013). *Human metapneumovirus* (hMPV) associated with respiratory infection in children hospitalized with acute lower respiratory tract infection in Hilla, Iraq. Int. J. Dis. Dis.; 1 (2): 20 – 23.

31-Esper, F.; Martinello, R.A.; Boucher, D.; Weibel, C.; Ferguson, D.; Landry, M. L. and Kahn, J. S. (2004). A 1-year experience with *human metapneumovirus* in children aged < 5 years. J. Infect. Dis.; 189: 1388 – 1396.

32-Aziz, T. A. G.; Salmo, N. and Bayati, A. H. (2014). Seroprevalence of anti – *human metapneumovirus* antibodies in hospitalized children in Suleimani city/Iraq. British Microbiol. Res. J.; 4 (12): 1325 – 1334.

33-Schaller, M.; Hogaboam, C. M.; Lukacs, N. and Kunkel, S. L. (2006). Respiratory viral infections drive chemokine expression and exacerbate the asthmatic response. J. Allergy. Clin. Immunol.; 118 (2): 295 – 302.

34-Al – Marzoqi, A H.; Al – Janabi, D. K. F.; Sh emmran, A. R. and Al – Taee, Z. M. (2013). Etiology of respiratory tract infections using physiological markers patterns for diagnosis in Hillah patients. Al-Qadisiyah J. Pure sci.; 15 (3): 1 – 8.

35-Odisho, S. M.; Al – Bana, A. S. and Yaassen, N.Y. (2009). Detection of *Respiratory syncytial virus* infection in a sample of infants in Iraq. Iraqi J. Med. Sci.; 7 (4): 11 – 19.

36- Khadadah, M.; Essa, S.; Higazi, Z.; Behbehani, N. and Al – Nakib, W. (2010). *Respiratory syncytial virus* and *human rhinovirus* are the major causes of severe lower respiratory tract infections in Kuwait. J. Med. Virol.; 82: 1462 – 1467.

37-Mahalingam,S. Schwarze, J.; Zaid, A.; nissen, M.; Sloots, T.; Tauro, S.; Storer, J.; Alvarez, R. and Tripp, R. A. (2006). Perspective on the host response to *human metapneumovirus* infection: what can we learn from respiratory syncytial virus infection?. Microbes and Infect.; 8: 285 – 293.

38-Lazar, I.; Weibel, C.; Dziura, J.; Ferguson, D.; Landry, M. L. and Kahn, J. S. (2004). Human

metapneumovirus and severity of *repiratory syncytial virus* disease. Emerg. Infect. Dis.; 10 (7): 1318 – 1320.

39- Leung, T. F.; To, M. Y.; Yeung, A. C. M.; Wong, Y. S.; Wong, G. W. K. and Chan, P. K. S. (2010). Multiplex molecular detection of respiratory pathogens in children with asthma exacerbation. Chest.; 137 (2): 348 – 354.

40-Toivonen, L.; Schez-Havupalo, L.; Rulli, M.; Ilonen, J.; Pelkonen, J.; Melen, K. and Waris, M. (2015). Blood MxA protein as marker for respiratory virus infection in young children. J. Clin. Virol.; 62: 8 – 13.

41-Foy, H. M. (1993). Infections caused by *Mycoplasma pneumoniae* and possible carrier state in different populations of patients. Clin. Infect. Dis.; suppl.1: S37 – S46.

42-Thurman K.; Walter, N.; Schwartz, S.; Metchell, S.; Dillon, M. and Baughman, A. (2009). Comparison of laboratory diagnosis procedures for detection of *M. pneumoniae* in community outbreaks. Clin. Infect. Dis.; 48: 1244 – 1249.

43- Falsey, A. R.; Hennessey, P. A.; Formica, M. A.; Cox, C. and Walsh, E. E. (2005). *Respiratory syncytial virus* infection in elderly and high – risk adults. New England J. Med.; 352 (17): 1749 – 1759.

44- Henrickson, K. J. and Hall, C. B. (2007). Diagnostic assay for *respiratory syncytial virus* disease. Pediatr. Infect. Dis. J.; 26 (11 suppl.): S36 – S40.

45-Jartti, T.; Lehtinen, P.; Vuorinen, T.; Koskenvuo, M. and Ruuskanen, O. (2004). Persistence of *rhinovirus* and *enterovirus* RNA after acute respiratory illness in children. J. Med. Virol.; 72 (4): 695 – 699.

46-Sung, R.; Chan, P.; Tsen, T.; Li, A.; Larn, W.; Yeung, A. C. and Nelson, E. A. (2009). Identification of viral and atypical bacterial pathogens in children hospitalized with acute respiratory infections in Hong Kong by multiplex PCR assay. J. Med. Virol.; 81: 153 – 159.

47- Shameran, A. R. and Al – Mola, G. A. (2014). Detection of *respiratory syncytial virus* (hRSV) by (PCR) technique in lower respiratory tract infection (LRTI) in infants and children under Babylon city. World Academy of Science, Engineering and Technology. Med. Helth. Sci.; 1 (9).

48- Ali, L. F.; Al – Suhail, R. G. and Nasir, F. G. (2014). Expression, extraction and purification of *respiratory syncytial virus* matrix protein from transformed *E. coli* (BL21). Int.J. Curr. Microbial. App. Sci.; 3 (8): 45 – 49.