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Detection of Nucleoprotein gene of Human Metapneumovirus and Chemokines & Histopathology study

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Abstract : Of a total 420 suspected Human Metapneumovirus infected cases only 388 positive cases were detected with RT-PCR. Study showed that number of male two hundred and fifty samples, while females were one hundred and thirty eight samples. Population groups studied samples subject groups were distribution into (3) groups including (10-20, 21-31 and 32-42) year, this distribution was made depending on their age and clinical status of both gender. The 21-31 year of infected patient high infection of virus at a rate 47.59% whilst 10-20 year percentage (36.59%) and 32-42 year percentage (15.46%).The samples were isolated from the al-Sadr hospital in Najaf. Chemokine are considered pro-inflammatory through infection was reduced as compared to concentration anti-inflammatory .The study appear high titer of Homeostatic after 7-21 day including (CCL27, CCL19 and CXCL13) comper Inflammatory including (CCL3, CCL11 and CXCL8).Yet CCL27 after 14 day (0.82±0.22) high titer camper with CCL19(0.80±0.24) and CXCL13(0.78±0.22)of virus infected group in the level of probability (P <0.05) while, CCL11, CCL3 and CXCL8 were dcreased to reach (0.76±0.25, 0.74±0.24 and 0.72±0.26) pg/ml respectively in the plasm of patients compared with the control group. Histopathological sections of infected lung was appear changes of cells and bleeding comper of laboratory animal control.
Key word: CEFCC, TCID, CCL, CXCL.

Introduction

Metapneumovirus had been characters including : (enveloped, family: Paramyxoviridae, subfamily: Pneumovirus. with single-stranded negative-sense , eight genes[1].The elderly and adult patients infected more cellular immune response[2]. Transmitted by direct contact with secretions, involving droplets and saliva[3,4].Symptoms begin after the secretion of RNA from 5-14 day[5.] During the acute phase of infection appear on the patient (fever, wheezing, cough, pneumonia , nasal congestion, sore, Purulent cough, bronchitis, otitis media, dyspnoea, conjunctivitis)[6]. Three to six days incubation period of the virus[7].While persons and adults during the presence of the virus have symptoms may be unclear but the typical symptoms of acute bronchitis[8]. persons older more serious especially for cases as they had chronic diseases such as cardiovascular disease , add children more susceptible to infection[9,10].

Chemokines have imported in infected patients after three day to fourteen day , especially eosinophils, monocytes and T-helper cells of bronchiolitis[11].

Cellular-viral effects on cells has been studied by the virus to tissue culture of CEFCC, addition to histological sections of the lung.

2 .Material and Methods

2.1 Samples Collection

Four hander twenty clinical samples were randomly collected from different areas of AL-Najaf province. Samples were collected during a period extended from 16 December 2015upto2of April 2016.Population groups studied samples subject groups were distribution into three groups This distribution was made depending on their age and clinical status of both gender. Two hundred and ten for females, while males amounted to one hundred and seventy-eight cases.

2.2 Real Time PCR Technique

Diagnosis of Human Metapneumovirus including type 1,2 and 3 by RT-PCR design relying primers in table (1).RNA Extraction Viral RNA was extracted by using kit (Cat. No.E0007, PowerPrep™ Viral DNA/RNA Extraction kit, spin).

Table(1): Nucleoprotein gene of Human Metapneumovirus[12].

Primer	Sequence	Target ergion Nucleotide position		Expected size of fragment
MPVN-3f	**5-GAGAAGAGCTGGGTAGAAG-3**	**Nucleoprotein gene**	**397–415**	**389**
MPVN-3r	**5-CAAACAAACTTTCTGCT- 3**			**786–770**

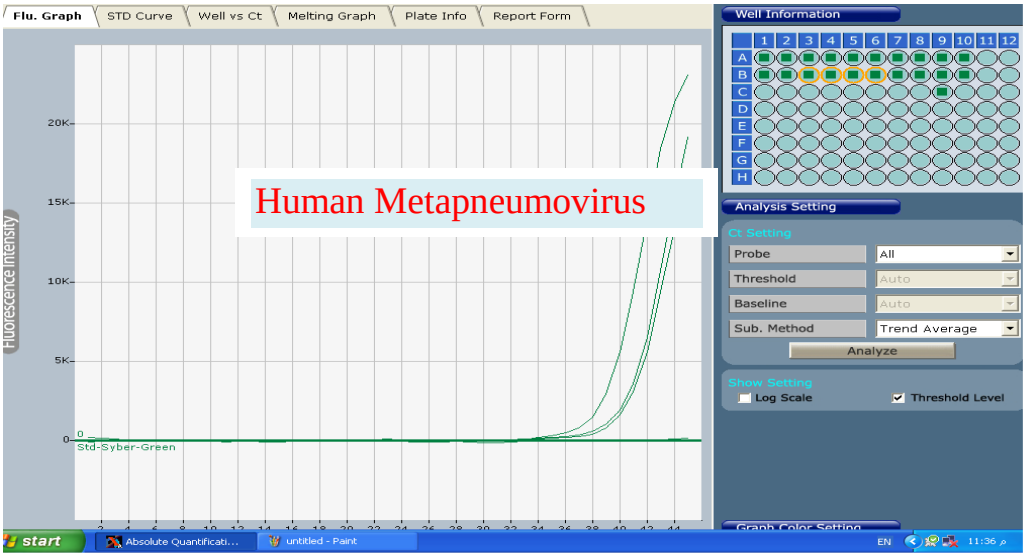
2.3 Tissue Processing

Procedure(13) was conducted in this study , which included the following steps (Dehydration, Clearing , Infiltration, Embedding , Sectioning, Staining).

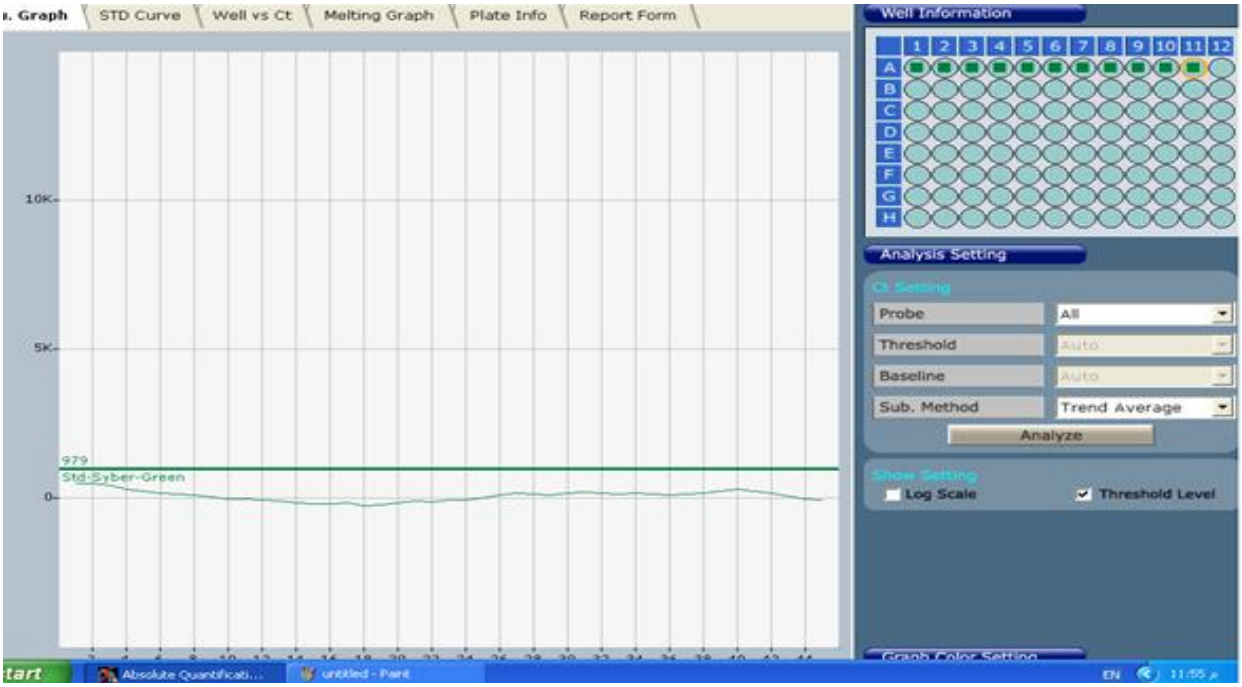
3.Results :

3.1Diagnosis viruses:

Real Time PCR Of a total 388 suspected of virus infected cases All the positive cases were undergone diagnosis with real -time – technique in figure(4).



A. Result is positive of three type of viruses



B. Result is negative

Figure(1): (A). Real T- PCR for detection Nucleoprotein gene of Human Metapneumovirus(B). Negative samples

3.2 Assess Chemokines of Human Metapneumovirus

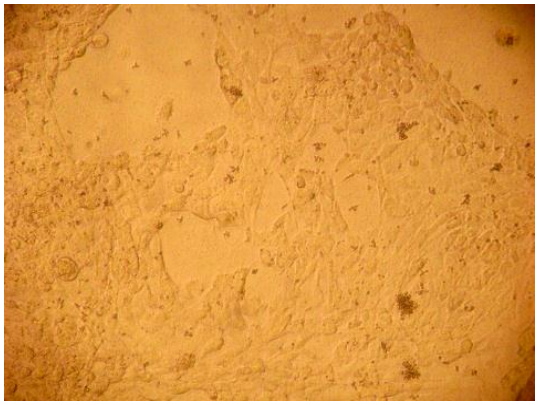
Study showed high titer of [CCL 27(0.82±0.22) & CCL 11(0.76±0.25) pg/ml],when compared after a fourteen day mentioned in table(2).Whilst after a twenty-one day diminution concentricity comprised Homeostatic : [CXCL 13(0.74±0.24), CCL 19(0.76±0.28) & CCL 27(0.78±0.24)pg/ml] , Inflammatory:[CXCL 8(0.64±0.28), CCL 11(0.69±0.26)& CCL 3(0.70±0.26)pg/ml] for the infected groups in comparison with healthy human (0.96±0.16)pg/ml

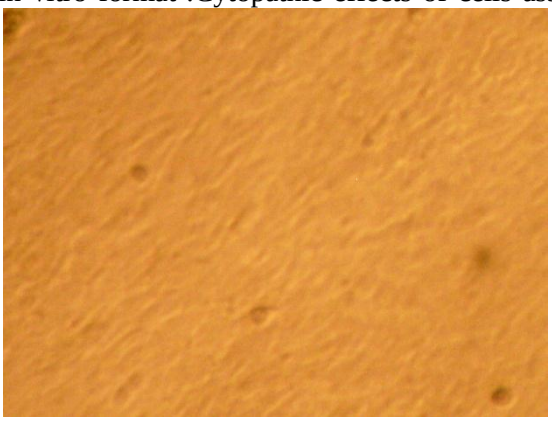
Table (2):Estimation Chemokines of Human Metapneumovirus

Chemokines		7day	14day	21day
Homeostatic	CCL 19			
		0.78±0.20	0.80±0.24	0.76±0.28
	CCL 27			
		0.80±0.20	0.82±0.22	0.78±0.24
	CXCL 13			
		0.76±0.20	0.78±0.22	0.74±0.24
Inflammatory	CCL 3			
		0.72±0.22	0.74±0.24	0.70±0.26
	CCL 11			
		0.74±0.20	0.76±0.25	0.69±0.26
	CXCL 8			
		0.69±0.24	0.72±0.26	0.64±0.28
Control Healthy human (n.20)		0.96±0.16	0. 96±0.16	0. 96±0.16

3.3 Elaboration of cell culture :

Adopted the manner of (14)to cell culture of viruses. As well Knockeash of[14-16] as illustrated the impact of HIV on the cells, which have been prepared in vitro format[2].Cytopathic effects of cells assemble hotbeds clear and recycled irregularly



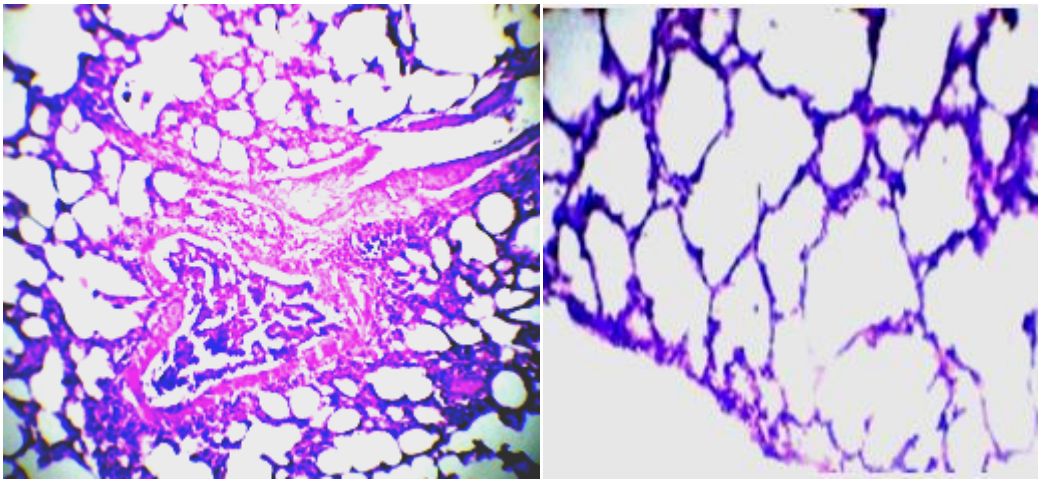


A: Human Metapneumovirus **B: Normal**

Figure (2): Human Metapneumovirus (A) illustrates cytopathic influences ofCEFCC, (D) normal cell culture.

3.4: Histopathlogical study:

The tissue sections of rat laboratory study is injected with the virus multiplier on fibroblasts fourth passsg$TCID_{50}$/0.5 ml was found equal to $10^{8.3}$ / 0.1 ml, which showed a higher concentration and then dosaged to laboratory animals for a week and then taken lung sectionsappear histological effects of virus showed thickening and bleeding of the cells in Figure (3) compared normal rat animal. laboratory animals infected has been monitored after three days showed symptoms clearly.



A: Human Metapneumovirus **B: Normal**

Figure(3): Lung sections of rats libratory after inoculation of Human Metapneumovirus in [CEFCC] .

Discussion

Four hundred and twenty infected of virus in the study for various age groups where the 21-31 age group most more exposed to injured a comparison of the age groups for study were randomly collect samples and by region, which have been isolated in addition to the surrounding environmental factors based on the

diagnosis of the virus laboratory, While the study showed both[17,18]has shown more than seventeen years hit by the virus. The percentage of females (55.87 %) and male (45.87%) which resembled with the[19],ratio amounting female (55.2%) to male (44.5%) respectively Homeostaticchemokines of human observed change in titer of [CXCL 13, CCL 19& CCL 27] at different stages from one up to three weeks ,as well Inflammatory [CXCL 8, CCL 11& CCL 3] also concentration alteration in plasma , but a study[20]conductedhuman MPV infected miceof CCL5 after twelve to twenty four hours, while CCL3lasted till 72 hours appear in concentricity covariance of animal groups. Results of[21] harmonization with our study. chemokines were studied because of an important role of the migration control cellularIn a location infection[22].The search of [23] correspond to cellular impact of the virus in tissue culturenext 48 h. Histological changes of infected rats the identical to[24]had been specified Supported on alveolar redness, interstitial perivascular, and peribronchiolar[25,26].

Conclusions:

1. The 21-31 year of infected patient high infection of virus.
2. Change in the level ofHomeostaticand Inflammatory.
3. Histopathology changes of lung sections , added of CEFCC.

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