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STUDY THE ROLE OF B19 VIRUS CAUSES THE INFECTION OF RESPIRATORY SYSTEM IN CHILDREN AND RELATIONSHIP WITH IL-6, IL-10 USING PCR AND ELISA TECHNIQUE IN DIYALA PROVINCE, IRAQ

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Abstract

Parvovirus B19 (B19v) is a small non enveloped single stranded DNA (ss DNA) virus of the family Parvoviridae, the subfamily parvovirinae, the genus erythrovirus. B19 was known as one of the possible cause of mild respiratory tract diseases. This study was aimed to detect prevalence of parvovirus B19 in patients with respiratory infections using the Real time polymerase chain reaction (RT-PCR) and study the immunological responses of cytokines IL-6, IL-10 using the Enzyme linked immunosorbent assay (ELISA) method. This study included 96 samples from both genders and ages ranging from (1-10years) samples were collected from respiratory system patients from 76 patients and 20 controls. Therefore, Results of current study showed that infection rate of B19 virus was 21.05% and all controls were healthy. The age group (4-6 years) recorded highest rate of infection with percentage of 26.9%. The results also showed that the infection rate of B19 virus in the rural was higher than in the urban with percentage of 22.2%. It was noticed that the concentration of IL-6 in patients which was infected with virus higher than in control. The concentration of IL-10 was also higher than in controls. The result also showed that high concentration level of IL-6, IL-10 in the age group (4-6 years). The results also proved that high concentration level of IL-6, IL-10 in the rural areas than in urban areas.

Key words: parvovirus B19, real time PCR, Cytokine, IL-6, IL-10

Introduction

Parvovirus B19 was discovered in 1975 by the scientist Cossart by examining the serological samples of normal individuals when evaluating the hepatitis B virus antibody tests using serological samples plates. B19 virus was a small, non-enveloped, icosahedral virus of about 23-26 nm in diameter containing a single stranded DNA genome of 5.6 KDa (Qiu *et al.,* 2017). B19 virus was most common during late winter and early spring. The prevalence of antibodies of B19 virus depends on age increasing from 2-20% in children less than 5 years, 15-40 % in children from 5 to 18 years and 40-80% in adults. infection with B19 virus was common in childhood and adulthood and over time the elder were the most seropositive people (Mor *et al.,* 2016). The virus was transmitted mainly through the respiratory secretions of infected person and the transfusion of blood components and through the placenta from the infected mother to the fetus (Lamont *et al.,* 2011). B19 virus causes many diseases in human including fifth diseases in children, transient aplastic crisis, hydropsfetalis, persistent anemia, arthritis, cardiomyopathy and infections in various other tissues. It also causes mild respiratory tract illness without rash, as well as acute obstructive airway disease and self-limiting obstructive airway disease (Romero- espinoza *et al.,* 2018) (Adamsonsal *et al.,* 2014). B19 virus associated with latent tissue infection. During the initial injury there is contentious latent injury to the bone marrow, skin, tonsils, liver and myocardial tissue (Norja *et al.,* 2008). The aim of this study is to determining the prevalence of the virus among children suffering from respiratory system infection using real time polymerase chain reaction (RT-PCR) and study the relationship between the virus and some immunological indicators IL-6 and IL-10 in Diyala province.

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Materials and methods

Subjects

This study included 96 blood samples, 20 healthy person as a control group and 76 patients with respiratory system infections (45 males and 31 females) with ages ranging from 1-10 years. The samples were collected from children emergency hall in Al Battol teaching hospital in baquba city during the period from the beginning of October 2019 to the beginning of January 2020.

Blood sampling

5 ml of venous blood was drawn by disposable syringe and collected in gel tube and allowed to clot, serum was obtained by use centrifugation with speed of 1500 rpm for 5 minute and the serum was divided into equal quantities (100 µl) into eppendroff tube and kept frozen at -20 c until analysis.

Methods

The genomic DNA was extracted from sera using viral nucleic acid extractionkit supplied by ABIO pure company. The primer used was the same sequence used by Arabzadeh (Arabzadeh *et al.,* 2017). RT-PCR (promega, go taq probe qPCR master mix, USA) was used to detect viral DNA. also ELISA was used to measure immunological responses for IL-6 and IL-10 depending on manufacture standards (My bio source, USA).

Statistical analysis

Statistical analysis software (SPSS) was used, the chi square test was used between groups. ANOVA test was used for IL-6 and IL-10, the statistical difference test was set to $P< 0.05$.

Results and Discussions

The results showed that viral infection in 16 children infected with respiratory system infection from 76 patients with percentage 21.05% with significance difference P.v = 0.025.

A study conducted in Iran in 2015 using RT-PCR indicated that the prevalence of virus among 63 infected out of a total of 583 patients with percentage 10.81 % (Rezaei *et al.,* 2015). Another study conducted in Turkey in 2014 using RT-PCR showed that the presence of the virus in 21 infected person of a total 56 patients with percentage of 21.4% (Sahiner *et al.,* 2014). Also there was a study conducted in Syria in 2015 using conventional PCR showed that the presence of the virus was found in 7 of the infected person of a total 50 patients with percentage 14% (Debs and Buhtori, 2015). The difference in proportions between societies was due to several factors including the method of transmitting the B19 virus in air drops through the respiratory tract, difference in geographical areas, age, social and cultural status, hereditary status, in addition to the type of laboratory technology used to diagnose B19 virus (He *et al.,* 2012).

Table 1: Viral infection in the study group.

Study groups	Total	Infected subject	P.v
Respiratory system patients	76	16(21.05%)	0.025
Control	20	0	0

Table 2: Viral infection in genders.

Study groups	Total	Infected subject
Females	31	4 (12.9%)
Males	45	12 (26.6%)
Control	20	0

Table 2 showed that the prevalence of the virus in males was more than in females, the infection was found among 12 males out of a total of 45 patients with a percentage of 26.6% and in 4 females of a total 31 patients with a percentage of 12.9%. The results of the current study were in agreement with a study conducted in Iran in 2018 using RT-PCR and showed that a higher incidence of males than females, the virus was found in 5 males of a total 84 patients with a rate of 5.9% and in 3 females of a total 72 patients with a rate of 14% (Tavakoli *et al.,* 2018). The results of the current study were also in agreement with another study conducted in Turkey in 2014 using the same technique, where the researcher found that the prevalence of the virus in males was 8 infections of a total 32 patients with a rate of 25% and in 4 infected females from a total of 24 patients with a rate of 16.7% (Sahiner *et al.,* 2014).

Table 3 showed that the most ages affected by the virus were within the age group 4-6 years with a rate of 26.9% followed by the age group 7-10 years with a rate of 20% and lowest ages of the virus infection are within the age group 1-3% with a rate of 17.1%. A study conducted in Iran in 2015 using RT-PCR inducted that the prevalence of virus is more among the age group 6-11 years as the virus was found in 24 infected person of a total 63 patients with a percentage of 13.8% and less prevalence in age group less than one years, the virus was found among 5 infected person of a total 63 patients

Table 3: Viral infection in age groups.

Age groups	Total	Infected subject
1-3 years	35	6 (17.1%)
4-6 years	26	7 (26.9%)
7-10 years	15	3 (20%)
Control	20	0

with a rate of 5% (Rezaei *et al.,* 2015). Another study conducted in Bulgaria in 2016 using PCR technique showed that the prevalence of the virus is more in the age group 5-9 years when the virus was found in 76 infected person of a total 280 patients with a rate of 27% and less prevalence in adults over 44 years, the virus was found in 5 infected person from a total of 280 patients with a rate of 2% (Lvanova *et al.,* 2016).

Table 4 showed that increase of virus infection in rural more than in urban and there were 10 infected person of a total 45 patients with a rate of 22.2% and in 6 infected person in the urban of a total 31 patients with a rate of 19.35%. The percentage of positivity in the rural is more than in urban areas this may be due to the low educational and cultural level of parents, poor health and social services, distance from the city center.

Table 5 showed that concentration of IL-6 was 17.824±15.948 which was higher than in controls with significance difference P.v = 0.001 and the concentration of IL-10 in patients was 52.009±31.528 which was higher than in controls with significance difference P.v = 0.002. The results of the current study were identical to a study conducted in the united states (London) in 2004 the high level of interleukin 6 among 78 patients of a total 84 patients and its concentration was 719.4±41.7. it also indicated that the high level of interleukin 6 is associated with acute virus infection (Kerr *et al.,* 2004). Another study conducted in Taiwan in 2006 showed an elevated concentration of IL-6 in person infected with B19 virus with a concentration of 190.2±17.2 (Hus *et al.,* 2006). The elevated level of interleukin 6 was associated with the non-structural proteins of B19 as these proteins regulate the reproduction of the IL-6 gene by binding to the NF-Kβ site in the IL-6 promoter (Moffatt *et al.,*1996).

A study conducted in Lativa in 2017 indicated that the concentration of interleukin 10 was higher in those infected with the virus with a concentration of 54.2±35.4 relative to the control group with concentration of 25.5±22.4 (Naciute *et al.,* 2017). Another study conducted in Finland in 2004 indicated a higher concentration of IL-10 among those infected with the virus with a concentration of 33.1±28.9 compared with a control group with a concentration of 6.3±5.4 (Franssil *et al.,* 2004).

Table 6 showed that the highest level of IL-6 in serum of patients for ages 4-6 years with a concentration of 21.974±20.327 and the lowest level was for ages 1-3 years with a concentration of 10.037±1.994. A study conducted in the united states in 2004 showed an increase in concentration of IL-6 in patients aged less than 20 year with a concentration of 33.8±1.9 (Kerr *et al.,* 2004). Table 6 also showed that the highest level of IL-10 in serum of those infected with the virus for ages 4-6 years and its concentration was 64.71±21.951 and the lowest level was for ages 1-3 years and its concentration was 32.446±21.951.

Table 7 showed that high level of IL-6 in the serum of infected females with a concentration of 25.101±19.259 compared to that of males with concentration of 16.145±12.608. A study conducted in the united states in 2004 showed a higher level of IL-6 in the serum of infected females with a concentration of 62.8±2.7 compared with males (Kerr *et al.,* 2004). Table 7 also showed that high level of IL-10 in the serum of infected females and its concentration was 83.084 ±57.450 compared to that of males and its concentration was 44.838±19.884.

Table 8 showed that an increase of IL-6 in serum of

Table 4: Viral infection with residential area.

Study groups	Total	Infected subject
Rural	45	10 (22.2%)
Urban	31	6 (19.35%)
control	20	0

Table 5: Cytokines concentration and viral infections.

Study groups	Total	Infected subject	IL-6	IL-10
Respiratory system patients	76	16 (21.05%)	17.824± 15.948	52.009± 31.528
Control	20	0	10.153± 5.238	34.743± 21.471

Table 6: Cytokines concentration and viral infection in age groups.

Age groups	No. patients	IL-6	IL-10
1-3 (34)	5	10.037±1.994	32.446±21.951
4-6 (27)	9	21.974±20.327	64.711±34.427
7-10 (15)	2	18.616±5.238	34.743±21.471

Table 7: Cytokines concentration and viral infections in genders.

Genders	No. patients	IL-6	IL-10
Males (46)	13	16.145±12.608	44.838±19.884
Females (30)	3	25.101±19.259	83.084±57.450
Control (20)	0	10.153±5.238	34.743±21.471

Table 8: Cytokines concentrations and viral infections in the residential area.

Study Grups	total	IL-6	IL-10
Rural	42	19.320±19.557	48.603±20.225
Urban	34	15.330±7.917	46.733±5.686
Control	20	10.153±5.238	34.743±21.471

infected person in the rural area and its concentration was 19.557±19.320 compared to that in urban areas and its concentration was 15.330±7.917. Table 8 also showed that an increase in the level of IL-10 in serum of infected person in the rural areas and its concentration was 48.603±20.225 compared to that in urban areas and its concentration was 46.733 ±5.686.

Conclusions

The prevalence of the virus was high using linear polymerase chain reaction technique in patients with respiratory system infection in Diyala province, the highest infection rate was in the age group 4-6 years, B19 infection in males is higher than in females, the highest level of IL-6 was in the age group 4-6 years, lowest level was in the age group 1-3 years and the highest level of IL-10 was in the age group 4-6 years and the lowest level was in the age group 1-3 years. infection with the virus constitutes a high rate of respiratory infection in children compared with other microorganisms.

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