Research Article

Detection viral load of Parvovirus B19 in patients with Chronic Renal Failure

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Abstract

Background : Because of Parvovirus B19 that occur in patients with chronic renal failure ; Parvovirus B19 was found to be associated with or might be an aggravation factor of renal failure , we tried to find out the rate of occurrence of this virus in dialysis and non dialysis patients.

Objective: The aim of the study was to investigate viral load for Parvovirus B19 was play an important role in the etiology of chronic renal failure patients

Subjects and methods :A cross sectional study included Serum samples were collected from 50 dialysis patients and 50 without dialysis patients 50 normal subjects as control, and subjected for ELISA technique for detection of parvovirus IgG antibody, and multiplex Real time PCR for detection virus and viral load of this virus

Results: Thirteen (26%) out of these 50 dialysis patients were positive by RT-PCR and, 16% (8/50) were positive by ELISA, while none of the controls was positive neither by RT-PCR nor by ELISA. The results of this study showed highly significant differences ($p<0.001$) on comparing between the median viral load in patients who had dialysis ($2.38x10^6$) copies / ml of Parvovirus B19 DNA and those who without dialysis($1.73x10^4$) copies/ml of Parvovirus B19 DNA. Also the results of B19 by real time PCR analysis showed a significant difference ($p<0.001$) in median viral load in patients who had acute infection($2.87x10^6$) copies/ml of Parvovirus B19 DNA ,than those who had chronic infection ($2.29x10^5$) copies/ml of Parvovirus B19 DNA ,

Conclusions Parvovirus B19 could be an important co-factor that play a role in CKD, which was higher rate in dialysis patients.

Key words: RT-PCR test , ELISA test , dialysis and non dialysis patients,

INTRODUCTION

The parvovirus B19 is the only member of the family Parvoviridae known to be pathogenic in humans(1). This virus is widespread, and manifestations of infection vary with the immunologic and hematologic status of the host. (2)

The role for parvovirus B19 infection is based on the association of renal findings with viral infection, positive serology, and identification of the viral genome in the glomerulus.(3) Infection is occasionally, especially in adults, associated with an CKD.(4) Chronic kidney disease (CKD) is one of the major public health problems , In the United States there are approximately twenty six million adults having non-dialysis dependent kidney disease , the infection in individuals with an underlying hemolytic disorder causes transient a plastic crisis. In the immune compromised host, persistent Parvovirus B19 infection is manifested as pure red cell aplasia and chronic anemia.(5)

Viral infection is the most prevalent comorbidity in individuals with chronic kidney disease.[6] The Parvovirus B19 cannot autonomously replicate but must rely on the host cellular machinery to support their life cycle through natural selection.[7]

Parvovirus B19 have evolved strategies to coopt the host organism to be a better site for their propagation. One of these strategies are directed at the cellular machinery and involve complicated and ingenious solutions to optimize infection، replication and new virion assembly and shedding.[8,9] Factors that predispose this population to complications of parvovirus B19 infection include impaired immune response, deficient erythropoietin production, and decreased erythrocyte survival.[10] The clinical sign of parvovirus B19 infection in renal may be underestimated; these individuals may develop persistent viremia as a result of a dysfunctional immune response.[11] Chronic anemia and pure red blood cell aplasia are the most common complications of parvovirus B19 infection in the CKD patients.[12] After gaining access to the human host, parvovirus B19 targets the erythroid progenitors in the bone marrow by binding to the glycosphingolipid globoside (Gb4) , also known as blood group P antigen.[13,14] The link between parvovirus B19 infection and glomerular disease has been suggested from numerous case reports that describe onset of nephritis or nephritic syndrome after onset of parvovirus infection.[15,16] clinical presentations and histologic patterns have been described.[17] Acute nephritic syndrome following a prodromal of fever, rash, and arthritis is most common. [18]

PATIENTS AND METHODS

The study was performed on 100 patients, among them 50 patients were on dialysis 22 females and 28 males , 50 patients without dialysis 27females and 23 males kept in conservation measure and 50 apperantly healthy persons that served as control group 24 females and 26 males . Their ages (patients and controls) were ranged from (17 – 88) years. All patients were attended the Imamian Al-Khademian Medical city, Baghdad Medical city and Al-Karma hospital during the period from July 2014 till February 2015 .

Statistical analysis: All data were expressed as mean ± SD. Student's t test was used to analyze sample averages. One way analysis of variance (ANOVA) was used to evaluate differences of means between groups. Ratio was compared by the chi square test. Correlations between HOMA-IR , other parameters were analyzed by Pearson's correlation .P < 0.001 was accepted as statistical difference.

RESULTS

The results are summarized in table (1-1) reveled that in dialysis patients , five (10%) were found to be anti IgG-B19 positive and 45 (90%) were negative, two (4%) patients were found to be anti IgM-B19 positive and 48 (96%) were negative , while in patients without dialysis four (8%) patients were found to be anti –IgG B19 positive and 46(92%) which found to be anti-B19 negative and the fifty patients were found to be negative IgM- , all control group was negative for both anti –parvovirus B19 IgM and IgG. Thirteen (26%) patients were found to be positive by PCR and 37 (74%) were negative ,while in patients without dialysis 7 (14%) patients were positive and 43(86%)patients were found to be negative ,there is high significant difference between apparently healthy control and patients (P<0.001) groups as show in table (1- 2).

The Relative risk indicator that dialysis and without dialysis patients (2.351-2.163) were more susceptible for infection than in control .The result of (B19) RT-PCR was show in figure (1-2). The results of this study showed highly significant differences (p<0.001) on comparing between the mean viral load in patients who had dialysis **($2.38x10^6$)** Copies/ ml of parvovirus B19 DNA and those who without dialysis**($1.73x10^5$)** Copies/ml of parvovirus B19 DNA/ table (1-4) .

In addition, the results of parvovirus B19 real time PCR analysis showed a significantly higher(p<0.001) mean viral load in patients who had acut infection **($2.87x10^6$)** Copies/mL of parvovirus B19 DNA ,than those who had chronic infection **($2.29x10^5$)** Copies/mL of parvovirus B19 DNA of the study table (1-5).

also, the results of parvovirus B19 real time PCR analysis showed a non significantly mean viral load in patients who less than six months of infection**($2.12x10^6$)** Copies/mL of parvovirus B19 DNA and those who had more than 6 months of infection **($2.60x10^6$)** Copies/mL of B19 DNA of the study (table 1-6).

Table 1: Comparison between IgG and IgM in studied groups

Parameters	ELIA	Study groups			Study groups	
Immunioglobin	Result	Control	Dialysis	Non-dialysis	Control	CKD
IgG	Positive%	0(0%)	5(10%)	4(8 %)	0(0%)	9(18%)
	Negative%	50(100 %)	45(90%)	46(92%)	50(100%)	91(91%)
	p value		>0.05	>0.05		>0.05
IgM	Positive%	0 (0%)	2(4%)	0(0%)	0(0%)	2(4%)
	Negative%	50(100%)	48(96%)	50(100%)	50(100%)	98(98%)
	p value		0.495	1.000		0.556

Table 2: Detection of parvovirus B19 by q-RT- PCR studied groups.

PCR	Study groups			Study groups	
	Control	Dialysis	Without dialysis	Control	CKD
Positive%	0(0 %)	13(26%)	7(14%)	0(0 %)	20(20%)
Negative%	50(100%)	37(74%)	4386%	50100%	80(80%)
p value		.001**	.001**		.001**
Relative Risk		2.351	2.163		2.25
95% confidence interval		1.842 to 3.002	1.737 to 2.693		1.786 to 2.835

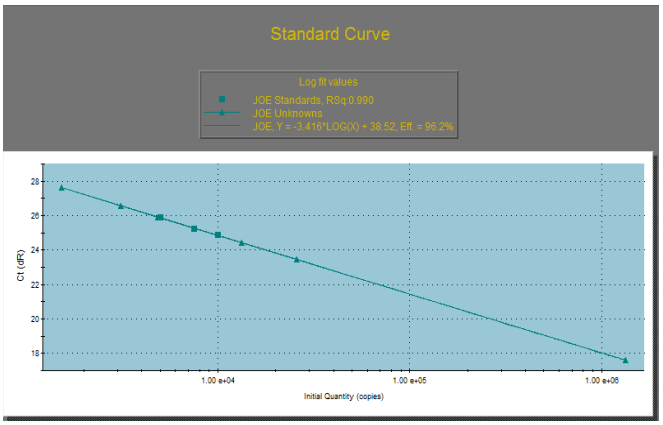

Figure 1:Real time PCR standard curve with positive parvovirus B19 infection, the squares are the standards and the triangles are the unknowns

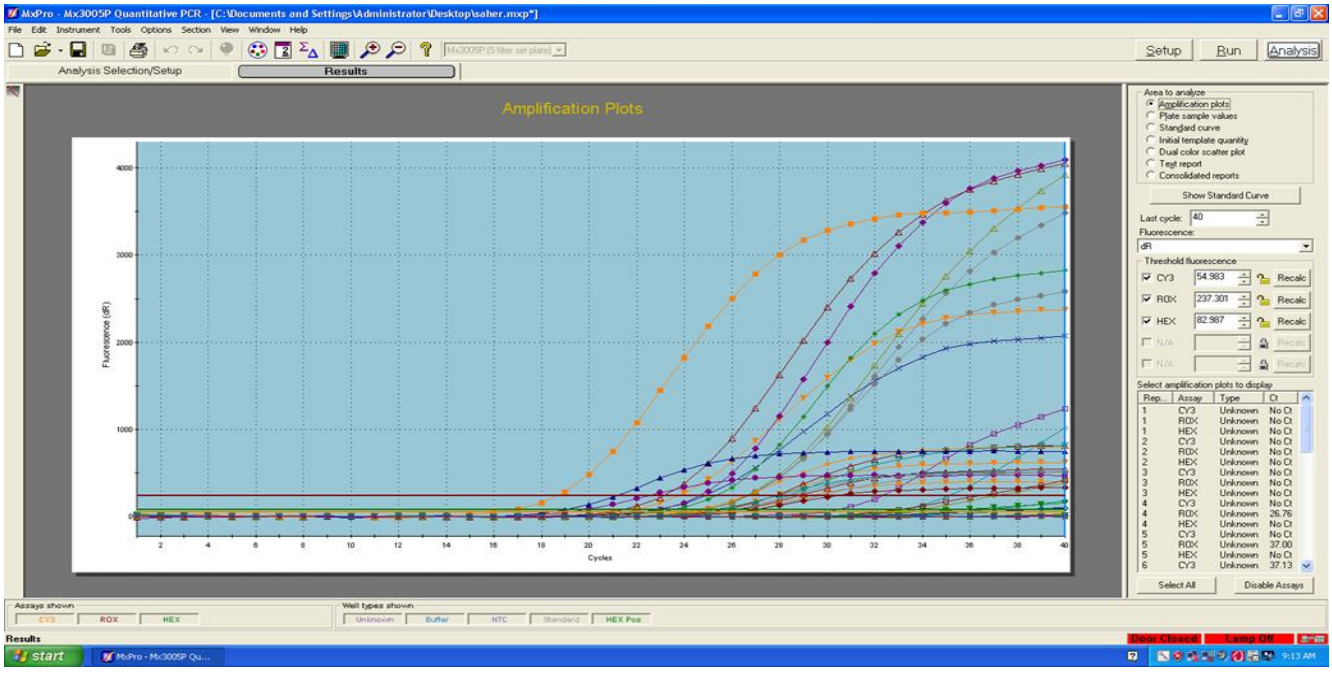

Figure 2: parvovirus B19, RT- PCR results; positive sample (over threshold), negative sample (on the line and under threshold), each one with internal control.

Table 3: Comparison between the mean viral load in CKD who had infection with and without dialysis of the studied group

Parameter	Without dialysis	With dialysis
Mean of viral load (Copies/mL)	1.73 x10^5	**2.383 x10^6**
Maximum	2.17 x10^6	**3.18 x10^6**
Minimum	1.4 x10^4	**1.89 x10^6**
P value	P<0.001	

Table 4: Comparison between the mean viral load in CKD who had acute infection and those who with chronic infection in the studied group

Parameter	Acute	Chronic
Mean of viral load(Copies/mL)	2.87 x10^6	**2.294 x10^5**
Maximum	3.18 x10^6	**1.89 x10^6**
Minimum	2.56 x10^6	**3.07 x10^4**
P value	P<0.001	

Table 5: Comparison between the mean viral load in CKD who had less than six months of dialysis and those with more than six months

Parameter	<6 months	>6 months
Mean of viral load (Copies/mL)	2.12 x10^6	**2.60 x10^6**
Maximum	2.32 x10^6	**3.18 x10^6**
Minimum	1.89 x10^6	**2.24 x10^6**
P value	P<0.001	

DISCUSSION

There are mean reasons to think the parvovirus may be an important pathogen in these populations. one of this reasons, erythropoiesis is maintained by erythropoiesis-stimulating agents, and red blood cells may have a shortened life span in the patient with anemia . This combination factors may predispose these patients to potentially associated with parvovirus infection . two reason ,the administration of erythropoietin during parvovirus B19 infection lead to facilitate viral replication by providing new target cells, thereby prolonging viremia and its associated complications. Three reason through blood transfusion spread of infection in an dialysis unit is a potential, emergency dialysis may be carried out in these centers, the patients whom unknown parvovirus B19 were referred to the centers for further management until the patients, conditions were stable and, shortage in health services, as a result of past situation, resulted in relatively improper cleaning and sterilization of medical instrument with shortage disposable filter. In this study, a significant association was found between the dialysis centers and the prevalence of parvovirus B19-infection . The nonrandom distribution of parvovirus B19-infected individuals among the centers indicates that local factors may a role in the epidemiology of parvovirus B19. This is in accordance with finding that the size of a dialysis center (i.e., the total number of patients treated) was related to the prevalence of parvovirus B19 infections is suggestive of remote infection of parvovirus B19 .

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