# GENOTYPING OF PARVOVIRUS B19 IN IRAQI PATIENTS WITH RENAL FAILURE

**Hanin F. Al-Asi[1], Jinan M. Al-Saffer[1] and Haidar A. Shamran[2]**

*[1]Biotechnology Department, School of Science, University of Baghdad, Baghdad 10066, Iraq*

*[2]Medical research Unit, School of Medicine, University of Al-Nahrain, Baghdad 10066, Iraq*

## ABSTRACT

Renal Failure (RF) is considered a public health problem worldwide with high incidence and prevalence rates. In Iraq, the patients' number with renal failure has increased gradually over the years. However, the immune suppression accompanied this increase the opportunity of several microorganisms to establish and introduce their harmful effects. Parvovirus is one of those microorganisms which were frequently reported to exploit the reduction of immunity of the host and reactive causing many health problems. This study aimed to investigate the prevalence and genotypes of parvovirus B19 infection among samples of Iraqi renal failure patients and evaluate some aspect of the immune status of those patients.Plasma and serum samples were obtained from a total of 50 RF patients and 30 age- and sex-matched apparently healthy controls. PCR techniques was used to amplify the seropositive samples and the resultant gene was subjected to direct sequence compared with reference sequence in NCBI to determine the genotyping of local isolates. Out of 50 RF patients, Viral DNA was successfully isolated, and accordingly, NS1gene amplified from six seropositive patients, but from none of controls. However, only 4 PCR products were sequenced with full length NS1 gene

**Key words:** Renal failure, Parvovirus B19, *NS1 gene*

| **No: of Tables: 04** | **No: of Figures: 01** | **No: of References: 10** |
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## INTRODUCTION

Parvovirus B19 is a non-enveloped icosahedral single-stranded linear DNA of either positive or negative polarity. The virus is a member of the *Parvoviridae* family and *Erythrovirus* genus [1]. So far, parvovirus B19 is divided into three genotypes (1, 2 and 3), which have about 10 % divergence in overall DNA sequence [2]. The virus has marked tropism to infect the erythroid progenitor cells in bone marrow, and diseases, like erythema infectiosum is a direct result for this tropism. Nevertheless, many other diseases like hydrops fetalis and arthropathies reflect the ability of the virus to infect different body tissues [3]. Thus, it is not surprising to encounter this virus associated with kidney infection or may be other organs especially with the body immunity becomes feeble. Renal failure patients are susceptible to viral infections because of their immune compromised background [4]. Eid *et.al*.[5] reported that 1-12% of adult renal transplant recipients have symptomatic parvovirus B19 infection during the first year after transplantation. The immune compromised state impedes the effective antiviral immune response, which can potentially lead to persistent viremia, and subsequent infection for different body organs.

### Material and Methods

***Study Population***: A total of 50 patients who were suffering from renal failure in Imamian Al-Khademian Medical city and some private laboratories during the period from September 2015 to February 2016 were recruited for this study. 30 apparently healthy individuals were selected to represent the control group. Complete data showed in table (1).

**Table (1)**: Complete Data of Study

| Risk Factor | Patients (50) | Control (30) | *P* Value |
|---|---|---|---|
| Age (year) mean±SD | 54.32 ±10.925 | 50.77 ±7.408 | 0.119 |
| Sex | | | |
| Male | 22 (44%) | 16 ( 53.3%) | 0.645 |
| Female | 28 (56%) | 14 (46.7%) | |
| Dwelling | | | |
| Rural | 23(46%) | 17 (56.7%) | 0.489 |
| Urban | 27(54%) | 13 (43.3%) | |
| Anemia | | | |
| Yes | 35(70%) | --------- | ------- |
| No | 15(30%) | | |
| Serum Urea (mg/dl) ±SD | 74.231 ±39.45688 | --------- | -------- |
| Serum Creatinine (mg/dl) ±SD | 1.7666 ±1.20689 | --------- | -------- |

***Estimation, Amplification and Sequencing of NS1 gene in plasma:*** The viral DNA was extracted according to the protocol provided by the Kit (viral nucleic acid extraction kit III/ Genaid / Taiwan). The presence of viral DNA was detected by gel electrophoresis then purity and concentration of the DNA was adopted by nano-drop. Polymerase chain reaction (PCR) carried out for all seropositive depending on ELISA test. Five pairs of primer previously described by Nguyen et. al. [6].

DNA sequencing was carried out for all purified PCR products (6 samples positive for parvovirus B19) by using ABI PRISMTM Big dye terminator cycle (Macrogen/Korea). phylogenetic tree was constructed with 1000 boots trap replicate using MEGAS software version 5.

***Statstical Analysis:*** The Statistical Package for the Social Sciences (SPSS, version 14) was used for statistical analysis. Chi- square was used to compare between categorical values. A *p*-value < 0.05was considered statistically significant.

## 3. Results

***Gene Amplification:*** viral DNA extracted from only 6 (35.29%), On the other hand none of the control group gave positive result for gene amplification. Figures (1) show the amplicons of the five regions in NS1 protein according the five primers. The P distance between the current isolates and reference isolates as among current isolates themselves is shown in table (2).

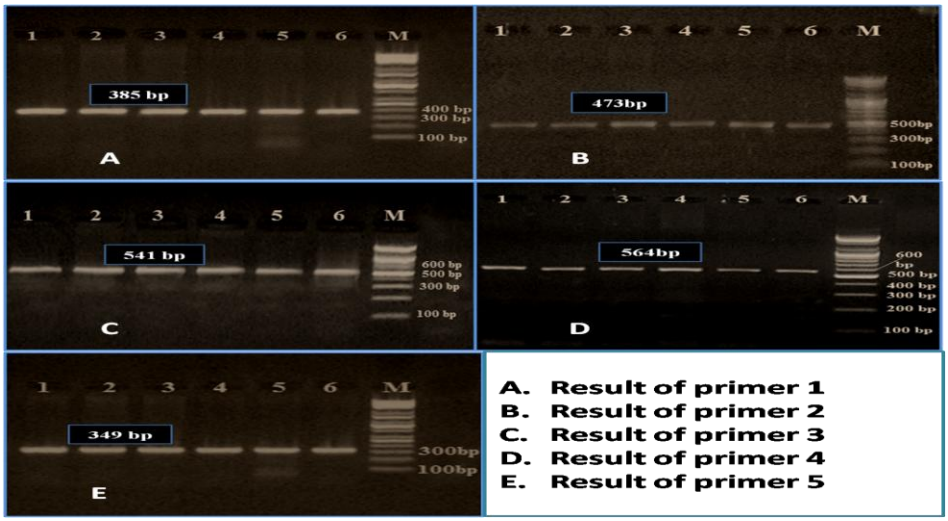


**Figure (1):** Gel electrophoresis of NS1 gene. Lane 1-6: positive amplification, M: DNA marker.

**Table (2)**: P-distance between the current isolates and reference isolates using MEGA software.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. M13178 | | | | | | | | | | | | |
| 2. AB030694.1 | 0.01 | | | | | | | | | | | |
| 3. AF162273.1 | 0.01 | 0.01 | | | | | | | | | | |
| 4. AF113323.1 | 0.01 | 0.01 | 0.01 | | | | | | | | | |
| 5. AY044266.1 | 0.13 | 0.13 | 0.13 | 0.13 | | | | | | | | |
| 6. AY064476.1 | 0.13 | 0.13 | 0.13 | 0.13 | 0.02 | | | | | | | |
| 7. AX003421.1 | 0.14 | 0.13 | 0.14 | 0.13 | 0.08 | 0.08 | | | | | | |
| 8. AY083234.1 | 0.13 | 0.13 | 0.13 | 0.13 | 0.07 | 0.08 | 0.05 | | | | | |
| 9. Isolate 1 | 0.01 | 0.02 | 0.02 | 0.02 | 0.14 | 0.13 | 0.14 | 0.13 | | | | |
| 10. Isolate 2 | 0.01 | 0.02 | 0.01 | 0.02 | 0.14 | 0.13 | 0.14 | 0.13 | 0.01 | | | |
| 11. Isolate 3 | 0.01 | 0.02 | 0.02 | 0.02 | 0.14 | 0.13 | 0.14 | 0.14 | 0.02 | 0.02 | | |
| 12. Isolate 4 | 0.01 | 0.02 | 0.02 | 0.02 | 0.14 | 0.13 | 0.14 | 0.13 | 0.00 | 0.01 | 0.02 | |

The sequence was found to extend from nucleotide number 436 to nucleotide number 2448 on viral nucleic acid. Table (3) shows the result of the comparison between the four isolates and M13178.

**Table (3)**:Amino acid alterations in NS1 protein of the four isolates compared to M13178 isolate (according to NCBI)

| Isolate | Position on NS1 gene | Base substitution | codon change | Amino Acid substitution |
|---|---|---|---|---|
| Isolate 1 | 501 | A→G | CAT→CGT | His→Arg |
| | 583<br>584 | A→C<br>G→C | AGT→CCT | Ser→Pro |
| | 814 | A→G | AAA→GAA | Lys→Glo |
| | 1038 | G→C | GAG→GAC | Glu→Asp |
| | 1151 | T→A | TTT→TAT | The→Tyr |
| | 1288 | A→C | ATG→CTG | Met→Leu |
| | 1465 | A→G | AGT→GGT | Ser→Gly |
| | 1969 | A→G | AGT→GGT | Ser→Gly |
| | 2008 | T→C | TTT→CTT | Phe→Leu |
| | 2053<br>2055 | C→A<br>C→G | CGC→AGG | Arg→Arg |
| | 2079<br>2081 | G→T<br>A→T | GAA→TAT | Glu→Tyr |
| | 2302 | G→T | GCT→TCT | Ale→Ser |
| Total sequence=2013 , total nt substitution=15(0.7%), total a.a substitution= 11(1.6%) | | | | |
| Isolate 2 | 603 | G→T | GGG→TGG | Gly→Try |
| | 639 | T→C | TTT→TTC | Phe→Phe |
| | 814 | A→G | AAA→GAA | Lys→Glu |
| | 850 | G→A | GAA→AAA | Glu→Lys |
| | 1038 | G→C | GAG→GAC | Glu→lu |
| | 1068 | G→T | AAG→AAT | Lys→Asp |
| | 1069 | G→T | GCT→TCT | Ala→Ser |
| | 1327 | T→A | TGT→AGT | Cys→Ser |
| | 1758 | G→C | AAG→AAC | Lys→Asp |
| | 1767 | T→G | TTT→TTG | Phe→Leu |
| | 2050<br>2052 | C→A<br>C→G | CGC→AGG | Arg→Arg |
| | 2299<br>3000 | G→A<br>G→T | GGG→ATG | Gly→Met |
| Total sequence=2013 , total nt substitution=14(0.7%), total a.a substitution= 10(1.5%) | | | | |
| Isolate 3 | 452 | G→A | GGG→GAG | Glu→Glu |
| | 478 | C→T | CTA→TTA | Leu→Leu |
| | 709 | A→T | AAC→TTT | Asn→Phe |

| | | | | |
|---|---|---|---|---|
| | 710<br>711 | A→T<br>C→T | | |
| | 981 | C→A | ACC→ACA | Thr→Thr |
| | 1003<br>1004 | A→T<br>G→T | AGT→TTT | Ser→Phe |
| | 1233 | A→G | GCA→GCG | Ala→Ala |
| | 1260 | T→A | TTT→TTA | Phe→Leu |
| | 1445 | T→C | TTG→TCG | Leu→Ser |
| | 1464 | A→G | AAA→AAG | Lys→Lys |
| | 1502<br>1503 | A→C<br>A→C | GAA→GCC | Glu→Ala |
| | 1670 | T→G | ATA→AGA | Ile→Arg |
| | 1902 | T→G | ATT→ATG | Ile→Met |
| | 1903 | A→G | AAT→GAT | Asn→Asp |
| | 2011 | T→C | TTT→CTT | Phe→Leu |
| | 2053<br>2055 | C→A<br>C→G | CGC→AGG | Arg→Arg |
| | 2079 | C→T | ACC→ACT | Thr→Thr |
| | 2404 | A→T | AAA→TAA | Lys→stop |
| Total sequence=2013 , total nt substitution=22(1.09%), total a.a substitution= 10(1.5%) | | | | |
| Isolate 4 | 551 | A→G | CAT→CGT | His→Arg |
| | 583<br>584 | A→C<br>G→C | AGT→CCT | Ser→Pro |
| | 814 | A→G | AAA→GAA | Lys→Glu |
| | 1038 | G→C | GAG→GAC | Glu→Asp |
| | 1151 | T→A | TTT→TAT | Phe→Tyr |
| | 1288 | A→C | ATG→CTG | Met→Leu |
| | 1465 | A→G | AGT→GGT | Ser→Gly |
| | 1969 | A→G | AGT→GGT | Ser→Gly |
| | 2008 | T→C | TTT→CTT | Phe→Leu |
| | 2053<br>2055 | C→A<br>C→G | CGC→AGG | Arg→Arg |
| | 2179<br>2181 | G→T<br>A→T | GAA→TAT | Glu→Tyr |
| | 2302 | G→A | GCT→ACT | Ala→Thr |
| | 2445 | T→C | TAT→TAC | Tyr→Tyr |
| Total sequence=2013 , total nt substitution=16(0.8%), total a.a substitution= 11(1.6%) | | | | |

**Discussion**

It appears that infection with B19V can occur even without immunosupresion, but the immunosuppressive status certainly enhances the establishment of infection as in case of elderly people and those with different pathological conditions associated with immune suppression.

The study shows that in Iraqi patients the virus belongs to genotype 1, which sounds to be the common genotype worldwide where as genotype 2 sporadically in European countries (such as Finland, Germany), the USA, and South America.

Obviously, there is few dramatic changes in DNA sequences between the four isolates and M13178 isolate and among the four isolates themselves, in that, there is no insertion/deletion or even the substitution of certain amino acid with stop codon except that in isolate 3 which located in the distal part of the NS1 gene. Results consent with previous studies has indicated that B19 sequences show a low degree of genetic diversity among B19 strains. While higher degree of variability could be observed in viral strains from distinct epidemiological settings and geographical areas ranging between 0.5 and 4.8% for the most distant isolates [7]. However, other study in Thailand reported that some isolates obtained from their patients with persistent infection exhibit a higher degree of variability in some parts of the NS1 genome.

In the 2013 nt sequenced in current study (NS1 gene), the total mutation sites were 67 accompanied with total amino acid substitutions 42. Similar Japanese study showed four subtypes (A–D) of B19 based on the amino acid substitutions of 10 B19 isolates in Japan. Their report showed that, in the 4145 nt studied (NS1, VP1 and VP2 genes) , 122 mutation sites were found in these B19 isolates, of which 24 were accompanied by amino acid substitution [8]. At the same level, a study on Vietnamese patients [9] confirmed that the gene encoding the NS1 protein are more divergent than the genes encoding the capsid proteins VP1 and VP2 and as expected, at the protein level, the NS1 region was most divergent, whilst the major capsid protein VP2 showed lower differences compared with the prototype B19- au (GenBank accession no. M13178).

The substitutions that emerged and were accompanied with amino acids substitution must be reflected on the nature of the resulting protein and thus affect its effectiveness. NS1 help to access to the limited, non-productive state when B19V infect non-permissive cells by over expression of the viral NS1 protein and little expression of genes for the structural proteins VP1 and VP2. NS1 is cytotoxic when transfected into erythroid cells, but cytotoxicity is abolished when single amino acid mutations are introduced in this domain [10]. In that, further studies are needed to understand the change resulting from these replacements in the protein and how they affect its function, especially on the apoptotic characteristic and the persistance of the virus. This change may also be related to the B19V related cancer.

## Conclusion

Patients with renal failure remain an important health concern and there is a significant association between the disease and the serostatus. The clinical significance of B19 infection in patients who have end stage renal failure and are on hemodialysis is not clear, but this population may be vulnerable to transient aplastic crisis or chronic anemia as a result of viral infection.

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