

PREVALENCE AND CIRCULATING GENOTYPES OF PARVOVIRUS B19 AMONG ADULT SICKLE CELL DISEASE PATIENTS AND BLOOD DONORS IN BAHRAIN

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Abstract: Parvovirus (PV) is a single-stranded DNA virus with a non-enveloped nucleus having a size of 18-26nm. It comprise 60 structural proteins which are of two types. PV B19 transmits through respiratory droplets or blood transfusion but some nosocomial infections also give a chance to PV B19 for causing an infection.

The study was particularly conducted on Sickle Cell Anemia (SCA) patients and focuses on the determination of parvovirus among Bahraini population by relying on their clinical status. Three groups have been taken for the serological study of PV B19. Moreover, around 100 healthy blood donors were taken in which both the males and females were included. However, samples were taken from the emergency unit of Salmania Medical Complex (SMC) and Ibrahim Khalil Kano Center (IKKC).

It is a cohort study based on total 250 people among which 150 were SCA patients, and 100 were healthy individuals. The findings reveal that approximately 40% patients suffered from VOC whereas, only 20% were detected in SCA patients with Non-Vaso-Occlusive Crises (NVOC). It was observed that the percentages were relatively same in both the male and female groups i.e. 33.2%. The percentage for the control group was 33.6 % in which 84 males and 16 females were included.

Parvovirus is a pathogenic virus and sometimes considered as life-threatening specifically for those individuals who have SCA due to which a risk of transient aplastic crisis increases. This virus is only associated with those patients who have some haematological disorders such as haemolytic anaemia and erythro-cytopenia. It is observed that just because of inappropriate features of testing the risk of PV B19 infection can increase. An effective screening test must be performed in the future to reduce the risk of PV B19 infection.

Keywords: Parvovirus B19, sickle cell anemia, prevalence, genotype

Introduction

Parvovirus (PV) B19 belongs to the family of Parvoviridae and is commonly known as PV-B19. The purpose of the current study is to find out the seroprevalence of Parvovirus B19 among the population of Bahrain As this issue has not been previously considered in this region. Moreover, the study has an aim to observe the circulating genotype of PV B19 in general population of Bahrain and the specific population having Sickle Cell Anemia (SCA)

Parvovirus is a single-stranded DNA virus with a non-enveloped nucleus having a size of 18-26nm (Adamson, 2013). The genome of the PV contains around 5000 nucleotides. It contains an icosahedral capsid which is comprised of 60 structural proteins. These structural proteins are of two types: first viral protein shows 5% contribution in making the capsid and the second viral protein makes about 95% of the entire capsid (Palinski, 2016). The epidemiological survey finds that half of the adult population has the immunoglobulin antibodies of this virus in their serum (Aminu & Koledade, 2014). PV B19 is the most common source of infection mostly seen after the winter season. The mode of transmission of infection is the respiratory droplets. However, some

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other routes of transmission also exist such as through blood transfusion and parental transfer. Moreover, some nosocomial infections also give a chance to PV B19 for causing an infection (Moschovi & Vlahopoulos, 2016).

In the human bone marrow and liver, Erythroid Progenitor Cells (EPCs) are found which are timely divided but PV B19 attacks on these cells. On the other hand, a hereditary blood disorder which is caused by the abnormal type of hemoglobin is commonly known as Sickle Cell Anemia (SCA) (DeBaun & Kirkham, 2012). The SCA condition is created due to less oxygen in which Red Blood Cells (RBCs) become rigid and Sickle-shaped and cannot be able to pass through the small capillaries of blood (Li & Karniadakis, 2016). It has been observed that the prevalence of SCA increases the rate of mortality and morbidity around the world. However, there are some hematological disorders which decreased the production of erythrocytes. These disorders are thalassemia, spherocytosis, and anemia which may increase the risk of transient aplastic crisis (Guillaud & Michel, 2012). However, it is commonly known as Reticulocytopenia and can be life-threatening if PV B19 is diagnosed as the source of infection.

According to the research of Koury (2014), PV B19 is responsible for several disease conditions, especially in blood-related infections. It has been found that the primary interest of PV is to replicate in the bone marrow where the division of EPCs is actively found (Claros & Andrades, 2012). As a result of this, the production of erythrocytes is terminated, and ultimately the hemoglobin concentration becomes reduced. However, this erythrovirus (PV) shows an adverse impact on those patients who have erythrocyte disorders either acquired or inherited (Rogo & Rezaei, 2014).

Methods

Study Design

It is a prospective study based on the serological findings and Neopterin detection from an erythrovirus which is commonly known as PV B19. The current study focuses on the determination of parvovirus among Bahraini population and relies on their clinical status. The study was particularly conducted on SCA patients, and the duration of this research was October (2012) -September (2013).

Three groups have been taken for the serological study of PV B19 among which 100 patients were associated with Vaso-Occlusive Crisis (VOC), and 50 were Non-Vaso-Occlusive Crisis (NVOC). Moreover, around 100 healthy blood donors were taken in which both the males and females were included. The target population includes patients from the emergency unit of Salmania Medical Complex (SMC) and Ibrahim Khalil Kano Center (IKKC). During the study, some patient's samples were excluded because they were non-Bahraini, below 18 years of age, and they had hereditary blood disorders such as thalassemia and other blood abnormalities.

Sample Collection And Its Processing

Two controls samples and two blood samples were taken from each patient. The phlebotomist took a sample of 2-3 ml venous blood into the first vacutainer tubes with an anticoagulant which prevents blood clotting. Ethylene Diamine Tetra-acetic Acid (EDTA) is used as an anti-coagulant. In the second vacutainer tube, 2-3 ml whole blood was collected without an anti-coagulant. The purpose of using EDTA is to find out the Complete Blood Count (CBC), erythrocyte count, Hemoglobin concentration (Hb), and the percentage of the reticulocytes. All these tests were performed on the same day when the blood sample was collected. The second sample remained untouched and was allowed to clot. After this, the tube was centrifuged by using Centaur Density Gradient Centrifugation (CDGC). It was set at 3500 rpm (revolution per minute) and the tube was centrifuged for only 10 minutes. After 10 minutes, the serum was separated from the blood and stored at -80 °C till the process started.

Research Methods

Detection Of Immunoglobulin By Using ELISA: ELISA technique was used for the detection of immunoglobulin in human serum or plasma. Here, two immunoglobulins were found by using the blood sample of patients infected with PV B19. IgG and IgM were the immunoglobulins detected by using the Immuno-enzymatic assay. The manufacturer of ELISA was the Nova Tech (Immundiagnostica GmbH, Nova Lisa™, Germany) and the product number of ELISA which was used for this procedure is PARG0370/PARM0370.

Measurement Of Neopterin Concentration: The concentration of Neopterin was also measured from the serum sample of patients and control samples by using ELISA technique. For measuring the concentration of Neopterin, an IBL International GMBH product was used. ELISA is a quantitative assay utilized for the

detection of Neopterin concentration in human serum, urine, and plasma. The test was performed as per manufacturer's instructions.

DNA Extraction: The process of DNA extraction was performed by using molecular techniques. For this purpose, the QIAGEN DNA extraction kit was used which contained two separate kits, first was QIAamp DNA mini and the second was QIAamp DNA blood mini kit. This kit was made in Germany in which the extracted DNA was stored for later use at -20 °C.

Polymerase Chain Reaction Method (PCR): It is a technique used for making copies of small segments of DNA. This method was used for the detection of PV B19 according to the protocol illustrated by Aebischer and Beer (2014). The samples of SCA patients were screened by using the consensus PCR assay with the help of primers present in the NS1 gene. This screening was specifically performed for the detection of erythrovirus DNA. In the current study, the serological and molecular test were performed, and as a result of this, it was found that 100 SCA patients were suffering from VOC, 50 SCA patients were detected as NVOC group, and the remaining 100 were set as control samples. On the other hand, Neopterin concentration was only measured from 88 samples of SCA patients with VOC, and 32 controlled samples. Only four samples were analyzed for detecting the genotype and they showed positive results for PV B19.

Results and Findings

It is a cohort study based on total 250 people among whom 150 were SCA patients and 100 were healthy individuals, who served as control group. The SCA patients were further divided into two groups: one group comprised SCA patients with VOC and the second group consisted of SCA patients with NVOC. The findings reveal that approximately 40% patients suffered from VOC whereas, only 20% of SCA patients were detected to have NVOC. In the VOC patients, the age extended from 18 to 68 years whereas, in the NVOC group, patient's age ranged from 18-71. All patients were Bahrainis only, no other ethnic group was found. The age range of the control group was 21 to 61 years.

The sample distribution was also based on gender differences among the population enrolled in the study. The samples of 51 male patients were found as SCA with VOC whereas, 32 males were found as SCA with NVOC. Around 49 females were found as SCA with VOC, and 18 females have SCA with NVOC. It was observed that the percentages were relatively same in both the male and female groups i.e. 33.2%. The percentage for the control group was 33.6 % in which 84 males and 16 females were included.

Parvovirus Antibodies in the Serum Sample

The samples which showed positive results for PV B19 containing IgG immunoglobulin were 165 (66%) whereas, 9 (3.6%) samples showed the presence of IgM immunoglobulin. The total number of positive SCA samples was 108 (72%) in which IgG was found. Among these SCA samples, 70 suffered from VOC and 38 were NVOC samples, and 57 samples were taken from the control group. The comparison showed the importance of IgG among SCA patients which is commonly known as the anti-parvovirus B19. In contrast, another anti-parvovirus B19 that is IgM was detected only in 6 samples of SCA patients with VOC whereas; IgM was not detected in the SCA patients with NVOC. Around six samples with both the IgG and IgM antibodies showed positive results of PV B19 among which four samples were taken from SCA patients with VOC and two samples from the control group.

After setting the findings mentioned above, all 250 samples were moved towards the molecular DNA extraction of PV B19. When PCR was performed, four samples were found to be positive for DNA presence, two samples were taken from VOC group, one from the NVOC group, and the last sample was taken from the control group. The given table was based on the relationship of parvovirus infection with IgM which is considered as an anti-parvovirus immunoglobulin. These results were compared by molecular findings of genotype with anti-IgM serology. The comparison between SCA patients with VOC and NVOC is presented in the table given below (Table 1).

Table 1. Molecular detection and response of antibodies in serum samples that showed positive results of PV B19

Group tested	PV B19- IgG positive n (%)	PV B19- IgM positive n (%)	PV B19- IgG & IgM positive n (%)	PV B19 viral DNA n (%)

SCD vaso-occlusive crisis	70 (70)	6 (6)	4 (4)	2 (2)
SCD non-vaso-occlusive crisis	38 (76)	0 (0)	0 (0)	1 (2)
100 control	57 (57)	3 (3)	2 (2)	1 (1)

The statistical analysis was also presented below for the correlation among molecular detection and anti-parvovirus B19 (IgM) (Figure 1).

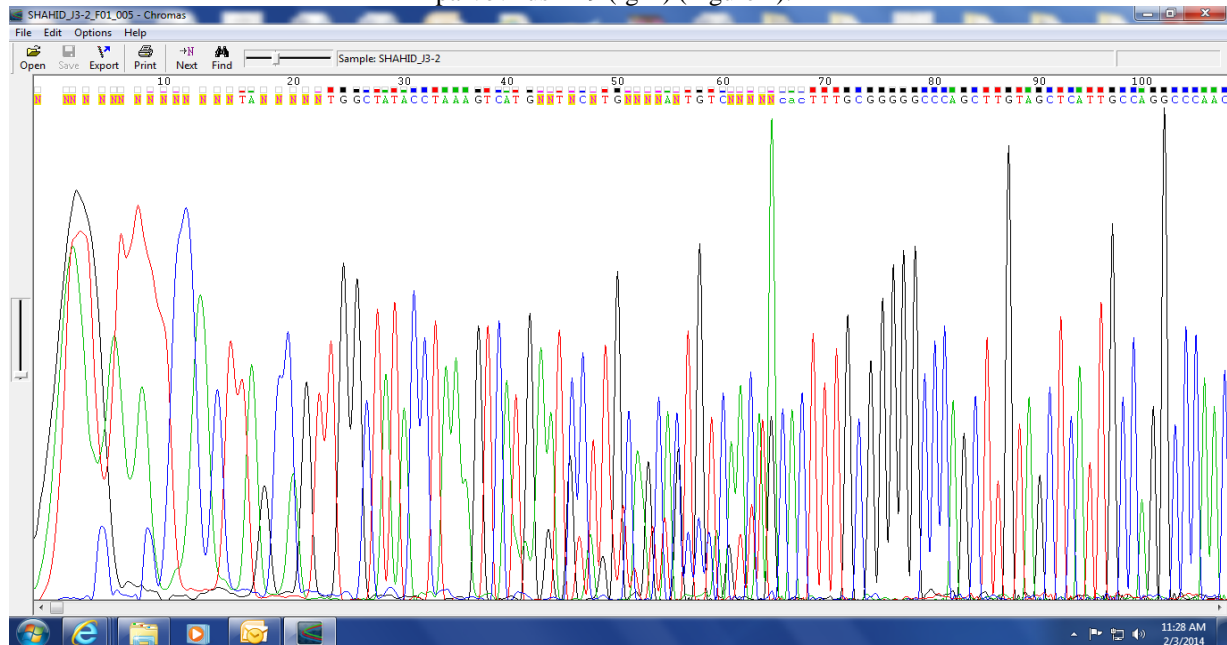


Figure 1. A chromatogram Of PV B19 Genotype 1. The figure analyzed A representative sequence by using the chroma software.

Neopterin Concentration

The concentration of Neopterin was measured with the help of a quantitative assay commonly known as ELISA technique. It was observed that about 88 SCA patients with VOC and 32 patient's sample were taken as control from people with same age and gender. After performing this, the Neopterin level was correlated with the laboratory findings of SCA with VOC group. When comparing the bacterial and viral lab findings, it was found that viral infections showed limited results in SCA vaso-occlusive crisis patients whereas, bacterial infection was found to be more in quantity. Only four samples showed viral infection findings but bacterial infection was more visible among 11 patients, but the Neopterin concentration was observed to be lower.

PCR Results

The total number of SCA patients was 150, but only four samples went through the molecular technique which is most commonly used for the detection of viral DNA or RNA. The findings of PCR showed that all four samples were positive for viral DNA and then they were passed through the process of genotyping and the restriction digestion which revealed that two bands showed the restriction for NS1. NS1 is a viral non-structured protein that is coded with NS gene segments. A figure is given below in which the representation of genotyping and the restriction bands is presented (**Figure 2**). Another figure of PCR is given which represents the process of gel electrophoresis for NS1 and their two restriction bands (**Figure 3**). All the four samples showed positive results and amplified by the Polymerase Chain Reaction (PCR). Further, these amplified segments were sent for the sequencing process by their application on Chroma Software where it was found that sequences were related to

Genotype 1.

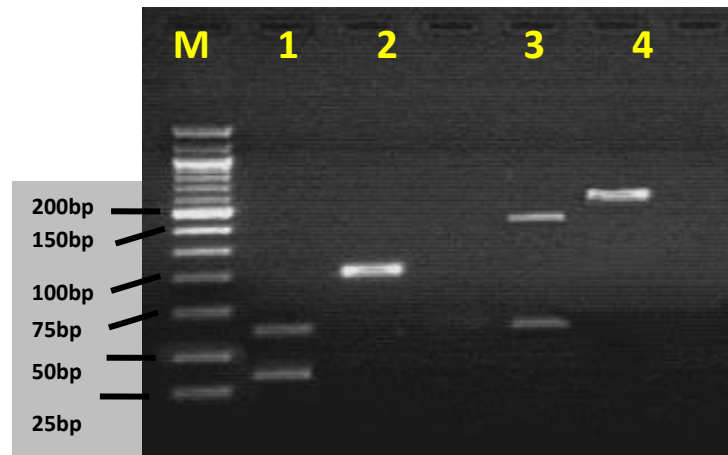


Figure 2. The genotyping and restriction digestion process in which ns1 gene is shown into small segments known as amplicons. *mfel* and *apal* restriction enzymes were used for the amplification of DNA. Two digested segments of NS1 were shown and their size was 67bp and 36bp.

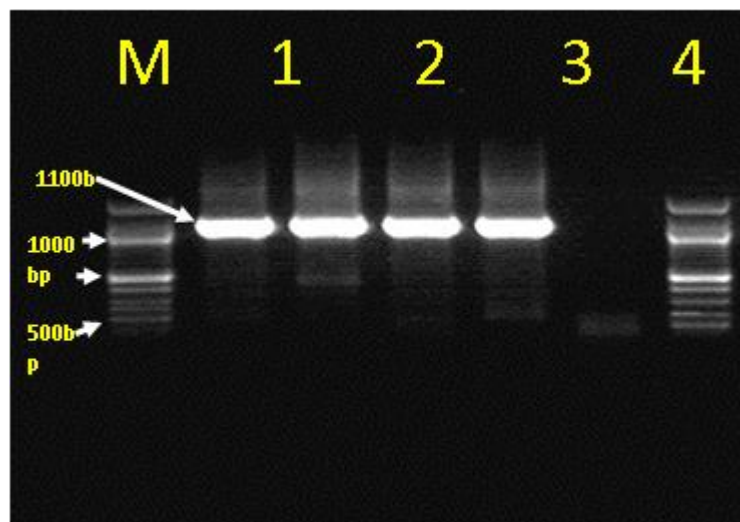


Figure 3. The sequencing process of amplified segments and the sequences were related to genotype 1. The sequencing of amplicons was performed for those patients whose test results found positive to PV B19 by the help of molecular and screening test.

In the end, it was also found that all the positive samples of infected patients in the current study were taken from adults. A 52 years old female and 31 years old male showed SCA condition with VOC whereas, a 31 years old female belonged to NVOC group. All four patients were found to be infected with parvovirus B19; however, these patients did not present the specification of aplastic crisis. However, three samples had anti-parvovirus B19 antibodies IgG and IgM. The lab findings of these three patients revealed that they all had erythro-cytopenia, anemia, and high rate of reticulocytes. The remaining sample was found healthy and gave blood donation at the blood bank of SMC (Salmania Medical Complex).

Discussion

Parvovirus is a pathogenic virus which is occasionally considered as life-threatening specifically for those individuals who have SCA due to which a risk of transient aplastic crisis increases (Turkeltaub & Tying, 2017). It has an ability to destroy the erythroid progenitor cells in human and cause destruction of these cells specifically in the bone marrow which may result in Erythropoiesis (Eaves, 2015). PV B19 was first identified in 1981 and its association with the disease was reported when a patient of sickle cell anaemia went through transient aplastic crisis (Williams & Jarreau, 2012). However, this virus is only associated with those patients who have some haematological disorders such as haemolytic anaemia and erythro-cytopenia. Since the detection of erythrovirus, it has been found that PV B19 is also associated with other diseases including: purpuric eruption on hands and feet in adults, erythema infectiosum, and spontaneous abortion in pregnant women (Bello & Lapadula, 2013). Moreover, PV B19 increases the risk of infection in the intrauterine cavity that can cause asymptomatic effects among females and may also cause several fatal complications (Ornoy & Ergaz, 2017).

Chronic anaemia was also reported in some immune-compromised patients and aplastic crisis in Sickle cell anaemic patients (Al-Najjar, 2013). A study was conducted in the capital of Saudi Arabia at the Armed Forces Hospital (AFH) in March 2001. The purpose of this study was to find out the exposure of PV B19 among patients with haemolytic disorders. For this purpose, lab records of 73 patient's serum were taken and sent for the detection of IgG and IgM by using ELISA technique. The findings revealed that 68% patients showed serological evidence of PV B19 which was due to previous exposure. The study concluded that 68% of PV B19 infected patients could be considered at risk of chronic haemolytic disease (Sener & Afsar, 2012).

Parvovirus can cause interruption in the production of RBCs which could be life-threatening sometimes but not in every case. At the initial stage, it is necessary to transfuse multiple blood bags so that the patients show recovery within two weeks (Hess, 2012). IgG and IgM are the two antibodies found in human body and they are commonly known as the anti-parvovirus immunoglobulin. In the current study, these two antibodies were identified by using a quantitative enzyme linked immunosorbent assay which is commonly known as ELISA. Furthermore, another molecular method was used for targeting the specific segment of the genome of virus; however, this molecular method is known as Polymerase chain reaction (Deng & Wu, 2012). In the current study, Neopterin level was also found in the serum samples of patients because it is an inflammatory marker and plays a significant role in the detection of cellular immune response (Parker & Oh, 2013).

According to the research of Gulf Cooperation Council, it is revealed that many countries including Saudi Arabia, Bahrain, and Kuwait showed high prevalence of Sickle cell anaemia (Barakat-Haddad, 2013). The current study was conducted in Bahrain where inherited haemoglobin disorders are frequently reported among which two most commonly found are; Sickle cell disease and Thalassemia. According to Tsitsikas and Amos (2014), SCA patients are found to be more susceptible for the recurrent infection of PV B19. The prevalence and complications of PV B19 in SCA and thalassemia patients have been reported around the world and it needs urgent development and strategies which provide the preventive measures to those patients who have haemolytic disorders (Chou & Thompson, 2012). These prevention strategies may also require reducing the burden of life-threatening complications linked with parvovirus infections. Therefore, routine inspection of the blood samples should be performed either by general screening method such as by measuring the Neopterin concentration or molecular method like PCR (Skvarc & Kaasch, 2013).

Conclusion

This study reflected the health status of Bahraini population among which high prevalence of PV B19 has been found. This study concluded that 70% SCD patients suffered from vaso-occlusive crisis and 76% patients belonged to non-vaso-occlusive group patients. Further observation revealed that serological indication of parvovirus was also found in high frequency that is 57% in the control group which was consisted of healthy individuals. The current study also observed high frequency of anti-parvovirus immunoglobulin both in the SCA patients and control group. Among the population of Bahrain, the identification of IgM was found to be significantly lower as compared to IgG. Due to the low frequency of IgM, the risk of fatal crisis may increase in such anaemic patients. After screening of blood donor's sample, it was concluded that only 3% donors have IgM antibody which can prevent them from the infection of PV B19. However, it is observed that just because of inappropriate features of testing, the presence of parvovirus DNA has been found highly in the SCA and Thalassemia patients.

Recommendations

In the end, it is recommended from the study that an effective screening test must be performed in the future to reduce the risk of PV B19 infection.

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