Conference Paper

The Serological Findings of Parvo Virus B19 and Neopterin Detection Among Sickle Cell Disease Patients and Blood Doners in the Kingdom of Bahrain

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Abstract

Introduction. Parvovirus B19 (PV B19) is a small, non-enveloped, ss DNA virus with an icosahedral capsid having a size of 18–26 nm. PV B19 transmits through respiratory droplets, blood transfusion and nosocomial infections that have also been documented recently. The virus targets the actively dividing Erythroid Progenitor Cells (EPCs) that are found in the human bone marrow, fetal liver and human umbilical cord.

Methods. The study was particularly conducted on Sickle Cell Disease (SCD) patients and focuses on the determination of parvovirus B19 among Bahraini population by relying on their clinical status. The serological study of PV B19 was performed using Enzyme Linked Immunosorbent Assay (ELISA) technique and includes 150 SCD patients and 100 healthy blood donors in which both males and females were employed. The samples were taken from the emergency unit of Salmania Medical Complex (SMC) and Ibrahim Khalil Kano Center (IKKC).

Results. Of the 150 SCD patients, 100 were with vaso-occlusive crisis(VOC) and 50 non-vaso-occlusive crisis (NVOC). The three groups showed significantly higher percentages of PV B19 IgG but the percentage in SCD was relatively high compared to the control group of age-matched healthy donors – 70% of the VOC patients, 76% of the NVOC cases and 57% of blood donors were found to be IgG sero-positive.

Discussion and Conclusion. PV B19 is a pathogenic virus and sometimes considered as life-threatening specifically for those individuals who have SCD due to which a risk of transient aplastic crisis increases. This virus is only associated with those patients who have some hematological disorders such as hemolytic anemia and erythro-cytopenia. An effective screening test must be performed in the future to reduce the risk of PV B19 infection.

Keywords: Aplastic Crisis, Seroprevalence, Bahraini Population, Genotypes, Vaso-occlusive crisis, Neopterin

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1. Introduction

Human parvovirus B19 infections are prevalent worldwide; and, many studies confirmed its association with Aplastic Crisis (AC) in SCD patients, however, limited data about the virus prevalence have been published. Likewise, no such studies have been published for the Bahraini population despite the high burden of SCD in this area. Therefore, the present study was planned to analyze the prevalence of PV B19 infection in selective Bahraini population, hence, forming a platform for better management of SCD in Bahrain.

PV B19 is a small, non-enveloped, ss DNA virus with an icosahedral capsid having a size of 18-26nm. The capsid is constituted of 60 structural viral proteins (VP) and are of two types: a minor structural protein VP1, that makes up about 5% of the capsid and a major structural protein VP2 that form the bulk of the total capsid composition (Adamson, 2013) and the genome of the PV B19 contains around 5000 nucleotides. (Palinski, 2016). An epidemiological survey observed 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 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).

The actively dividing EPCs are in the human bone marrow and liver, PV B19 attacks on these cells and thus causing erythrocytopenia, decreased erythrocyte production. The hereditary hematological disorders such as spherocytosis, thalassemia, or SCD in particular are at risk of transient aplastic crisis if infected by PV B19 (DeBaun & Kirkham, 2012). SCD is caused by the abnormal type of hemoglobin (Hb), hemoglobin S (HbS). The hemoglobin of red blood cells (RBCs) is distorted as a result of homozygosity for a mutant gene that causes the normal hemoglobin (HbA) to alter to hemoglobin S. The SCD condition is created due to less oxygen in which RBCs become rigid and Sickle-shaped and cannot be able to pass through the small blood capillaries (Li & Karniadakis, 2016). It has been observed that the prevalence of SCD increases the rate of mortality and morbidity around the world. These disorders increase the risk of transient aplastic crisis and can be life-threatening if PV B19 is the source of infection (Guillaud & Michel, 2012).

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 EPCs is actively dividing (Claros & Andrades, 2012). As a result of this, the production of erythrocytes is terminated, and ultimately the hemoglobin concentration becomes reduced. However, the virus shows an adverse impact on those patients who have another erythrocyte disorders either acquired or inherited (Rogo & Rezaei, 2014)

2. Methodology

2.1. Study design

It is a prospective study based on the serological findings and Neopterin detection for the erythrovirus PV B19. The current study focuses on the determination of parvovirus among Bahraini population. The study was particularly conducted on SCD 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 VOC, and 50 were NVOC and 100 healthy blood donors in which both the males and females were included. The target population includes patients from the emergency unit of SMC and IKKC. During the study, some patient's samples were excluded because they were non-Bahraini, below 18 years of age, and they had other hereditary blood disorders such as thalassemia and other blood abnormalities.

2.2. Sample collection and its processing

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, 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.

2.3. 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^{TM}, Germany) and the product number of ELISA which was used for this procedure is PARG0370/PARM0370.

2.4. Measurement of Neopterin concentration

The concentration of Neopterin (NPT) was also measured from the serum sample of patients and control samples by using ELISA technique, for 88 SCD vaso-occlusive crisis, and 32 of the age and gender matching control group. NPT level of SCD vaso-occlusive crisis patients were correlated to the patients' laboratory findings. Laboratory tests for bacterial and viral infections in blood culture, urine culture and respiratory profile were recorded as (present, absent or not done). 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.

2.5. 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.

2.6. Polymerase chain reaction method (PCR)

It is a technique used for making copies of small segments of DNA. This method was used to confirm the detection of PV B19, and done according to the protocol illustrated by Aebischer and Beer (2014). The samples of SCD patients were screened by using the consensus PCR assay with the help of primers present in the NS1 gene. This screening was specifically for the detection of the erythrovirus DNA. Thus, in the current study, the serological and molecular test were performed, On the other

hand, Neopterin concentration was only measured from 88 samples of SCD patients with VOC, and 32 controlled samples. Furthermore, the current study investigated the circulating genotype of PV B19 among the Bahraini population.

3. Results

In the VOC patients, the age extended from 18 to 68 years whereas, in the NVOC group, patient's age ranged from 18-71 years. 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 SCD with VOC whereas, 32 males were found as SCD with NVOC. Around 49 females were found as SCA with VOC, and 18 females have SCD with NVOC. It was observed that the percentages were 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.

3.1. 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 SCD samples was 108 (72%) in which IgG was found. Among these SCD samples, 70 suffered from VOC and 38 were NVOC samples, and 57 samples were IgG positive from the control group. The comparison showed the importance of IgG among SCD 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 SCD patients with VOC. IgM was not detected in the SCD 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 SCD patients with VOC and two samples from the control group.

Based on 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 from VOC group, one from the NVOC group, and the last sample was 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 SCD patients with VOC, NVOC and the control group 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)

3.2. Neopterin concentration

Upon comparison between bacterial and viral laboratory findings in SCD vaso-occlusive crisis patients, it was found that the number of viral infections tested in those patients were limited, 4 only (5.7 %), but, the NPT concentrations were significantly high (p=0.037). On the other hand, bacterial testing were more in number 11 (15.7%), nevertheless, the NPT concentrations were lower. **Table 2** shows the comparison between NPT positive samples of SCD vaso-occlusive

crisis patients and the healthy controls samples, the correlation was significantly high (p=0.000), as, all control samples were negative for NPT incidence.

Table 2: The comparison between NPT positive samples of SCD vaso-occlusive crisis patients and the healthy controls (p=0.00 by Fisher's Exact Test).

NPT	% SCD (n) vaso-occlusive crisis	% Control (n)
Positive	80.7 (71)	0 (0)
Negative	19.3 (17)	100 (32)
Total	100 (88)	100 (32)

3.3. PCR results

All the 250 clinical samples were extracted, DNAs from the serum samples were amplified by PCR using consensus primers. Out of 150 SCD patient 3 (2%), (2 from SCD vaso-occlusive crisis patients and 1 from SCD non-vaso-occlusive crisis patients) and 1% (1/100) of the controls recruited for this study were found positive for viral DNA by PCR. All the 4 positive samples were subjected to genotyping procedure and the restriction

digestion revealed 2 bands each for NS1 restriction (36 & 67 bp of the 103 bp uncleaved fragment and VP1u restriction 149 and 55 bp of the 204 bp uncleaved fragment).

Representative gel electrophoresis for NS1, VP1u bands and their restriction products were photographed and are shown in **(Figs. 1)** The 4 positive results were amplified by nested PCR **(Fig. 2)** and sent for sequencing. A representative chromatogram of the sequences received from the Genoscreen is shown as **(Fig. 3)**. Analyses of our sequences with "Chromas", "Blast" and "Clustal W" revealed that the sequences belonged to Genotype 1.

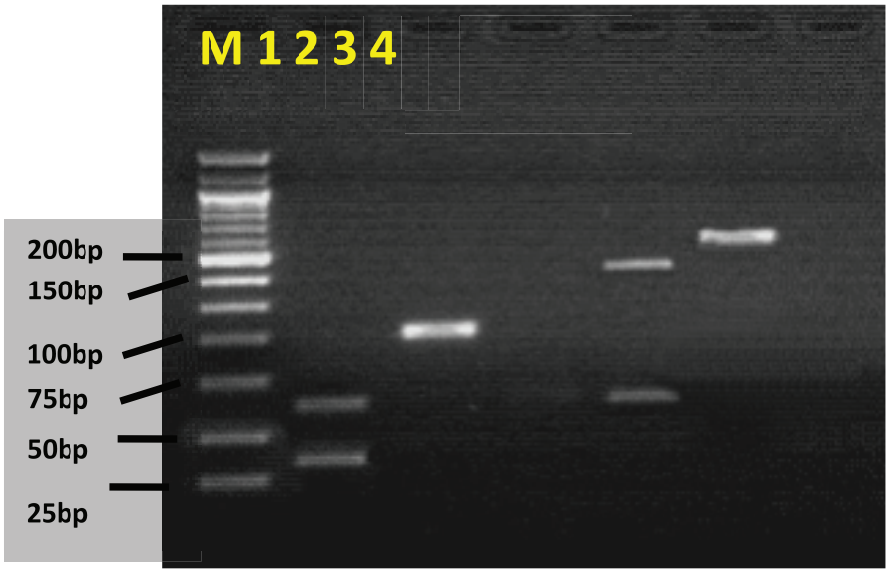

Figure 1: The genotyping and restriction digestion process in which NS1 gene is shown into small segments known as amplicons. *Mfel* and *Apal* restriction enzymes were used for the amplification of DNA. Two digested segments of NS1 were shown and their size was 67bp and 36bp.

4. 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 & Tyring, 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 anemia went through transient aplastic crisis (Williams & Jarreau, 2012). However, this virus is only associated with those patients who have some hematological disorders such as hemolytic anemia and erythro-cytopenia. Since the detection of erythrovirus, it has been found that PV B19 is also associated with other diseases including: purpuric

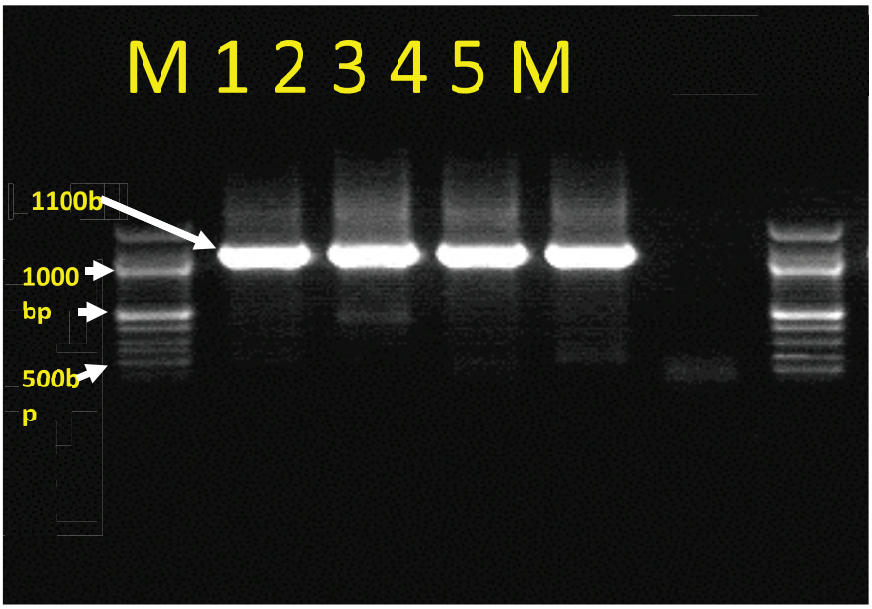

Figure 2: The sequencing process of amplified segments and the sequences were related to genotype 1. The sequencing of amplicons was performed for those patients whose test results found positive to PV B19 by the help of molecular and screening test.

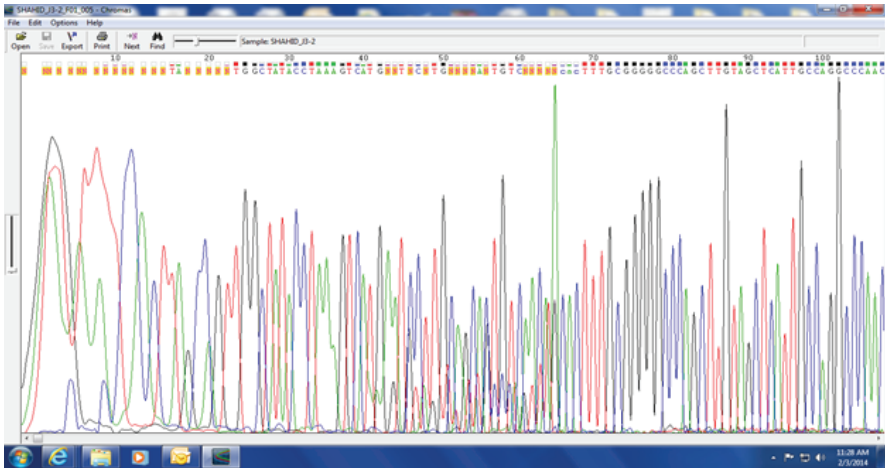

Figure 3: **Representative Chromatogram of PV B19 Genotype 1.** The figure shows a representative sequence analyzed using the **Chromas software.**

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 anemia was also reported in some immune-compromised patients and aplastic crisis in Sickle cell anemic patients (Al-Najjar, 2013). A study was conducted in the capital of Saudi Arabia at the Armed Forces Hospital (AFH) in March 2001, to find out the exposure of PV B19 among patients with hemolytic 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 hemolytic 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 against PV B19 and they are commonly known as the anti-parvovirus immunoglobulin. In the current study, these two antibodies were identified by using ELISA. Furthermore, another molecular method was used for targeting the specific segment of the genome of virus. 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 anemia (Barakat-Haddad, 2013). The current study was conducted in Bahrain where inherited hemoglobin 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 SCD 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 hemolytic 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).

5. 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 consisted of healthy individuals. The current study also observed high frequency of anti-parvovirus immunoglobulin both in the SCD 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 frequency of IgM, the risk of fatal crisis may increase in such anemic 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 to be high in the SCD and Thalassemia patients. It is concluded 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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