Introduction

Human parvovirus (B19) is member of genus Erythrovirus belonging to family Parvoviridae encoding non-structural protein (NS-1) along two viral capsid protein, VP1 and VP2. VP1 protein is found in lymphocytes, neutrophils, macrophages and lymphocytes. B19 since its accidental discovery during healthy blood donors screening for hepatitis B [1] has been documented as significant cause of morbidity and mortality among various patients of different age groups [2]. B19 is the causal agent for diseases like transient aplastic crisis, arthralgia and chronic pure red cell aplasia [3]. B19 can be found in respiratory secretions and blood of infected persons. B19 transmission may occur by transfusion and infectious blood products [4]. In pregnant women, B19 infection occurs vertically from mother to fetus resulting in fetal red blood cell lysis, hydrops fetalis, spontaneous abortion and fetal mortality [5]. B19 causes erythema infectiosum in children that leads to different clinical complications [6]. B19 infection may lead to glomerulonephritis, myocarditis, hepatic failure, peripheral neuropathies [7] and it may also lead to red cell aplasia and less frequently neutropenia and thrombocytopenia in immunocompromised patients [8]. Few reports highlight the role of B19 infection in association with various clinical syndromes and neurological disorders. However, its role is unclear and not yet completely understood. Literature review revealed 89 articles describing 129 myalgic encephalomyelitis patients related to central 79 (61.2%) and peripheral nervous 41 (31.8%) manifestations [9]. In another report, it was concluded that acute encephalitis and encephalopathy are most common reason accounting an overall 38.8% of all B19-associated neurological manifestations [10]. Specific antibodies (IgA, IgG and IgM) are produced in response to any infection. IgG antibody sustain probably for several months [11]. B19 infection diagnosis is possible in case of initial infection specifically by IgM detection [12]. An immuno-histochemical approach is routinely used in diagnosis of B19 infection [13]. Apart from immunoglobulin detection, different molecular approaches like dot blot hybridization and PCR is commonly used for B19 DNA detection [14]. Nested PCR, a reliable, sensitive and rapid approach is used in B19 infection detection. Though the association between B19 and neurological manifestations has been explained, still there is lack of studies regarding B19 prevalence and associated neurological risk factors among individuals particularly in Saudi Arabia. Current
study aimed to assess the prevalence of IgG and IgM using ELISA and PCR based approach in B19 neurological infected patients. This study also aimed to assess the B19 associated risk factors among neurological patients.

Materials and Methods

Ethical approval

Ethical approval of study was obtained from the Institutional Review Board (IRB) Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia. The patients enrolled in study were informed about purpose of study. Informed consents were obtained from all the patients enrolled in study.

Study population and sample collection

One hundred and forty randomly collected blood samples without known genders from different hospitals of Makkah were enrolled in this study. Sampling was performed between February and August 2015. All randomly selected patients in this cross sectional study were Saudi national (age ranged between 1-70 years; mean age 23 + 5 years). From each enrolled patient, 10 mL blood was collected in sterile tubes. Each sample was further aliquoted in 1.5 mL tube containing 50 µl of 10% Tween-20 (Tw20). All blood collection vials were thoroughly mixed by inverting 15-20 times and then kept at room temperature for 15 minutes. All samples were centrifuged (2000g, 10min) at room temperature. Supernatant was transferred into another sterile tube and stored immediately at -80°C until used for immunoglobulin testing and nested PCR analysis.

Detection of B19 specific IgG and IgM antibodies

All the 140 consecutive samples were screened for both IgG and IgM antibodies using recombinant antigens (MIKROGEN DIAGNOSTIK recomLine Parvovirus B19 IgG Cat# 4472, recomLine Parvovirus B19 IgM Cat# 4473, Germany) as instructed by manufacturer guidelines. The samples for immunoglobulins detection were tested using ELISA system (Human Diagnostics, Germany). Values were measured as ratio of serum sample optical density (OD 450nm) to cutoff’s OD450nm. An index value > 1.0 or <0.9 indicated sample positivity or negativity respectively.

Amplification of viral DNA

Viral DNA extraction was performed from serum samples using viral DNA extraction kit (QIAamp DNA Mini Kit, Cat# 51304, Germany) as per manufacturer instructions. Extracted DNA samples were further cleaned up before PCR to avoid any inhibitors, enzymes and others contaminations using DNA Clean and concentrator Kit (ZYMO RESEARCH kit DNA Clean and Concentrator-25 Cat# D4033, USA) following manufacturer guidelines. Eluted DNA was then stored immediately at −20°C.

Samples were amplified through nested based PCR approach. First round nested PCR was performed in 25 µl reaction volume containing 5 µl DNA with 2 µl (10pmol) of each primer; outer sense oligonucleotide (5’AATACACTGTGTTTTATGGGCG 3’) (position1392–1422) and outer antisense oligonucleotide (5’CCATGCTGGTTATAAC CACAGGT 3’) (position 1659-1682), 0.5 µl of 2mM dNTPs mix, 0.75 µl of 25mM MgCl₂, 1.25U Taq polymerase (Thermo Scientific™ DreamTaq DNA Polymerase, Cat# EP0702), 2.5 µl 10x buffer using thermocycler (Applied Biosystems, Veriti 96 well thermal cycler, USA). First round of nested PCR was performed at 5min at 94°C, followed by 35 cycles of denaturation at 95 °C (40 s), annealing at 54 °C (45 s) and extension at 72 °C (30 sec). A volume of 4 µl from first round nested PCR product was used subsequently in second round of nested PCR. Second round of nested PCR was performed by 35 amplification cycles with similar amplification profile as that of first round. Second round of nested PCR was performed with inner sense nucleotide (5’ATGAAAACCTTCCATATATGATGTA G 3’) (position 1498-1525) and inner antisense nucleotide (5’CTAAATGCTTTGCGAGCT CTAC 3’) (position 1576-1600). Amplicons were resolved using 2% agarose gel electrophoresis and analyzed by UV light.

Statistical analysis

Data was statistically analyzed using SPSS (ver 16.0). To check the significance, 95% confidence interval (CI) was used and P-values < 0.05 were considered significant statistically.

Results

The study included total 140 neurological patients. The age distribution of our cases included 20 patients (14.2%) between 31-40, and 41-60 followed by 19 (13.5%) patients less
than 1 year old while 11-20, 21-30, 51-60, 1-10 and 61-70 years comprised 12.8%, 12% and 15% of study population respectively. Initially all the 140 samples were screened for both antibodies (IgG and IgM) detection.

The prevalence of IgG and IgM were analyzed according to the age groups. IgG and IgM displayed similar proportion among different age group <1 year (10.5%), 1-10 (8.3%), 11-20 (5.6%), 21-30 (11.1%) while IgG proportion of 10% and 5.6% measured between 31-40 and 51-60 age group respectively. Out of total 140 samples tested, prevalence of IgG and IgM in our study was 6.4% and 3.5% respectively (Table 1). Presence of viral proteins terminals i.e., VP-N (N-terminal half of the structural proteins) and VP-C (C terminal half of structural proteins), NS-1 (non-structural protein) and VP-1S (unique region of VP1 (VP1u)) by recom Line dot blot assays confirmed the samples positivity and negativity (Figure 1). Subsequently, out of 140 serum samples, 9 samples positive for IgG and IGM were confirmed by nested PCR assay.

Molecular detection confirmed 6.42% B19 DNA seropositive samples of neurological patients. Our study showed that history of blood transfusion is key factor for B19 infection in neurological patients who have had previously undergone blood transfusion (95% CI=0.4-4.2, OR=1.9:1) (Table 2). Other related risk factors like organ transplantation, pregnancy and thalassemia disclosed no significant association found with B19 in neurological patients enrolled in this study.

### Discussion

B19 is an infectious agent and its transmission occurs through the blood, respiratory route and blood products [17]. The aim of the current study was the assessment of B19 prevalence among neurological patients in western region of Saudi Arabia. Current study reported an overall less prevalence of IgG and IgM like similar studies reported in past [18]. This difference may be due the fact that our results are based upon small sample size. Limited sample size may not provide accurate prevalence information of that population. Hence large sample size is desirable to get the more reliable and higher confidence interval.

| Age groups (years) | Number (n) | IgG positive n (%) | IgG negative n (%) | 95% CI | P |
|-------------------|------------|-------------------|-------------------|-------|---|
| <1 year           | 19         | 2 (10.5)          | 17 (89.4)         | 35-62 | 0.475 |
| 1-10              | 12         | 1 (8.3)           | 11 (91.6)         | 31-59 |     |
| 11-20             | 18         | 1 (5.6)           | 17 (94.5)         | 37-61 |     |
| 21-30             | 18         | 2 (11.1)          | 16 (88.8)         | 41-67 |     |
| 31-40             | 20         | 2 (10)            | 18 (90)           | 42-69 |     |
| 41-50             | 20         | 0 (0)             | 20 (100)          | 21-52 |     |
| 51-60             | 18         | 1 (5.6)           | 17 (94.5)         | 39-70 |     |
| 61-70             | 15         | 0 (0)             | 15 (100)          | 18-46 |     |
| Total             | 140        | 9 (6.4)           | 131 (93.5)        |      |     |

| Number (n) | IgM positive n (%) | IgM negative n (%) | 95% CI | P |
|------------|-------------------|-------------------|-------|---|
| <1 year    | 19                | 2 (10.5)          | 17 (89.4) | 35-62 | 0.381 |
| 1-10       | 12                | 1 (8.3)           | 11 (91.6) | 31-59 |     |
| 11-20      | 18                | 0 (0)             | 18 (100) | 22-51 |     |
| 21-30      | 18                | 0 (0)             | 18 (100) | 22-51 |     |
| 31-40      | 20                | 1 (5)             | 19 (95) | 38-69 |     |
| 41-50      | 20                | 0 (0)             | 20 (100) | 21-52 |     |
| 51-60      | 18                | 1 (5)             | 17 (94.4) | 39-70 |     |
| 61-70      | 15                | 0 (0)             | 15 (100) | 18-46 |     |
| Total      | 140               | 5 (3.5)           | 135 (96.4) |      |     |

Note: n=frequency (number); CI=confidence interval

Previous studies elucidated the B19 associated risk factors during infection and transmission among neurological patients [19].

The overall prevalence rate of B19 IgG antibody reported in this study was 6.4%, which is less than any studies reported so far. Previous studies also reported low prevalence of IgG (16.2%) [20]. These discrepancies in results could be probably due to the B19 replication and its tropism. Such alterations in prevalence can be attributed to differences in the sensitivity and specificity of the assays and detection methods used.
Current Health Sciences Journal  
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Figure 1. PCR for HPVB19. Lane 1 and 13 show 100bp DNA ladder, Lane 2-3, 5-11 indicating total 9 PCR positive samples with product size of 102bp. Lane 4 and Lane 12 represent positive and negative control respectively.

Table 2. Possible risk factors associated with HPVB19 among patients.

| Variable              | Number (n) | Positive (%) | p-value | OR  | 95% CI |
|-----------------------|------------|--------------|---------|-----|--------|
| Organ transplant      |            |              |         |     |        |
| No                    | 132        | 12 (9.0)     | 0.1     | 1   | 0.7-1.8|
| Yes                   | 8          | 5 (62.5)     |         | 1.2 |        |
| History of transfusion|            |              |         |     |        |
| No                    | 112        | 25 (22.3)    | 0.04    | 1   | 0.4-4.2|
| Yes                   | 28         | 11 (40)      |         | 1.9 |        |
| Pregnancy             |            |              |         |     |        |
| No                    | 128        | 15 (11.7)    | 0.80    | 1   | 0.7-1.6|
| Yes                   | 12         | 02 (16.6)    |         | 0.9 |        |
| Thalassemia           |            |              |         |     |        |
| No                    | 133        | 9 (6.7)      | 0.1     | 1   | 0.7-1.8|
| Yes                   | 7          | 3 (42.8)     |         | 1.2 |        |

Note: OR=Odds ratio; CI=Confidence interval

A study performed in India revealed the IgG antibody prevalence of 34% in hematologic malignancies like lymphoma and leukemia, which is comparable to present study [24].

An overall prevalence of IgM in our study was 3.5%, it may attributed due to short appearance of IgM during an infection [25] resulting in low B19 prevalence or its low seropositivity as in case of our study. Our study showed that IgM prevalence was higher in age groups of 1-10, 31-40 and 51-60 years. It can be concluded that IgM prevalence in current study reflects prevalence of acute infection while IgG sero-positivity along IgM sero-negativity indicate previous B19 infection [26]. Detection of IgM in serum samples is supported by previous reports on blood, transfusion, pregnancy and as well as neoplasm cases [27]. Studies conducted in past unveiled higher B19 prevalence rates of 61.4% and 75.6% in pregnant women in countries like Norway [28] and Iran [29] respectively. But current study divulged minimum prevalence of 16.6% in pregnant women than previous study done in Saudi Arabia with prevalence of 26.3% (< 20 years age) and 62.5% (>38 years) [22]. This low prevalence rate of B19 in pregnant women in our study showed that IgG prevalence may be recognized as an age dependent factor [30]. Epidemiological studies based upon blood

Our study also demonstrated that IgG seropositivity changes among different age groups. B19 infection is transmitted from mother to fetus so its incidence seems to be higher in pregnant women (21-30 years) and children <1 year [21-22], 21-30 and 31-40 years age group [18]. Previous reports suggested an overall low IgG prevalence but our studies indicate its higher incidence in 51-60 years age group that is probably due to blood transfusion, which plays key role in virus transmission [23].
donations conducted in past revealed variable prevalence of B19 infection among different countries [31]. Earlier studies reported wide range of B19 infection with minimum B19 prevalence in beta thalassemia and hemophilia patients with similar mean age groups as in case of our studies [18, 33]. In our survey, mean age was 30 years with an overall prevalence of 42.8%, associated with thalassemia B19 infected patients. The higher prevalence of B19 IgG antibodies in thalassemia patients in our study may be because of multiple blood transfusion and furthermore interprets its role in B19 infection [23].

The molecular approaches are the most sensitive and reliable diagnostic methods for detection of B19 DNA. Most reported and established PCR procedures have capability to detect B19 DNA (1-100 copies/ml) [34]. In contrast to single primer set simplification, using nested PCR it can reveal between 10-100 copies of B19 DNA. Risk of cross over carrying contamination during sample handling from first round to second round poses important issue in nested PCR [35]. In present study, all 140 serum samples either sero-positive or sero-negative were analyzed through nested PCR for the confirmation of B19 DNA. In our study, B19 was not confirmed in all samples by PCR, which were even positive for IgG and IgM ELISA simultaneously suggesting the low risk of B19 transmission via blood transfusion and organ transplant [33]. Previous studies from symptomatic patients analyzed by the two approaches PCR and ELISA reported 8.8% positive simultaneously [36]. Another study performed using nested PCR approach also reported single sample positive for B19 DNA after testing 100 samples [37]. Both ELISA and PCR suggests that B19 DNA positive patients especially both IgG and IgM antibodies display acute infection in neurological patients, however those with only IgG show persistent and chronic B19 infection while others with IgM only show no more persistent infection [38]. In view of previous studies, our findings agree with previous studies reported PCR positivity of less than 10% in symptomatic individuals [11]. Our results showed no significant association regarding thalassemia and pregnancy associated risk factors. Further studies with larger sample size are required to evaluate the B19 associated risk factors. It has been concluded that B19 persistence mechanism may be due to lack of antibody production against B19 or possibly due to defect in antigen presenting cells. Our study may have limitations like small population size, demographic variables, nationality and gender. These probably lessen the results significance and study power and hence the results may not be generalized to the entire Saudi population regarding B19 infection in neurological patients.

Conclusion

In conclusion, B19 prevalence was detected at very low rates among patients with neurological infections. There are no guidelines for B19 screening during blood donations. Preventive measures using serology approaches like ELISA coupled with sensitive nucleic acids testing PCR should be implemented while screening during blood donations to neurological patients in order to avoid nosocomial transmission of B19. More studies are needed in Saudi Arabia to understand the etiological role of B19 prevalence among patients with underlying neurological infections.

Author Contributions

AKJ Conceived the study, designed the experiments and wrote first draft of manuscript, AJ wrote, reviewed and approved final manuscript, SSA. Analyzed the data and interpreted results, FRA and MHK assisted in sampling and medical history of participants. All authors have read and approved the final version of manuscript with no allegations.

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Conflict of interests

None to declare.

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