ORIGINAL ARTICLE

Detection of Human Parvovirus B19 Infection in Cases of Systemic Lupus Erythematosus Attending Benha University Hospitals

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ABSTRACT

Background*: Systemic lupus erythematosus is an autoimmune disease. Parvovirus B19 infection can break tolerance to self-DNA and promote pathogenesis of autoimmunity and might induce either idiopathic SLE in a person who is genetically susceptibl or it might induce a SLE-like picture.* ***Objectives****: Detection of PV-B19 DNA and it's IgM and IgG antibodies in the serum of SLE patients and in apparently healthy volunteer in Benha University Hospitals.* ***Methodology****: The study was conducted on 60 subjects classified into 2 groups: Group I: Including 40 SLE patients fulfilling SLICC Classification Criteria , 20 of them were in exacerbation state and the other 20 cases were in remission state and Group II: Including 20 age and sex matched apparently healthy volunteers serve as a control group . All patients were subjected to full history taking, clinical examination and laboratory investigations. PV- B19 IgM and IgG were measured using anti PV- B19 ELISA kits and PV-B19 DNA was detected by using real time polymerase chain reaction (PCR).* ***Results:*** *PV- B19 DNA was detected in 9 cases (45%) with active disease, Out of the 9 patients with PV- B19 DNA, only 1 had positive PCR results alone , 1 had positive PCR and IgM results and 7 had positive PCR, IgM and IgG results. on the other hand, PV- B19 DNA was detected in only 1 case (5%) out of 20 remittent cases (p=0.003). Active cases were significantly associated with higher frequency of IgM and IgG when compared to remittent cases (p=0.010, 0.020, respectively).There was significant differences in SLEDAI between active and remitted cases (p<0.001).* ***Conclusion****: Combined use of PCR and ELISA are needed for accurate diagnosis of PV- B19 infection.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is the prototype of autoimmune disease of unknown etiology[1].

Genetic, epigenetic, hormonal and environmental factors alter the function of practically every cell of the adaptive and innate immune response to advance the autoimmune response and cause inflammation and eventually damage in multiple organs[2] .

Infection is one of the major causes of morbidity and mortality in SLE patients[3]. Infection can act as an environmental trigger for onset of SLE in a genetically susceptible individual or can lead to flares in a known case of SLE , Certain microbial agents (viral, bacterial, parasitic or fungal) may play a role in the pathogenesis of SLE , There are multiple mechanisms by which host infection by a pathogen can lead to autoimmunity in SLE, e.g : molecular mimicry, bystander phenomenon, epitope spreading or other mechanisms[4].

Parvovirus B19 (*PV-B19*) is a small, non-enveloped virus that has a diameter of approximately 23–26 nm and contains a linear single-stranded DNA genome of 5.6 kb, flanked by two identical terminal hairpin structures. PV- B19V belongs to Erythroparvovirus of the Parvoviridae family [5].

The transmission of parvovirus B19 occurs mainly via respiratory droplets but it can also spread by contaminated blood, organ transplantation and from mother to fetus [6] .The high-risk period for spread is early in the acute phase of infection. Infected patients are most contagious during this phase &patients can be asymptomatic or present with non-specific flu-like illness[7]. While the typical symptoms attributed to PV-B19 infection can be the results of other infective processes[8]. PV- B19 has the potential to induce transient autoimmune response manifesting as lupus-like symptoms such as malar rash, fever, arthropathy, myalgia, cytopenia, pleuritis, myopericarditis and acute hepatitis[9]. The family history, a self-limiting disease

course , absence of discoid lesions, alopecia, Raynaud phenomenon and autoimmune hemolytic anemia and presence of short-lived, low levels of antibodies (particularly ANA& anti-dsDNA) may help in diagnosis of PV-B19 infection but a laboratory diagnosis is required for accurate diagnosis of PV-B19 infection [10].

The present work aims to detect human PV-B19 DNA and it's IgM and IgG antibodies in the serum of cases with SLE and in apparently healthy volunteers in Benha University Hospitals.

METHODOLOGY

This study was carried out in Medical Microbiology and Immunology Department of Benha Faculty of Medicine . The patients were selected from the inpatients and the outpatients clinics of the Rheumatology, Rehabilitation and Physical Medicine Department of Benha University Hospitals during the period from December 2019 to march 2021. Approval of the Ethical Committee was obtained from Faculty of Medicine , Benha University and an informed consent was obtained from all subjects enrolled in the study.

Subjects and samples:

This study was conducted on 60 subjects (40 patients and 20 apparently healthy controls) classified into two groups as follow:

- *Group I:* Including 40 SLE patients sub divided in to two sub groups:
  - A: Including 20 SLE patients in exacerbation state fulfilling SLICC Classification Criteria for SLE, they were 2 (10%) males and 18 (90%) females .Their ages ranged between 16-55 years with mean of 33 years.
  - B: Including 20 SLE patients (in remission state) fulfilling SLICC Classification Criteria for SLE. They were 6 (30%) males and14 (70%) females .Their ages ranged between 10-65 years with mean of 22.5 years.
- *Group II:* Including 20 age and sex matched apparently healthy volunteers serve as a control group.

All SLE patients and controls were subjected to: full history taking, thorough clinical examination, laboratory assessment: CBC, ESR, ANA, Anti- ds DNS, C3, C4, 24 h protein (gm/24 hrs), serum creatinine, serum urea, assessment of disease activity SLEDAI score, detection of antiparvo B19IgM and antiparvo B19 IgG by Enzme Linke Immunosorbant Assay (ELISA) and detection of PV-B19 DNA by real time-PCR

***Blood sampling*:** Venous blood samples were taken under sterile conditions in serum separator tubes from each participant. Samples were centrifuged for 15 minutes at 1000 x g. The samples were divided into three aliquotes for anti-parvovirus B19 IgM antibodies quantification, anti-parvovirus B19 IgG antibodies quantification and real time PCR analysis and stored at -80°C until further use.

Quantification of anti-parvovirus B19 IgM antibodies serum levels:

Anti-parvovirus B19 IgM antibodies serum levels were quantitated by using human antiparvovirus B19 IgM ELISA Kit (DRG Instruments GmbH) and procedure was done according to the manufacturer's instructions.

Quantification of anti-parvovirus B19 IgG antibodies serum levels:

Anti-parvovirus B19 IgG antibodies serum levels were quantitated by using human antiparvovirus B19 IgG ELISA Kit (DRG Instruments GmbH) and procedure was done according to the manufacturer's instructions.

Detection of parvovirus B19-DNA by real time PCR:

First , viral DNA extraction using **G-Spine^{TM} Total DNA Blood Extraction Kit (iNTRON Biotechnology$^{®}$)** and procedure was done according to the manufacturer's instructions then Viral DNA was amplified by real time-PCR, the primers used for amplifications are shown in (table 1).

Table1: Sequences of the primers used in real time PCR for detection of Parvovirus B19:

Name	Sequences (5' > 3')	Tm	Primer length
B19V forward primer	**5** TGCAGATGCCCTCCACCCA 3'	**50,4**	**19**
B19V reverse primer	**5′-** GCTGCTTTCACTGAGTTCTTC 3	**50,4**	**21**

The reactions were set up with Universal PCR Master Mix (**Real MODTM Green W2 2X q PCR mix (iNTRON Biotechnology ®**)

The PCR reaction mix was prepared as follow (25 ml reaction) :

10µl of single strand cDNA was mixed with 12.5µl of QuaniTect SYBR Green master mix, 1.25 µl of forward primer and 1.25 µl of reverse primer.

***Amplification was done on a Rotor-Gene Q real-time PCR machine (Qiagen; Germany) using the following PCR thermal cycle conditions*:**

Initial hold at 95ºC for 10 min ,followed by 40 cycles(including : denaturation for 20 sec at 95° C, annealing for 40 sec at 58° C and elongation for 30 sec at 72° C).

Standard curve:

Absolute quantification was determined by generating a synthetic standard curve that was designed based on the 104bp fragment from NS1viral region of PV-B19 (5'TGCAGA TG CCC TCCACCCAGACCTCCAAACCACCCCAATTGTCA CAGACACAGTATCAGCAGCAGTGGTGGTGAAA GCTCTGAAGAACTCAGTGAAAGCAGCT-3´).Serial dilutions from 10^8 to 10^1 copies /mL were used to generate the calibration curves for the qPCR assays .

The number of copies in the samples was calculated with Rotor-Gene Q Software taking into account the average of CT values obtained with respect to the standard curve. Viral load are expressed in copies per milliliter (cps/mL) of serum.

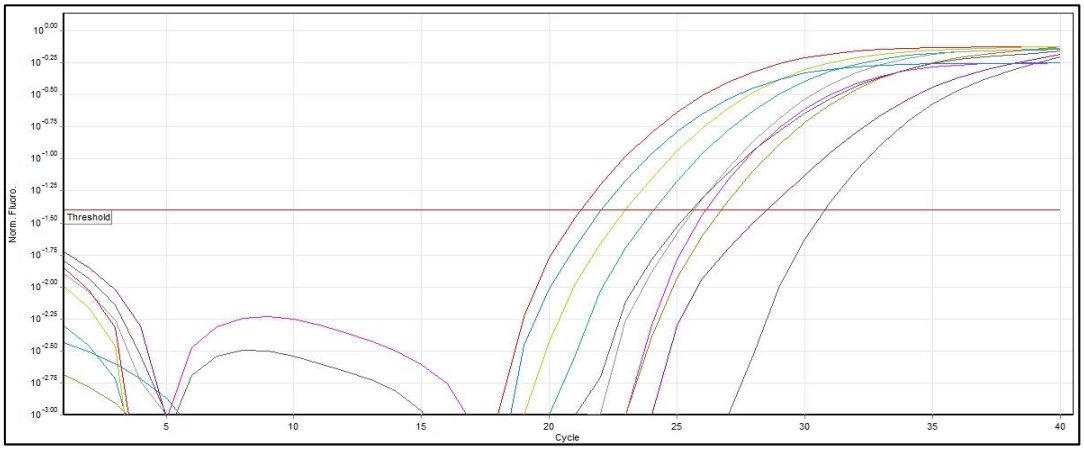

Fig. 1: Amplification curve of samples using SYBR Green in real time PCR

Statistical Analysis

The collected data was revised, coded, tabulated using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Data were presented and suitable analysis was done according to the type of data obtained for each parameter

RESULTS

Group	Activity	Markers	Number
Control		Negative PCR+IgM+IgG	16
		Positive IgG only	2
		Positive PCR &IgG	1
		Positive PCR &IgM	1
SLE	Remission	Negative PCR &IgM &IgG	15
		Positive IgM only	1
		Positive IgG only	3
		Positive PCR &IgG	1
	Active	Negative PCR&IgM&IgG	6
		Positive IgG only	5
		Positive PCR only	1
		Positive PCR&IgM	1
		Positive PCR&IgM&IgG	7

Fig. 2: Stratification of studied subjects according to Parvo virus B19 results

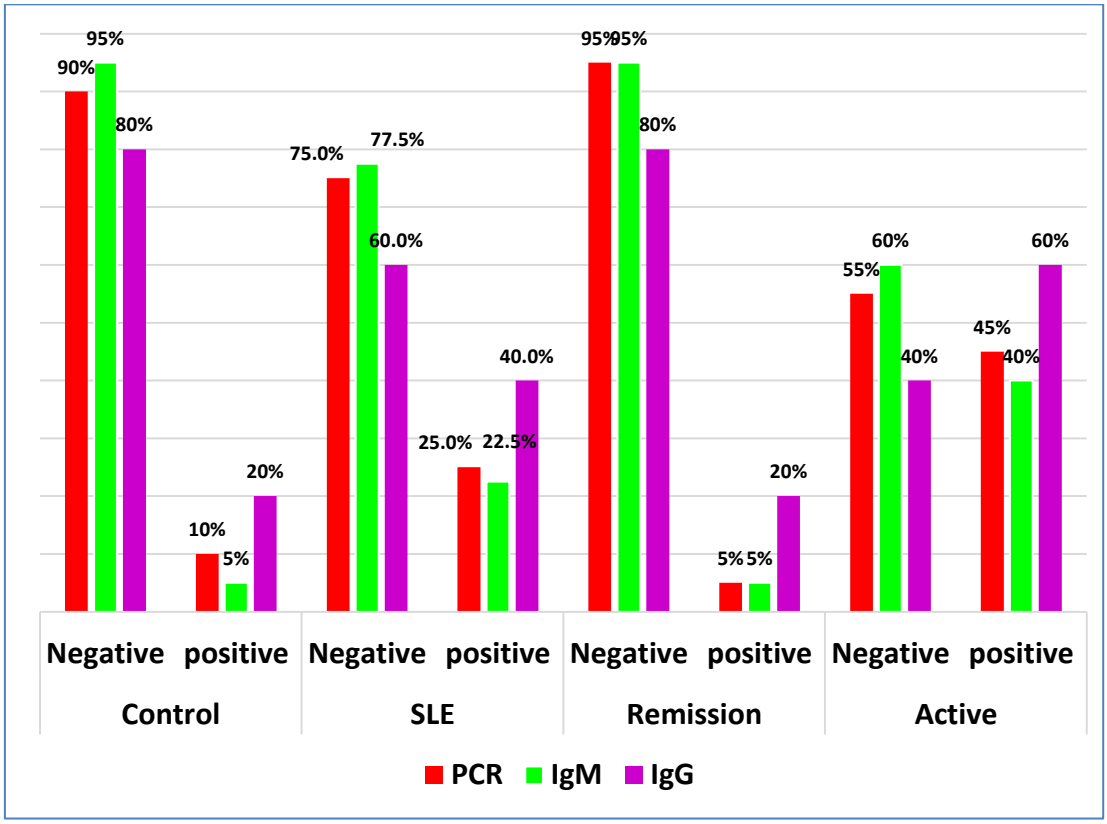

Fig. 3: PCR, IgG and IgM positivity in all studied groups

- Regarding the control group : 2 subjets had positive PCR (one of them had positive PCR and IgG results, the other subject had positive PCR and IgM results) ,2 subjects had positive IgG results only and 16 subjects had negative (PCR ,IgM and IgG) results.
- Regarding the remittent SLE cases: 1 case had positive PCR and IgG results ,15 cases had Negative (PCR ,IgM and IgG) results, 1 case had positive IgM results only and 3 cases had Positive IgG results only
- Regarding the active SLE cases : 9 cases had positive PCR (1 had positive PCR results only,1 had positive PCR and IgM results and 7 had positive PCR,IgM and IgG results) , 6 cases had negative (PCR ,IgM and IgG) results and 5 cases had positive IgG results only .

Table 2: Comparison of IgG and IgM in relation to viral load .

			All subjects N=60				*p*
			PCR below detection limit N=48		Positive PCR N=12		
PCR viral load		**Median (range)**	-	-	108347	3629-1951433	-
IgM	**Negative**	**N, %**	47	97.9%	3	25%	**<0.001**
	Positive	**N, %**	1	2.1%	9	75%	
IgG	**Negative**	**N, %**	38	79.2%	2	16.7%	**<0.001**
	Positive	**N, %**	10	20.8%	10	83.3%	

- With in all studied subjects, 12 cases had detected Parvo virus B19 DNA, viral load median was 108347, ranged from 3629 to 1951433.
- Higher frequency of positive IgM antibodies were significantly associated with positive PCR ($p<0.001$)& Higher frequency of positive IgG antibodies were significantly associated with positive PCR ($p<0.001$).

Table 3: Correlation of Parvovirus B19 viral load with age, SLEDIA, IgM titre &IgG titre in SLE cases.

	Parvo virus load	
	rs	***p***
Age	0.030	0.934
SLEDAI	0.745	**0.013**
IgM titre	0.758	**0.011**
IgG titre	0.03	0.934

rs, correlation coefficient.

PV-B19 viral load showed significant positive correlation with SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) as well as IgM titre, but not with age and IgG titre.

Table (4): Regression analysis for prediction of SLE activity (SLEDAI).

	Univariable		Multivariable	
	β	***p***	***β***	***p***
Age	-0.127	0.149		
Gender	1.187	0.169		
Duration	-0.233	0.275		
PCR expression (log_{10})	1.842	**<0.001**	1.661	**<0.001**
IgM titre	0.004	**0.024**	0.121	**0.019**
IgG titre	0.006	**<0.001**	0.114	**0.043**

B, regression coefficient.

Regression analysis was conducted for prediction of SLE activity (higher SLEDAI) using age, gender, duration, viral load, IgM and IgG titres as covariates.

Higher viral load, IgM and IgG titres were associated with higher SLEDAI in univariable analysis. However, using significant covariates in univariable analysis into multivariable analysis revealed that only Parvo virusB19 viral load, IgM and IgG titres were considered as independent predictors for more active SLE cases (higher SLEDAI).

DISCUSSION

The present study was conducted on 40 SLE cases. Their median age was 29 years, ranged from 10 to 65 years. They were 10 children (25%), 30 adult cases (75%); 8 males (20%) and 32 females (80%). In addition to 20 healthy control group of matched age and gender .Their median age was 23 years, ranged from 10 to 44 years ,They were 7children (35%), 13 adult cases (65%); 6 males (30%) and 14 females (70%)

This result was in agreement with that reported by Hot et al [11]. in Israel who reported that the majority of study subjects were females (88%), with a mean age of 41 years (range 16–72 years). Also the Gazareen, et al [12]. in Egypt reported that majority of study patients were females(96,7%) The mean age of patients was 24.2 years (range 15- 40years). El-mak et al [13] also noticed the high incidence of SLE among women (89%) rather than male. Their median age was 29 years, ranged from 10 to 65 years. This result may attributed to the predominance of the SLE among females. Quintero et al.[14] & Cunningham et al[15] suggested that the sex hormones such as estrogen play roles in autoimmunity including SLE.

In contrast to our results,Gazareen et al[12] found that most individuals becoming infected with PV- B19 during their school years. Patients included in their study were adults, the least age was 15 years old for females and 16 in males

PV-B19 infection evolution is characterized by a 5-day phase with high viremia .This viremia is neutralized with antibodies directed against two structural viral proteins VP1 and VP2 .The viremia decreases with the synthesis of IgA and IgM, followed by the synthesis of IgG anti-PV-B19V [16].

In this study, Parvovirus B19 DNA was detected in 10 (25%) of the 40 patients with SLE . Out of the ten patients with PV- B19 DNA, only one patient had positive IgG antiPV-B19 antibody , one patient had positive IgM antiPV-B19 antibody and seven patients had positive IgM and IgG antibodies. Where as in the other 30 SLE patients PV- B19 DNA wasn't detected , Out of the 30 patients with out PV- B19 DNA, only one patient had positive IgM anti PV-B19 antibody and eight patients had positive IgG antibodies. Regarding the control group, Parvovirus B19 DNA was detected in 2 (10%) out of the 20 subjects of healthy control , One of them had positive IgM antibody , and one had positive IgG antibody. whereas IgG anti PV-B19 antibodies were detected in 2 out of 18 subjects of healthy control with out PV- B19 DNA .

The result of this study was in agreement with El Saadany et al[17] in Egypt who detected Parvovirus B19 DNA in 11 of the 30 patients with SLE (33.3%). Out of

the eleven patients with B19 DNA, only two had IgG anti-B19 antibody and one had IgM anti-B19 antibody, whereas IgG and IgM anti-B19 antibodies were detected in 11 (57.8%) and 9 (47.3%) of 19 SLE patients without PV- B19 DNA respectively. In contrast to our result they reported that PV- B19 DNA was not detected in any of the normal controls.

Also a study done by Valencia et al[18].in Mexico , reported the high prevalence of PV-B19 in Yucatan, and the presence of IgM, IgG, and viral load in Mayan women with established SLE suggest that PV-B19 infection could be an environmental factor to trigger or reactivate SLE .

Another study done by Hod et al[11] reported that elevated titre of IgG Abs to PV- B19 were found in 25 (49%) SLE patients, and slightly elevated IgM Abs titre to PV- B19 were detected in 8 (15.7%) , but Only two of 51 patients were positive for PV- B19 DNA .

In contrast to our results , Gazareen et al [12]. reported that no comparable positivity rates for viral DNA in SLE patients and healthy controls.

In our result there was non significant differences between patients with positive IgG antibodies compared to negative cases regarding age, and gender ($p>0.05$ for each).

El-mak et al[13]. noticed that the high level of IgG was observed among the age group between 15-25 years, the study subjects in this age group represent the students' category, the crowdedness and the close areas for long time of the day such as classes in schools may lead to increase the transmission of the virus from infected to non infected student . Also, Qiu et al [19]. demonstrated that human parvovirus B19 infection was thought to be an infection transmitted most frequently by school-aged children.

In our result there was no significant differences between patients with PV- B19 DNA positive compared to negative cases, regarding age, and gender

In contrast to our result Gazareen et al[12] .reported that patients with B19 DNA positive were more likely to be young age (mean 22.08 years) compared to negative cases (mean 25.67 years).

In our work , twenty cases were in active state and twenty cases were in remission state , Parvovirus B19 DNA was detected in 9 cases (45%) with active disease, Out of the 9 patients with B19 DNA, only one had positive PCR results only, one had positive PCR and IgM results and seven had positive PCR,IgM and IgG results , and in the remaining 11 active cases , 5 cases had positive IgG results & 6 cases had negative (PCR ,IgM and IgG) results. on the other hand , Parvovirus B19 DNA was detected in only one case (5%) out of 20 remittent cases and in the remaining 19 remittent cases , one case had positive IgM results only , three cases had positive IgG results only and 15 cases had negative (PCR & IgM & IgG) results with significant differences between both subgroups ($p=0.003$).

Several studies and case reports have implicated PV-B19 in inducing flares of SLE and that treatment for B19 in SLE patients with this infection might be useful to reduce flare-ups of such a serious disease, and administration of immunoglobulins could be helpful to control infection if discontinuation of the immunosuppressive therapy is not feasible [10] .

On studying different disease activity of SLE patients, we found that there was statistically significant differences in SLEDAI between active and remitted cases ($p<0.001$)

In our result ,the average SLEDAI score was 7.5 (range from 0 to 28) in active cases and was 3 (range from 0 to 4) in remitted cases. Moreover, Higher SLEDAI was significantly associated with patients with positive IgM, patients with positive IgG and patients with positive PCR ($p <0.001$ for each)

In contrast to our results, Gasareen et al [12] found that there was non significant difference between PV- B19 DNA positive and negative cases as regard activity of SLE.

In our work there was a significant positive correlation between Viral load and SLEDAI as well as IgM titre, but not with IgG titre. In contrast to our results Valencia et al [18] .reported no correlation of IgM or IgG titre with viral load in both groups. However, viral load was significantly higher in the controls with IgG

We also reported that Parvo virus B19 IgG titre showed significant Positive correlation with SLEDAI, but on the other hand Parvo virus IgM titre showed non significant correlation with SLEDAI . In contrast to our result, Hod et al [11] .reported no difference in the SLEDAI scores between patients with and without elevated IgG or IgM level.

CONCLUSION

Our results support the hypothesis that PV-B19 infection could be an environmental factor to trigger or reactivate SLE so that screening for PV-B19 infection in SLE patients might be useful to reduce the incidence and prevalence of this infection and so reduce flare-ups of such a serious disease and for accurate diagnosis combined use of PCR and ELISA are needed

Recommendations:

Longitudinal studies and a large sample are required to confirm the association of PV-B19 with the development of SLE. Moreover, the rheumatologist should investigate their SLE patients for parvovirus B19 routinely

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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