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HUMAN PARVOVIRUS B19 ANTIBODY AMONG ARTHROPATHIC PATIENTS WITH ESPECIAL EMPHASIS ON SICKLE CELL DISEASES IN BASRAH

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Abstract

A case control study was carried out during the period from October 2006 till September 2007 in Basrah Governorate. To estimate the overall prevalence of Human Parvovirus B19 (HPV B19) antibody and its association to Rheumatoid factor seropositivity among sickler and non sickler arthropathic patients.

A total of 182 blood samples were collected. Ninety (90) from arthropathic patients with or without sickle cell diseases (SCD), who attended the orthopaedic, rheumatology and internal medicine consultant clinics in Basrah General Hospital and Hemoglobinopathies Center at the Maternity and Children Hospital. Ninety two (92) from control group, non arthropathic with or without SCD.

Human parvovirus B19 IgG antibodies were serologically detected by an Enzyme- Linked Immunosorbent Assay (ELISA). The rheumatoid factor was detected by Latex agglutination test.

The overall prevalence of HPV B19 antibody in Basrah among study population was 68.7% . The prevalence of this antibody was 76.9% among arthropathic non sickler and 65.8% in sickler arthropathic patients. These differences were shown statistically not significant P> 0.05 compared to control group where prevalence was 63.2% in non arthropathic sickler and 66.7% among non arthropathic non sickler individuals.

Eighty percent of arthropathic patients who had positive rheumatoid factor were also positive for HPV B19 antibody, which indicate a significant association (P<0.05).

In the present study the seropositivity of HPV B19 was shown to be increased with age. In relation to site of joint affected, the small joints of the hand and foot were the commonest site of manifestation 78.1%.

HPV B19 antibody was significantly more (79.7%) among persons with history of blood transfusion. The types of SCD had no significant effect on the prevalence of HPV B19 antibody (P>0.05). However there was a positive relation between HPV B19 seropositivity and the duration of illness (P<0.0%).

In conclusion, HPV B19 is common with high prevalence in our region. There is clear association between HPV B19 infection and rheumatoid factor positivity. Individuals with sickle cell diseases regardless the type and those with history of blood transfusion were considered as risk groups for acquiring HPV B19 infections.

HPV B19: Human Parvovirus B19. **SCD**: Sickle Cell Diseases. **RA**: Rheumatoid Factor.

Introduction

Human Parvovirus B19 (HPV B19) is belong to parvoviridae family and the genus is erythrovirus, which accounts for almost all human parvovirus infections. It causes a broad spectrum of diseases[1]. The severity of the diseases depends on the immunological and hematological status of the host2. In normal children HPV B19 infection is common and usually asymptomatic or mild with short duration[3]. The disease characterized by erythematous rash known as erythema infectiosum clinically similar to presentation of rubella which results in misdiagnosis. In adults, especially women, HPV B19 infection is associated with arthralgia or frank arthritis. The joint may be painful, often accompanied with swelling and stiffness. These

symptoms usually continued for 1-3 weeks but sometimes lasted more than 2 years[2].

A person with underlying chronic hemolytic anemia, such as sickle cell disease is suffered from a serious complications of aplastic crisis due to HPV B19 infection[4]. The virus suppressed erythropoiesis transiently and the condition is self-limited with bone marrow recovery occurring within 7-10 days[5]. Epidemiological data reveals that the prevalence rate of anti-HPV B19 IgG antibodies increased with age[6].

This study aimed on estimation of HPV B19 antibody prevalence in Basrah general population, to determine the prevalence of B19 antibody among arthropathic patient with or without SCD and to evaluate the association of HPV B19 infection with rheumatoid factor seropositivity.

Materials and methods

A total of 182 blood samples were collected from arthropathic patients attending the orthopaedic, rheumatology and internal medicine consultant clinics in Basrah General Hospital and Hemoglobinopathies Center in Maternity and Children Hospital.

The study group include 90 (28 males and 62 females) patients complained from arthropathies subdivided into two subgroups:

A- Arthropathic patients, non sickler comprised 52 patients (16 males and 36 females).

B- Arthropathic patients with sickle cell diseases (SCD) identified according to conventional slide sickling test and Hb-electrophoresis results, comprised 38 patients (12 males and 26 females). The clinical criterias for characterizations of arthropathies includes: Arthralgia or arthritis, at least one joint affected, both acute and chronic illness. Involvement of symmetrical or asymmetrical joint distribution and joint symptom with sickle cell diseases. Traumal joints definitely were excluded.

The control group include 92 individuals (37 males and 55 females) who were obtained from the medical students, oil company employees. The control group was subdivided to: Individuals who were healthy and never complained from arthropathies with or without SCD.

Non arthropathic with sickle cell diseases (38: 14 males and 24 females).

Non arthropathic without SCD (54:23 males and 31 females). Blood collection and laboratory investigations: Venus blood (5 ml) was taken from both patients and control groups. Each sample was divided into two parts: 1 ml in ethylene di-amine tetra acetic acid (EDTA) tube for hematological examination (Hb–electrophoresis and direct sickling tests, and the remaining part kept at room temperature for one hour in sterile disposable test tube allowed to clot and serum was separated and stored frozen at –20 C° which was used for rheumatoid factor test by slide agglutination test kit* and for detection of anti-HPV B19 IgG by DRG parvovirus B19 ELISA Kit** using recombinant parvovirus B19 antigen which is minor structural virus protein (VP1 proteins). All procedures were done according to the manufacturer s instructions. Hb-electrophoresis was done to determine the type of hemoglobinopathy on cellulose acetate membrane in alkaline buffer pH: 8.6[7,8].

*Plasmatic RA Latex kit, catalogue No. 010.**DRG-Novum-Germany,catalogue No.3503 .

Results

The overall prevalence of HPV-B19 antibody among study population was 68.7% (Table I). The highest prevalence rate was observed among arthropathic non-sickler patients (76.9%), while the rate among arthropathic patients with SCD was 65.8%. There were no statistically differences between the

prevalence in these groups (P>0.05). However, HPV-B19 antibody prevalence was less among non arthropathic sickler patients (63.2%) and normal individuals (non arthropathic non-sickler (66.7%). None of these groups showed significant differences (P>0.05).

Analysis of data to determine the association of rheumatoid factor seropositivity to the presence of HPV-B19 antibody (Table II), showed that 80% of the patients with rheumatoid factor positive results were positive to HPV-B19 antibody in contrast to 63.1% observed among rheumatoid factor negative patients. These differences was statistically significant (P<0.05).

The prevalence of HPV-B19 antibody varies with age groups. Table (IIIa) present the gradual increase in the prevalence of HPV-B19 antibody with increasing age among arthropathic patients with SCD: in age groups of 1-20, 21-40 and 41-60 years which was 57.1%, 70% and 86% respectively. Almost the same trend was found among non-sickler with arthropathies. However, non-arthropathic patients whether with SCD or normal individuals shows the same trend of age related increase (Table 3b). Although normal individuals group showed greater rates of exposure to viruses at age group 21-40 years (75%).

The presence of HPV-B19 antibody almost the same in patients with different joint sites involved with average 67.6% (Table IV). Except for those with ankle joint showed lower percentage (57.1%), while the small joints are slightly more affected 78.1%.

The association of HPV-B19 antibody to the history of blood transfusion showed that individuals who had a history of blood transfusion showed higher percentages of HPV-B19 seropositivity (79.7%) than those not received blood at all (63.4%). This differences was statistically significant (P<0.05) (Table V).

The pattern of HPV-B19 antibody responses in various underlying types of SCD was shown in table VI. The highest seropositivity was observed among arthropathic patients with normal hemoglobin which was 77% compared to 50% among arthropathic patients with sickle-thalassemia. For patients with sickle cell anemia or sickle-cell trait, the seropositivity to HPV-B19 was 73.7% and 66.7% respectively. These differences were statistically not significant (P>0.05).

The seropositivity to HPV-B19 was increased with the longer duration of arthropathic illness (Table VIII). In patients with short duration of illness, the seropositivity rate was 53.8% compared to those with long durations (75.8%). The maximum HPV-B19 seropositivity was observed among 15-30 days duration of illness (83.9%). There were a positive relation between the duration of arthropathic illness and the presence of HPV-B19 seropositivity (P<0.05%).

Table I: Prevalence of HPV-B19 antibody in various groups of the study populations

Study group	HPV-B19 antibody
	No. positive / Test (%)
Basrah study population✷	125 / 182 (68.7)
Arthropathic patients with SCD	25 / 38 (65.8)
Arthropathic non-sickler	40 / 52 (76.9)
Non-arthropathic with SCD	24 / 38 (63.2)
Normal individuals	36 / 54 (66.7)

✷Overall prevalence in the study population P > 0.05

Table II: The association of HPV-B19 antibody and Rheumatoid factor (RF) test

Rheumatoid factor	HPV-B19 antibodies		Total No. (%)
	No. Positive (%)	No. Negative (%)	
Positive	48 (80)	12 (20)	60 (33)
Negative	77 (63.1)	45 (36.9)	122 (67)
Total	125 (68.7)	57 (31.3)	182 (100)

χ^2= 5.33 df = 1 P < 0.05

Table IIIa: Prevalence of HPV-B19 antibodies among arthropathic patients with SCD in relation to age groups

Age groups (years)	Arthropathic patients	
	SCD patients Positive/Total (%)	Non-sickler Positive/Total (%)
1-20	12/21 (57.1)	-------
21-40	7/10 (70.0)	10/14 (71.4)
41-60	6/7 (86.0)	30/38 (78.9)
Total	25/38 (65.8)	40/52 (76.9)

χ^2 = 24.806 (below 20/above20) df =1 (P<0.05)

Table IIIb: Prevalence of HPV-B19 antibodies among non-arthropathic patients with SCD in relation to age groups

Age groups (years)	Normal individuals(non-arthropathic)	
	SCD patients Positive/Total (%)	Non-sickler Positive/Total (%)
1-20	14/27 (51.85)	--------
21-40	7/8 (87.50)	12/16 (75.0)
41-60	3/3 (100)	24/38 (63.2)
Total	24/38 (63.15)	36/54 (66.7)

χ^2= 28.36 (below 20/above 20)df = 1 (P<0.05)

Table IV: Distribution of HPV-B19 antibodies in relation to the site of joints affected

Joint	HPV-B19 antibody
	Positive/No. tested (%)
Small joints*	25/32 (78.1)
Wrist	15/20 (75)
Knee	37/51 (72.5)
Elbow	13/18 (72.2)
Ankle	12/21 (57.1)
Others**	17/24 (70.8)
Total	110/166 (71.7)

χ^2= 2.9 df = 5 P > 0.05

* Small joints : include metacarpal phalangeal joints , interphalangeal joints and metatarsal joints.
** Others : include spine , hip and shoulder joints.
Note: more than one affected joint may be seen in the same patient.

Table V: The association of HPV-B19 antibodies and history of blood transfusion

Blood Transfusion	HPV-B19 antibody
	No. Positive/No. tested (%)
Received	47/59 (79.7)
Not received	78/123 (63.4)
Total	125/182 (68.7)

χ^2= 4.89 df = 1 P < 0.05

Table VI: Pattern of HPV B19 antibodies response among arthropathic patients in relation to underlying types of hemoglobin

SCD*type	HPV-B19 antibody
	No. Positive /No. tested (%)
SS**	14/19 (73.7)
SF***	5/10 (50.0)
AS****	6/9 (66.7)
A*****	40/52 (77.0)
Total	65/90 (72.2)

χ^2= 3.2 df = 3 P > 0.05

SCD* : sickle cell diseases
SS** : sickle cell anemia
SF*** : sickle-thalassemia
AS**** : sickle cell trait
A***** : normal hemoglobin

Table VII: The relation of HPV-B19 antibodies prevalence to the duration of present arthropathic illness

Duration of illness (days)	HPV-B19 antibody
	No. Positive/No. tested (%)
<15	14/26 (53.8)
15-30	26/31 (83.9)
>30	25/33 (75.8)
Total	65/90 (72.2)

χ^2= 6.67 df = 2 P < 0.05

Discussion

There is a considerable evidence that viruses may be an important factor in the pathogenesis of autoimmune rheumatic diseases[9]. HPV-B19 is a common pathogen in humans, it is thought to be one of causative agent for rheumatic diseases in adults and children[10,11]. Also it is considered as predisposing factor of transient a plastic crisis (TAC) in patients with hemolytic disorders[12].

Serologic studies on HPV-B19 antibodies as a trigger for rheumatic diseases and the most common predisposing agent for TAC in SCD patients are very limited in Iraqi society. There is no information to determine the prevalence of HPV-B19 antibody among those group of patients despite that rheumatic symptoms and SCD are common in our society. Therefore, this study presents the association of HPV-

B19 infections in these groups of patients in Basrah Governorate.

The present study showed that the seropositivity to HPV-B19 is high in Basrah population whether in diseased patients (arthropathic with SCD) or normal individuals. This is because HPV-B19 infections is common, highly contagious and still no awareness to this viral infection, since it is mild and mostly asymptomatic. These results is in consistent with other studies where 50-75% of adults in United Kingdom[13] and 79.1% of Italian blood donors[14] were seropositive to HPV-B19 antibodies. Our results showed that no significant differences in the percentage of positive HPV-B19 antibody among arthropathic patients with SCD as compared to control group (non arthropathic with or without SCD), this is in contrast to a study carried out in Northern Brazil that showed HPV-B19 was an important agent among arthropathies[15]. However, this study is in agreement with a serological study carried out in Togo[16] which was found no difference in HPV-B19 antibody prevalence among SCD patients compared to control group.

The current study showed clear association between HPV-B19 antibodies and rheumatoid factor which is in consistent with studies carried out on transgenic animals[17]. However, these observations can be explained on the bases that chronic arthropathies fulfilled the criteria of classifications of rheumatoid arthritis, but acute type resemble rheumatoid arthritis in term of rheumatoid factor production[17,18]. When HPV-B19 infected macrophage cell line or bone marrow cells lead to increased secretion of interleukin 6 (IL-6) and tumor necrosis factor (TNF) which are mediated in response to viral protein non structural protein (NS1) that responsible for activation of IL-6 and TNF in the pathogenesis of rheumatoid arthritis, since IL-6 responsible for the activation of auto-reactive T-cell and appearance of auto-antibodies including rheumatoid factor[19].

The current study showed that the seroprevalence of HPV-B19 is increased with age. This trend of age relation was also reported by other serological studies in United State of America and several other countries[10,16,20,21]. There were a significant differences in the prevalence of HPV-B19 antibody among SCD patients in various age groups, this may be attributed to the abnormal immune response, bone marrow turn over and immaturity of immune system in children compared to adults resulting in low level of antibody production. Also with progressing age, patients with SCD at increased demand for blood transfusion which is considered as a mode of transmission for HPV-B19 infections leading to increased risk of exposure to virus. However, a study carried out in Jamaica on SCD patients showed that 70.7% of patients reported seroconversion to HPV-B19 by age of 20 years[22].

History of blood transfusion have a significant effect of HPV-B19 seropositivity. This might be due to the lack of screening procedures for the virus to blood donors and ineffective inactivation and purification measures which are important to avoid iatrogenic infection especially among patients at risk. These finding is in agreement with study of HPV-B19 among blood donors[23] and studies that found increased prevalence of HPV-B19 antibodies among hemophiliacs[24,25].

Production of HPV-B19 specific IgG is not detectable within two weeks duration of illness and reaching it's maximum titer by month[26]. Arthropathic patients with prolonged symptoms showed no corresponding increase in the amount /or duration of anti-B19 IgM[19]. Long duration of disease is significantly affect the rate of HPV-B19 seropositivity which coincide with the pattern of immune responses detected in

immunocompetent patients. These observation is in consistent with that described by others[27]. However, patients complained from prolonged symptoms (more than 15 days) are more suggestive of HPV-B19 infection that agreed with the other studies[10,19].

References

1- Brown KE, Young NS. Parvoviruses and Bone Marrow Failure. Stem Cells. 1996; 14(2): 151-163.
2- Alexandrova R, Shikova E. Parvovirus B19. Experimental Pathology and Parasitology. 1999; 38-44.
3- Zepf B, Young NS, Brown KE. Parvovirus B19. New England Journal of Medicine. 2004; 350: 586-597.
4- Ziyaeyan M, Rasouli M, Alborzi A. The Seroprevalence of Parvovirus B19 Infection among To-Be-Married Girls, Pregnant Women and Their Neonates in Shiraz, Iran. Japanese Journal of Infectious Diseases. 2005; 58: 95-97.
5- Weir E. Parvovirus B19 infection: fifth disease and more. Canadian Medical Association Journal. 2005; 172(6): 743.
6- Kelly HA, Siebert D, Hammond R, et al. The age specific prevalence of human parvovirus immunity in Victoria, Australia compared with other parts of the world. Epidemiology and infection. 2000; 124: 449-457.
7- Lewis SM, Bain BJ, Bates I. Practical haematology. 9th ed. Churchil Livingstone, London. 2001; 241.
8- Mohammed M. Bacterial skin infections among patients with hemoglobinopathies. MSc thesis, Basrah, Iraq 2003.
9- Perl A. Mechanisms of viral pathogenesis in rheumatic disease. Annals of the Rheumatic Diseases. 1999; 58: 454-461.
10- Sabbella C, Goldfarb J. Parvovirus B19 Infections. American Family Physician .1999; 60(5): 1455-1460.
11- Heegaard DE, Brown KE. Human parvovirus B19. Clinical Microbiology Reviews. 2002; 15(3): 485-505.
12- Badr MA. Human Parvovirus B19 infection among patients with chronic blood disorders. Saudi Med J. 2002; 23(3): 295-297.
13- Longhurst HJ. Parvovirus arthropathy masquerading as the arthritis of Behcets disease. Annals of the Rheumatic Diseases. 2001; 60:1080.
14- Manaresi E, Gallinella G, Morselli Labate AM, et al. Seroprevalence of IgG against conformational and linear capsid antigens of parvovirus B19 in Italian blood donors. Epidemiology and Infection. 2004; 132: 857-862.
15- Freitas RB, Monteiro TAF, Silva Filho MG, et al. Association Between Human Parvovirus B19 and Arthropathy in Belem, Para, North Brazil. Revista do Instituto de Medicina Tropical de Sao paulo. 2002; 44(1): 17-22.
16- Teuscher T, Baillod B, Holzer BR. Prevalence of human parvovirus B19 in sickle cell disease and healthy controls. Tropical and Geographical Medicine. 1991; 43(1-2): 108-110.
17- Takasawa N, Munakata Y, Ishii KK, et al. Human Parvovirus B19 Transgenic Mice Become Susceptible to Polyarthritis .The Journal of Immunology. 2004; 173: 4675-4683.
18- Stoll T, Bruhlmann P, Brunner U, et al. Parvovirus B19 – induced arthritis / arthropathy--an important differential diagnosis of chronic polyarthritis. Schweiz Med Wochenschr. 1995; 125(8): 347-354.
19- Franssila R. T-helper cell Immunity against Human parvovirus B19. PhD thesis submitted to Haartman Institute University of Helsinki, Finland. 2005.
20- Tsujimura M, Matsushita K, Shiraki H, et al. Human parvovirus B19 infection in blood donors. Vox Sang. 1995; 69: 206-212.
21- Zimmerman SA, Davis JS, Schultz WH, et al. Subclinical parvovirus B19 infection in children with sickle cell anemia. Journal of Pediatric Hematology / Oncology. 2003; 25(5): 387-389.
22- Serjeant BE, Hambleton IR, Kerr S, et al. Haematological response To parvovirus B19 infection in homozygous sickle - cell disease. Lancet. 2001; 358(9295): 1779-1780.
23- Letaief M, Vanham G, Boukef K, et al. Higher prevalence of parvovirus B19 in Belgium as compared to Tunisia blood donors: differential implications for prevention of transfusional transmission. Transfusion Science. 1997; 18(4): 523-530.
24- Cohen BJ. Letter to the editor: outbreaks caused by parvovirus B19. Euro Surveillance. 2005; 10(9): 3-4.
25- Ragni MV, Koch WC, Jordan JA. Parvovirus B19 infection in patients with hemophilia. Transfusion. 1996; 36(3): 238-241.
26- Serjeant GR, Topley JM, Mason K, et al. Outbreak of aplastic crisis in sickle cell anemia associated with parvovirus-like agent. Lancet. 1981; 2 : 595.
27- Corcoran A, Doyle S. Advances in the biology, diagnosis and host- pathogen interactions of parvovirus B19. Journal of Medical Microbiology. 2004; 53: 459-475.