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STUDY OF CHRONIC HEMOLYTIC ANAEMIA PATIENTS IN RIO DE JANEIRO: PREVALENCE OF ANTI-HUMAN PARVOVIRUS B19 IgG ANTIBODIES AND THE DEVELOPMENT OF TRANSIENT APLASTIC CRISES

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SUMMARY

The prevalence of anti-human parvovirus B19 IgG antibodies was determined in sera from 165 chronic hemolytic anemia patients, receiving medical care at Instituto Estadual de Hematologia (IEHE), Rio de Janeiro, during the year of 1994. This sample represents around 10% of the chronic hemolytic anemia patients attending at IEHE. Most of these patients (140) have sickle cell disease. Anti-B19 IgG antibodies were detected in 32.1% of patients. No statistically significant difference ($p > 0.05$) was seen between IgG antibody prevalence in male (27.8%) and female (35.5%) patients. Anti-B19 IgG antibodies were more frequent in older (37.6%) than younger (28.2%) than 20 years old patients, although this difference had no statistical significance ($p > 0.05$). Anti-B19 IgG antibody prevalence showed that 67.9% of patients enrolled in the study were susceptible to B19 acute infection. With the aim to detect acute B19 infection, patients follow up continued until February 1996. During this period four patients presented transient aplastic crisis due to human parvovirus B19 as confirmed by the detection of specific IgM antibodies. All four patients were younger than 20 years old, and 3 were younger than 10 years old. Three of them were sickle cell disease patients. Three of the four acute B19 infection occurred during 1994 springtime.

KEYWORDS: Human Parvovirus B19; Sickle-cell disease; Transient aplastic crisis

INTRODUCTION

Human parvovirus B19, discovered in 1974 by COSSART *et al.*[7], is now recognized as an important human pathogen causing erythema infectiosum or fifth disease in normal children and occurring worldwide. Other pathogenic presentations depending of host conditions are transient aplastic crisis (TAC) in chronic hemolytic anaemia patients, pure red blood cell aplasia (PRBCA) in immunosuppressed patients, and non-immune hydrops fetalis (NIHF) when infection occurs in pregnant women[5]. These clinical presentations are related to the tropism of the virus for bone marrow precursors, mainly erythroblasts[23]. TAC in chronic hemolytic anaemia patients is characterized by profound reticulocytopenia and worsening anaemia. Most patients require red blood cell transfusion and TAC is one of possible fatal events in sickle cell anemia[17,30]. Sickle cell disease is the most common chronic hemolytic anaemia in Brazil and one of the most important genetic diseases in this country[27]. We have studied the human parvovirus B19 circulation in Brazil since 1988, when we found an asymptomatic viremic blood donor in Rio de Janeiro[9]. Infection is widespread in Rio de Janeiro city where anti-B19 IgG antibodies were found in 73.7% of the sera collected during 1985 and 1986[25]. Clinical presentations such as TAC in hereditary spherocytosis and sickle cell patients[13], fifth disease[12,26], NIHF[11,16] were also described by us in Rio de Janeiro, and other Brazilian States during the last decade. We are reporting here a 20 months follow up, hold during 1994 to 1996, in a cohort of chronic hemolytic anemia patients attending at Instituto Estadual de Hematologia in Rio de Janeiro.

MATERIALS AND METHODS

Patients: 165 anemic patients were selected from a total of 1629 attending at IEHE during the period of June to December 1994, in order to verify the anti-B19 IgG antibodies prevalence. This sample represents around 10% of the patients and the description of this population is shown in Table 1. During the period from June 1994 to February 1996, nineteen patients suspecting to present TAC (HT < 20% or Hb < 8 mg%), were tested for acute human parvovirus B19 infection. Another five patients sharing the same ward of a B19 positive TAC patient during November 1994 were also included in this study. This project obtained the approval by the Ethical Research Council of IEHE.

Serum specimens: Blood samples were taken by vein puncture, and sera were stored at -20 °C until tested.

Laboratory diagnosis: Anti-B19 IgG and IgM antibodies were

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tested by *in house* enzyme immuno assays, using µ-antibody capture-MACEIA[10] and a direct IgG test[12]. Viremia was analyzed by B19 DNA detection by dot-blot hybridization assay using a biotin-labeled probe[22].

Statistical tests: The chi square test with Yates' correction was used to analyze anti-B19 IgG results.

RESULTS

From a total of 1629 anemic patients attending at IEHE during 1994, 165 blood samples were collected to study anti-B19 IgG antibody prevalence. Most of these patients (1125) have sickle cell disease so 140 from 165 blood samples were collected from SCD patients (Table 1). Only 25 blood samples were collected from patients presenting another type of hemolytic disease: chronic (21) or acquired (4). As shown in Table 2, 32.1% of these patients were immune to B19 parvovirus and 67.9 % remaining susceptible to the virus. In this group of patients 93 were females and 72 were males. No difference in IgG antibodies prevalence was noticed between these groups. The age distribution of the patients showed that the patients less than 20 years old had lower antibody prevalence than older patients. During the 20 months follow up, 19 patients were suspected to present TAC and anti-B19 IgM could be detected in only 4 (Table 3). None of them had B19 DNA in the sample tested. The B19 positive acute infections were detected during the months of September, October, November 1994 and May 1995. One of these patients has hereditary spherocytosis and the other three were sickle cell disease patients. All four patients were less than 20 years old and three of them were less than 10 years old. Table 4 shows the laboratory

Table 1
Description of the anemic patients attending at IEHE and the patients selected to this study during 1994

Chronic hemolytic anemia	No. of patients studied	Total no. of patients attending at IEHE
Sickle-cell disease	140	1125
Sickle-cell trait	1	270
Haemoglobin SD disease	2	12
Haemoglobin C disease	1	10
Haemoglobin SC disease	11	113
Thalassemia major	2	63
Thalassemia intermedia	2	6
Hereditary spherocytosis	2	21
Acquired hemolytic anemia	4	9
Total	165	1629

Table 3
Patients presenting TAC at IEHE from 1994 to 1996

Date of specimen collection	Patients' age (years)	Chronic hemolytic anemia	Anti-B19 IgM	Anti-B19 IgG	B19 DNA
23/09/94	**6**	**SCD[a]**	**pos**	**pos**	**neg**
06/10/94	12	SCD	neg	neg	neg
31/10/94	**5**	**SCD**	**pos**	**neg**	**neg**
03/11/94	17	SCD	neg	nd[f]	neg
14/11/94	**17**	**HS[b]**	**pos**	**neg**	**neg**
17/11/94	40	SCD	neg	neg	neg
17/11/94	32	HSCD[c]	neg	neg	neg
03/12/94	7	SCD	neg	neg	neg
08/12/94	10	SCD	neg	neg	neg
23/01/95	22	SCD	neg	neg	neg
23/01/95	7	SCD	neg	neg	neg
23/01/95	1	SCD	neg	pos	neg
08/03/95	1	SCD	neg	neg	neg
05/05/95	**4**	**SCD**	**pos**	**neg**	**neg**
09/05/95	27	HA[d]	neg	neg	neg
18/05/95	32	SCD	neg	nd	neg
11/08/95	29	T I [e]	neg	nd	neg
26/09/95	26	SCD	neg	neg	neg
06/02/96	11	SCD	neg	nd	neg

[a] Sickle-cell disease, [b]Hereditary spherocytosis, [c]Haemoglobin SC Disease; [d]Hypoplastic anaemia, [e]Thalassemia intermedia, [f]Not done

Table 4
Laboratory results obtained from contacts in a ward of one TAC patient at IEHE during November 1994

Disease	Age (years)	Anti-B19 IgM	Anti B19 IgG	B19 DNA
SCD[a]	12	Neg	Neg	Neg
AML[b]	69	Neg	Neg	Neg
AML	46	Neg	Pos	ND[d]
PNH[c]	62	Neg	Neg	Neg
AML	65	Neg	Pos	ND

[a] Sickle-cell disease, [b] Acute Myeloblastic Leukaemia, [c]Paroxysmal Nocturnal Hemoglobinuria, [d]Not done

Table 2
Anti-B19 IgG prevalence in chronic hemolytic anemia patients at IEHE during 1994

Anti-B19 IgG	Number of Patients	Sex		Age range (years)		
		Female	Male	1-9	10-20	>20
Positive	53 (32.1)[a]	33 (35.5)	20 (27.8)	13 (26.5)	14 (29.8)	26 (37.6)
Negative	112 (67.9)	60 (64.5)	52 (72.2)	36 (73.5)	33 (70.2)	43 (62.4)
Total	165 (100)	93	72	49	47	69

[a] No. in parenthesis means the percentage from the total number of cases

results of 5 patients that shared the same ward with one B19 positive TAC patient during November 1994. None of them became infected, although three were immunosuppressed and two of them were already immune to the virus.

DISCUSSION

Infection by the human parvovirus B19 is widespread and it occurs in all regions where it has been studied. Around 50% of people have anti-B19 antibodies by the age of 15 years old. Infection continues to occur in adult life and around 80% of the population is immune to the virus by the age of 50 years old[6]. The same antibody distribution was described in USA[3], France[8], Germany[33] and Japan[24], but for some countries like Brazil[25] and Africa[32] the antibody prevalence can be higher. Some isolated Indian populations showed lower antibody prevalence as described in Brazilian Amazon[15] and some regions in Africa[29]. We describe here anti-B19 antibody prevalence in a sample of 10% of chronic hemolytic anemia patients receiving medical care at IEHE, RJ. Previous immunity to B19 was detected in 32.1% of the patients; this number is very low as compared to previous results (73.7%) obtained in Rio de Janeiro general population[25] during 1985/1986. There is no difference regarding sex as also described in the previous study[25]. As shown in Table 2, 41.8% of the patients were more than 20 years old. It was expected that these older people should have higher antibodies prevalence if we compare with other prevalence studies[3,6,8,24,29,33] and knowing that B19 acute infection occurs mainly in scholars and teenagers[6]. In our group of patients, age was not associated with seropositivity to B19. Antibody prevalence increased with the age group from 26.6 (less than 10 years old) to 37.6 (more than 20 years old), although suggestive this difference did not achieve statistical significance ($p > 0.05$). EI outbreaks occur in intervals of 3 to 4 years[3,6], during the end of the winter, springtime and beginning of summer[2]. In Asian countries the epidemiology of B19 infection is quite distinct. The antibody prevalence is very low in Hong Kong[19], Singapore[21] and Taiwan[20], when compared to countries in Occident. Intervals of 10 years between outbreaks have been described in Japan[34]. These results suggested that anti-B19 antibody prevalence could be lower during pre-epidemic periods. In our study we use sera collected during 1994 (end of winter to beginning of summer), when we had an EI outbreak in Rio de Janeiro city[12,26]. This can explain why we had so low antibody prevalence in our patients. Another possibility to explain our data is the severity of infection in this group that leads to TAC being fatal when blood transfusions were not performed on time. The risk of death by TAC is described in SCD patients[17,30] although a study hold in Africa did not show any difference in anti-B19 IgG antibody prevalence between SCD patients and normal population in sera collected during the same epidemiological period[31]. Table 3 shows the results of 19 patients showing TAC during the studied period (June, 1994 to February, 1996). All but 7 patients were less than 20 years old, as also the 4 patients that had laboratorial confirmation (by specific IgM detection) of B19 acute infection. Three of these 4 patients were less than 10 years old according to other papers, which report that B19 acute infection is more frequent at this age range[3,6,8,24,29,33]. All 4 but one confirmed cases occurred during 1994 springtime, the other case occurred in the beginning of winter 1995. These cases confirm the seasonality described for other countries, and also for Rio de Janeiro after 12 years of consecutive studies[9,10,13]. Among these 4 patients only one was also positive for anti-B19 IgG. It shows that sera were collected early during the infection but not during viremia (all 4 patients were B19 DNA negative), which normally occurs together with TAC symptoms[14]. B19 DNA detection is very important in TAC patients because anti-B19 IgM antibodies can be not found in the beginning of the symptoms. The laboratorial diagnosis of TAC is quite different from EI cases when anti-B19 antibodies (IgM and IgG) were almost present when rash mediated by antigen-antibodies complex appears[12]. During November 1994 a TAC patient were in a ward together with 5 other patients (Table 4). None of them developed TAC, although 3 of them was susceptible to the infection (anti-B19 IgG negative). For 3 of these patients the diagnosis was acute myeloblastic leukaemia (immunosuppressed), that could develop persistent B19 infection[18]. Therefore two of them were immune to the virus (anti-B19 IgG positive). TAC patients are highly infectious for hospital staff and other patients having contact with them[4]. Normally, TAC symptoms occur during viremia (peak of virus around 10^{14} particles/ml), and patients eliminate high quantity of virus by respiratory route[1].

We conclude that laboratorial diagnosis of acute B19 infections is very important in patients in haematology wards, making possible better prognosis of the cases, lower clinical cost and lower risk for inpatients. These will be more important if we consider that, during 1994, 67.9% of the patients were still susceptible to B19 infection.

RESUMO

Estudo de pacientes com anemia hemolítica crônica no Rio de Janeiro: prevalência de anticorpos IgG anti-parvovirus humano B19 e desenvolvimento de crise aplástica transitória

A prevalência de anticorpos anti-parvovirus humano B19 foi determinada em soros de 165 pacientes portadores de anemia hemolítica crônica, atendidos no Instituto Estadual de Hematologia (IEHE), Rio de Janeiro, durante o ano de 1994. Esta amostra representa cerca de 10% dos pacientes portadores de anemia hemolítica crônica atendidos no IEHE. A maioria destes pacientes (140) são portadores de anemia falciforme. Anticorpos IgG anti-parvovirus humano B19 foram detectados em 32,1% dos pacientes. Nenhuma diferença estatisticamente significante foi verificada entre a prevalência de anticorpos em pacientes do sexo masculino (27,8%) e feminino (35,5%). Anticorpos IgG anti-parvovirus humano B19 foram mais freqüentes em pacientes na faixa etária acima (37,8%) que abaixo (28,2%) de 20 anos de idade, embora esta diferença não tenha significado estatístico ($p > 0,05$). A prevalência de anticorpos IgG anti-B19 demonstrou que 67,9% dos pacientes incluídos no estudo eram ainda suscetíveis à infecção aguda pelo parvovirus humano B19. Com o objetivo de detectar infecção aguda por este vírus, o seguimento de pacientes continuou até fevereiro de 1996. Durante este período, 4 pacientes apresentaram crise de aplasia transitória devido ao parvovirus humano B19 conforme confirmado pela detecção de anticorpos IgM específicos. Todos 4 pacientes estavam na faixa etária abaixo de 20 anos, sendo que 3 tinham menos de 10 anos de idade. Três destes pacientes eram portadores de anemia falciforme. Em 3 dos 4 pacientes, a infecção aguda por B19 ocorreu durante a primavera de 1994.

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