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ARTIGO ORIGINAL

Estudo de associação entre moléculas HLA e toxoplasmose em pacientes em diálise e transplantados renais

Study of association between HLA molecules and toxoplasmosis in patients on dialysis and kidney transplant

Estudio de asociación entre moléculas HLA y toxoplasmosis en pacientes en diálisis y trasplantados renales

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RESUMO

Infecções primárias por *Toxoplasma gondii* são assintomáticas em decorrência de efetividade do sistema imunológico e indivíduos imunocomprometidos são alvos de infecções oportunistas. Está diretamente ligada à imunidade do indivíduo e o Complexo Principal de Histocompatibilidade (CPH) está envolvido nessa função. O objetivo do estudo foi investigar a frequência das especificidades HLA classe I (HLA-A e HLA-B) e classe II (HLA-DRB1) e realizar um estudo comparativo da frequência de anticorpos anti-*T. gondii* com a frequência das especificidades HLA no soro de pacientes em diálise e transplantados renais. Participaram do estudo 256 pacientes, sendo 203 em diálise e 53 transplantados renais, e como controles, 73 indivíduos saudáveis. O método utilizado para detecção e caracterização de anticorpos foi o ELISA quantitativo (ensaio imunoenzimático de micropartículas (MEIA-*Abbott AxSYM® SYSTEM*) e todos os pacientes foram tipados para as moléculas HLA, classe I e classe II. Em relação às moléculas HLA classe I, não foram detectados alelos envolvidos na suscetibilidade e/ou proteção para toxoplasmose. Quanto à freqüência das moléculas HLA classe II encontrou-se maior frequência dos alelos DRB1*17 e DRB1*07 sugerindo estar relacionado com marcador genético de suscetibilidade. Esse estudo sugere a participação das especificidades HLA classe I e classe II na resistência e suscetibilidade para toxoplasmose.

Descritores: *Toxoplasmose. Antígenos HLA. Insuficiência renal crônica.*

ABSTRACT

Toxoplasma gondii caused primary infections are asymptomatic after activation of the immunological system and immunocompromised people are the target of opportunist infections. They are directly linked to the individual´s immunity and the Main Histocompatibility Complex (MHC) is involved in the function. Current analysis investigates the frequency of HLA class I (HLA-A and HLA-B) and II (HLA-DRB1) specificities and compares anti-*T.gondii* antibodies and frequency of HLA specificities in the blood of patients undergoing dialysis and of kidney-transplant patients. Two hundred and fifty-six patients participated in current study, of which 203 were undergoing dialysis and 53 underwent kidney transplantation; control comprised 73 healthy individuals. Quantitative ELISA test (microparticle enzyme immunoassay – MEIA - Abbott AxSYM® SYSTEM) was employed for the detection and characterization of antibodies. All patients were typed for HLA Classes I and II. Alleles involved in the susceptibility and/or protection against toxoplasmosis were not detected for HLA class I. Since a higher frequency of alleles DRB1*17 and DRB1*07 in HLA Class II molecules was detected, a relationship with susceptibility genetic marker has been suggested. Current investigation suggests the participation of HLA Class I and II specificities in the resistance against and susceptibility to toxoplasmosis.

Keywords: toxoplasmosis; HLA antigens, chronic kidney failure.

RESUMEN

Infecciones primarias por *Toxoplasma gondii* son asintomáticas debido a la efectividad del sistema inmunológico, y huéspedes inmunocomprometidos son más susceptibles de infecciones oportunistas. Está directamente relacionada a la inmunidad del huésped, y el Complejo Principal de Histocom-

patibilidad (CPH) está relacionado a esta función. El objetivo del estudio fue de investigar la frecuencia de las especificidades HLA clase I (HLA-A y HLA-B) y clase II (HLA-DRB1) y realizar un estudio comparativo de la frecuencia de anticuerpos anti-T. gondii con la frecuencia de las especificidades HLA en el suero de pacientes en diálisis y trasplantados renales. Participaron del estudio 256 pacientes, siendo 203 en diálisis y 53 trasplantados renales, y como controles, 73 individuos saludables. El método utilizado para la detección y caracterización de anticuerpos fue el de ELISA cuantitativo (ensayo inmunoenzimático de micropartículas (MEIA-Abbott AxSYM® SYSTEM) y todos los pacientes fueron tipados para las moléculas HLA, clase I e clase II. En relación a las moléculas HLA clase I, no fueron detectados alelos relacionados en la susceptibilidad y/o protección para toxoplasmosis. Cuanto a la frecuencia de las moléculas HLA clase II se encontró mayor frecuencia de los alelos DRB1*17 y DRB1*07 sugiriendo estar relacionado con marcador genético de susceptibilidad. Este estudio sugiere la participación de las especificidades HLA clase I y clase II en la resistencia y susceptibilidad para toxoplasmosis.

Palabras clave: *Toxoplasmosis. Antígenos HLA. Insuficiencia renal crónica.*

INTRODUCTION

Toxoplasmosis is a disease caused by *Toxoplasma gondii* (*T. gondii*), an intercellular parasite belonging to the Apicomplexa phylum and to the Sporozoa class, with worldwide distribution, infecting up to a third of world human population and a great variety of other species such as mammals and birds.[1] Parasitosis manifests itself in a variety of ways although most *T. gondii*-caused primary infections are asymptomatic due to the activation of the immunological system. Immunocompromised people, such as patients undergoing dialysis and patients with kidney transplants, have been targeted by opportunist infections. Clinical cases become very serious, featuring high morbidity and mortality rates.[2] Infection is directly linked to the person´s immunity and the Main Histocompatibility Complex (MHC), a set of genes on the short arm of the human chromosome 6, is involved. Human MHC is divided into three regions, namely, Classes I (HLA-A, -B and -C), II (HLA-DR, -DQ and -DP) and III. In humans these molecules are called Human Leucocyte Antigen or HLA.[3] Molecules Classes I and II are involved in the immunomodulation of the immune response because they perceive and present their own peptides either tumoral or of infectious microorganisms to cells T, and make the immune system differentiate which is one´s own or not. Class II molecule has a more limited distribution in the organism. It lies on the surface of the cells and is directly related to immune response for the presentation of peptides to the auxiliary cells T (CD4+). Consequently, the specific markers that confer susceptibility or resistance to disease are detected.[4] Further, it is also involved in donor and receiver compatibility and in the presentation of antigens to lymphocytes T, with a relevant role in the pathogeny of several diseases.[5] Several studies were undertaken to detect the association between pathologies and HLA molecules, comprising self-immune endocrine diseases,[6,7] gastrointestinal diseases,[8] dermatological diseases,[9-11] psychiatric diseases[12,13] , ophthalmological diseases[14], hearing pathologies,[15,16] tuberculosis[17,18] and kidney diseases.[19]

The above diseases could be avoided when vaccines are discovered. Tachyzoites of mild *T. gondii* have been used in animal vaccines.[20-25]

Gene transcription-attenuated parasites have been recently been forwarded as a new type of vaccine[23-25] although live organism vaccine in humans may be restricted because of safety issues. The development of vaccines based on peptides bred in epitopes which induce INFN-g by T CD8+ cells is highly promisingas a strategy for mobilizing the immune system against *T. gondii* in humans.[26-30]

Since toxoplasmosis affects approximately one third of the world population,[31] it is highly relevant to evaluate, as a preliminary investigation, the involvement of genetic factors in the infection in a population dependent on hemo-derivatives and body organs.

Current investigation focuses on the association between HLA molecules Class I (HLA-A and HLA-B) and class II (HLA-DRB1) and anti-*T. gondii* antibodies in patients undergoing dialysis and in patients with kidney transplants.

MATERIAL AND METHODS

The descriptive, exploratory and co-relational study was performed with 203 patients undergoing dialysis and with 53 patients with kidney transplants, attended at the Hemodialysis Sector of the Hospital Santa Casa de Misericórdia and the Clínica do Rim, in Maringá PR Brazil.

All patients were typified for molecule HLA Class I (A, B) and Class II (DRB1) and evaluated for positivity for anti-*T. gondii* IgG and IgM antibodies. The biological material of patients undergoing dialysis was collected between January and March 2011. Transplant patients were invited to participate in the investigation during routine clinical visits. Research exclusion criteria were patients less than 18 years old, patients with difficulties in understanding the procedure, lack of stability during collection and those who refused to participate.

Biological material was collected from a 10 mL peripheral blood sample by puncture and placed in sterile vacuum tubes with anticoagulant EDTA. After serum separation, aliquots were retrieved and stored in a freezer at -80°C until use. After de-freezing, the material was centrifuged at 10.000 rpm for 10 minutes to eliminate fibrins, red globules and other suspended particles.

The detection and characterization of anti-*T. gondii* antibodies and the study of genetic polymorphism of HLA molecules were performed in the Laboratory of Immunogenetics of the Universidade Estadual de Maringá, Maringá PR Brazil.

Detection and Characterization of antibodies

The ELISA method with the application of quantitative microparticle enzyme immunoassay – MEIA - Abbott Diagnostics AxSYM® SYSTEM Toxoplasma IgG and IgM for *T. gondii*, detected and characterized anti-*T. gondii* antibodies.

Interpretation of results took into account reagent IgG (IgGR) when AXSYM Toxoplasm G was higher than or equal to 3UI/mL, indicating acute infection or infection transmitted to the parasite. On the other hand, IgG (IgGNR) was taken into account when AxSYM Toxoplasm G rates were lower than 2 UI/mL. Results of AxSYM Toxoplasm G equal to or higher than 2 UI/mL and lower than 3 UI/mL were within the grey zone and might have low IgG levels. In this case, a second sample was obtained and tested. Samples with index rates equal to or higher than 0.600 were considered reactive to antibody IgM; samples with index rates lower than or equal to 0.499 were not reactive for antibodies IgM; samples with index rates between 0.500 and 0.599 lay within the grey zone and the assay should be repeated. Test protocols and the calculation of reference rates were conducted according to the manufacturer (Abbott AxSYM® SYSTEM – Toxoplasm IgG and Toxoplasm IgM, 2009 and 2006 respectively).

DNA extraction

DNA was extracted from samples with 10mL peripheral blood, collected by venous puncture, in vacuum tubes and with anti-coagulant EDTA. They were centrifuged at 2500 rpm, during 10 minutes, to obtain the leukocyte layer. DNA was extracted from this layer by reagent EZ-DNA, following recommendations by manufacturer (Biological Industries, Kibbutz Beit, Haemek).

HLA typing

LABType® SSO One Lambda kit with Luminex technology was employed to typify HLA class I (HLA-A and -B) and class II (HLA-DRB1). The PCR method employs biotinylated primers. Amplified material is de-natured and hybridized with probes linked to microbeads of the Luminiex multi-analytic system. Each bead is marked by a specific fluorescence and has an oligonucleotide probe correspondent to an HLA allele or to a group of HLA alleles. After hybridization, the probes that hybridized with DNA are marked by solution Streptavidin phycoerythrin (SAPE). Flow cytometer LABScan® 100 recognizes bead fluorescence and probe-conjugated SAPE. Data are analyzed by HLA Fusion to determine HLA typing.

All patients were typified with regard to molecules HLA class I (A, B) and class II (DRB1).

Statistical treatment

Patients were divided into positive and negative for anti-*Toxoplasma gondii* IgG and anti-*Toxoplasma gondii* IgM antibodies for statistical evaluation. The number of times that a determined HLA specificity appeared (n) and its relative frequency were determined. Further, p was calculated by Fisher Exact test (P-rate); p rate was calculated by Bonferroni correction (Pc-rate) for p rates lower than 0.05. Odds Ratio (OR) and ConfidenceInterval (CI = 95%) were determined when p rate was less than 0.05.

Ethical aspects

After being informed on the aims of research, anonymity and doubts, the subjects signed the Term of Free Consent (Appendix B). Ethical aspects complied with resolution 196 of the 10th October 1996 of the Brazilian Health Council of the Ministry of Health which regulated research on humans.[32] Current research was approved by the Committee for Ethics in Research (COPEP) of the State University of Maringá, 725/2010 (Annex A).

RESULTS

The sampling of 203 patients undergoing dialysis was distributed into 129 (63.55%) males and 74 females (36.45%). Mean age of male patients was 52.6 years (DP±15.64 years), between 18 and 83 years, whereas mean age of female patients was 51.78 years (DP±14.98), between 18 and 84 years (p>0.05).

One hundred and forty (68.96%) out of 203 patients had anti-*T. gondii* IgG antibodies, or rather, 89 (68.99%) were males and 51 (68.92%) were females. Only 2 males (0.99%) were positive for anti-*T. gondii* IgM antibodies, whereas 61 (30.05%) under treatment were not blood positive.

When frequency of Class I (HLA-A and HLA-B) and Class II (DRB1) specificities in patients undergoing dialysis, with or without blood positivity for toxoplasmosis, was analyzed, it was found that HLA-A*02 was the most frequent for IgG in both groups, or rather, 66 were positive and 30 were negative. In the case of locus B specificities, B*51 was the most frequent, with 36 and 13 times respectively in positive and negative patients. According to statistical analysis, no specificity HLA Class I was significant for the protection and/or susceptibility for toxoplasmosis (Annex B).

With regard to Class II molecules in patients undergoing dialysis, specificity DRB1*11 was more frequent (39 times) in the blood positive group when compared with blood negative group (19 times), although difference was not statistically significant. Statistically significant difference existed in specificity DRB1*17, or rather, it was more frequent (15 times) in the blood positive group when compared to the blood negative group (once) at p<0.05 (p=0.0476). The above suggests a probable susceptibility for toxoplasmosis, as table 1 shows.

Table 1 – HLA-DRB1 allele frequency in blood positive patients undergoing dialysis and in blood negative patients for anti-*T. gondii* IgG antibodies.

Specificity	Reagent n=140	Non-reagent n=61			
HLA-DRB1	N	N	p-rate	pC-rate	OR (IC95%)
03	18	7	1.0000	1.0000	-
04	35	20	0.3435	1.0000	-
07	29	13	1.0000	1.0000	-
08	18	10	0.5271	1.0000	-
09	3	4	0.2064	1.0000	-
10	8	2	0.7299	1.0000	-
11	39	19	0.6469	1.0000	-
12	2	0	1.0000	1.0000	-
13	31	16	0.613	1.0000	-
14	12	8	0.3289	1.0000	-
15	28	9	0.4579	1.0000	-
16	11	4	1.0000	1.0000	-
17	15	1	0.0476	0.7616	6.83 (1.03; 290.33)
18	5	2	1.0000	1.0000	-
51	1	0	1.0000	1.0000	-

n = number of times that allele appeared; Fa = Allele frequency; P-rate = calculated by Fisher Exact Test; Pc-rate = rate of p corrected by Bonferroni correction; OR = odds ratio; IC (95%) = Confidence Interval at 95%; ns = not significant (p>0.05).

Table 2 – Allele frequency HLA-DRB1 in group of soropositive patients with transplants and soronegative patients for anti-*T. gondii* IgG antibodies.

Specificity	Reagent n=33	Non-reagent n=19			
HLA-DRB1	N	N	p-rate	pC-rate	OR (IC95%)
01	3	1	1.0000	1.0000	-
03	7	5	0.7520	1.0000	-
04	7	7	0.2476	1.0000	-
07	11	0	0.0069	0.1104	-
08	2	2	0.6166	1.0000	-
09	2	2	0.6166	1.0000	-
10	1	0	1.0000	1.0000	-
11	13	6	0.7944	1.0000	-
12	2	1	1.0000	1.0000	-
13	4	4	0.4533	1.0000	-
14	5	0	0.1573	1.0000	-
15	4	6	0.1622	1.0000	-
16	4	2	1.0000	1.0000	-
17	2	1	1.0000	1.0000	-
18	0	1	0.3585	1.0000	-
52	1	0	1.0000	1.0000	-

n = number of times that the allele is extant; Fa = allele frequency; P-rate = calculated by Fisher´s Exact Test; Pc-rate = p corrected by Bonferroni correction; OR = odds ratio; IC (95%) = Confidence Interval 95%; ns = not significant (p>0.05).

No specificity that would be involved in the protection and/or susceptibility for toxoplasmosis was reported when the frequency of HLA specificities with anti-*T. gondii* IgM antibodies in the group of patients undergoing dialysis was analyzed.

Fifty-three patients with kidney transplant, 32 males (60.38%) and 21 females (39.62%), were evaluated. Mean age for males was 46.28 years (DP±12.72), ranging between 22 and 69 years, whereas mean age for females was 40.1 years (DP±10.8), ranging between 18 and 64 years. Twenty-one (65.63%) male patients and 13 (61.90%) female patients manifested anti-*T. gondii* IgG antibodies.

When frequency of specificities class I in patients with transplants and the state of blood positive or negative IgG for toxoplasmosis were analyzed, it may be remarked that specificity A*02 was more frequent in blood positive patient group (20 times) when compared with blood negative patients (11 times), although there was no statistically significant difference with regard to protection and/or susceptibility for toxoplasmosis.

In the case of molecules Class II, specificity DRB1*11 was the most frequent in the two groups, with 13 times in blood positive patients and 6 times in blood negative ones, albeit with no statistically significant differences. Specificity DRB1*07 may be involved in susceptibility for toxoplasmosis since it was more frequent in the group of blood positive patients (11 times) when compared to the group of blood negative patients (nil times), $p<0.05$ ($p=0.0069$), as table 2 shows.

No specificities that would suggest susceptibilities and/or protection for acute infection (IgM) were found in the group of patients with kidney transplants.

Results suggest that specificity DRB1*07 may be involved in the susceptibility for toxoplasmosis.

DISCUSSION

Alpha and Beta chain regions are sites with the highest polymorphism of MHC genes and evidence the diversity of the immunological response against infectious pathogens. Interactions with the environment and with the individual´s genetic factors increase susceptibility to several infections and diseases in humans. Allele diversity may be a consequence of interaction with pathogenic genes since MHC molecules have an important role in the induction of immune responses, coupled to susceptibility or protection against disease.[33,34] During the literature review, we found only one study on HLA and nasal carriage of S. Association studies have been undertaken on several diseases for the effective search of specific markers for susceptibility and for resistance.[4]

Opportunist infections in immunocompromised individuals become more frequent. Bibliographical research have shown people with AIDS had encephalitis caused by *T. gondii*, and genes HLA-DQ*01 and HLA-DQ*03 seemed to be related to genetic susceptibility marker for the development of the disease.[35] The possibility that genes HLA-DQ*0402 andDRB1*08 may be related to susceptibility to encephalitis for toxoplasmosis was recorded in another study with HIV-positive patients and with neurotoxoplasmosis.[36] The above specificities have a relevant role in determining the immune response against *T. gondii* and as evidence of a genetic regulation for the susceptibility of these patients in undergoing these neurological disorders. In a study with 87 patients undergoing dialysis in the state of Minas Gerais, Brazil, to record frequency of genotypes HLA-A,- B* and DRB1* and the association with risk of terminal kidney disease, DRB1*07 was the most frequent specificity, with 14.67%, and thus corroborating current analysis.[37]

Congenital toxoplasmosis

Congenital toxoplasmosis, one of the most serious types of the disease, has been studied by many researchers. There may be an ocular involvement association with antigen HLA-Bw*62 in patients´ mothers that have ocular toxoplasmosis, since it is the main consequence of the disease.[38] Another association with the same type of disease has been found in newly-born children infected by hydrocephaly with a prevalence of HLA-DQ*03 when compared with infected children without hydrocephaly.[39]

The participation of molecules HLA in the presentation

of peptides foreign to immune system cells shows that infectious diseases may exert selective genetic pressure and that genes involved in the immune response are more numerous and more polymorphic within the human genome. The evolution of the immune response towards a wide diversity of pathogenic agents is thus suggested.[40]

Current analysis is thus a contribution on the involvement of molecules HLA-DRB1*17 as a possible genetic marker for toxoplasmosis susceptibility in patients undergoing dialysis and the involvement of molecule HLA-DRB1*07 in patients with kidney transplants.

CONCLUSION

Alleles involved in the susceptibility for and/or protection against toxoplasmosis were not detected in molecules HLA class I (HLA-A and HLA-B) either in patients undergoing dialysis or in patients with kidney transplants.

DRB1*17 and DRB1*07 in HLA class II molecules were more frequent in the two groups of patients with soropositivity for anti-IgG antibodies.

No specificities that would suggest susceptibility and/or protection against acute infection (IgM) were detected either in patients undergoing dialysis or in patients with kidney transplants.

Results suggest that DRB1*17 and DRB1*07 may be involved in susceptibility for toxoplasmosis. A multi-professional team has to be careful in its prevention when interrupting or slowing down toxoplasmosis progression.

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