A study on HLA-DR/DQ typing in adult Malay patients with acute amoebic liver abscess

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ABSTRACT

Introduction: Amoebiasis is a parasitic disease caused by *Entamoeba histolytica* that may lead to death in developing countries. Few important risk factors have been identified in the development of amoebic liver abscess (ALA). There are limited reports that suggest an association between antigens of the major histocompatibility complex (MHC) particularly class II antigens and ALA development. This present work aimed at studying the possible association of HLA antigens with ALA and disease severity. Results of the study may serve as a guide for further immunological studies dealing with *E. histolytica*.

Methods: This preliminary study involved two groups of subjects: 20 ALA patients in the experimental group and 40 healthy individuals in the control group. Cases were selected from adult Malay patients confirmed with ALA based on clinical signs and symptoms, radiological findings, microbiological findings and who were admitted to the medical or surgical ward, Hospital USM, Kelantan. Venous blood was obtained from each patient and HLA typing was then conducted using polymerase chain reaction specific primer sequence.

Results: HLA DR12 was most frequently found in the healthy control and ALA groups at 40% and 55% respectively. HLA DQ7 and DQ8 were found to have the highest percentage in the ALA group at 65%. In the control group, HLA DQ8 (57.5%) had the highest percentage.

Conclusion: HLA antigens play a role in acquisition of ALA and provide understanding of the disease outcome.

KEY WORDS:
Amoebic Liver Abscess, Entamoeba histolytica, HLA DR/DQ Typing, human leucocytes antigens, major histocompatibility complex

INTRODUCTION

Amoebiasis is one of parasitic diseases that may lead to death in developing countries. Amoebic liver abscess (ALA) is the most common extraintestinal manifestation which accounts for 8.5% of symptomatic patients infected with E. histolytica.[1] Few important risk factors have been identified in the development of ALA. It has been identified that people who originate from or travel to an endemic area followed by underlying severe immunosuppression state, chronic infection or malnourishment with severe hypoalbuminaemia are at risk.[2] Cases associated with alcoholism and diabetes mellitus had also been reported.[3]

Risk factors associated with ALA include alcoholism, malnutrition, and immunosuppression.[4] A few reports suggest that there are associations between ALA and antigens of the major histocompatibility complex (MHC), particularly class II antigens. In understanding the disease process, several studies demonstrate the presence of infarction, enzymatic hydrolysis and immunological reaction (either independently or in combination) as contributors in the formation of an abscess.[5]

The human leucocytes antigens (HLA) describe a region of genes located on chromosome 6 in humans and compose of a series of highly polymorphic genes based on multiple alleles. Among HLA class II genes, DRB1 coding for DRβ chains has the highest degree of polymorphism, and appears to be responsible for variations in the immune responses of different individuals to different antigens. Genetic variability influences susceptibility to several diseases, including ALA.

Antibody responses with both innate and acquired immunity indicate that CD4+ T cells play a role in protection against diseases or infection. Poor innate and acquired immunity increase susceptibility. The association of specific HLA alleles with susceptibility to infectious and autoimmune diseases is probably attributable to a direct involvement of the HLA molecules as an antigen presenter or possibly due to a close gene linkage.[6]

Thus, this study was conducted as a prevalence study for local immunological data and HLA typing in the Malay population. The research findings herein may guide further immunological studies pertaining to *E. Histolytica*. It promotes further understanding of the disease as well as initiates field studies on HLA typing. Findings from this study would help in identifying contributory or risk factors involved in ALA patients in our population particularly in terms of

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immunological predispositions. It would also serve as a guide in determining the probable outcome or prognosis of the disease or invasive amoebiasis based on underlying genetic predispositions.

MATERIALS AND METHODS

This is a comparative cross-sectional study. The present study aimed to compare the distribution of HLA DR/DQ between acute ALA patients and healthy individuals and to determine the association of HLA DR/DQ type with severity levels of acute ALA. Individuals for the experimental group were selected from adult Malay patients confirmed with ALA who were admitted to the surgical or medical ward, Hospital USM, Kelantan. Malay subjects were selected based on subjects' first and last names as well as their identification card. We did not further define the study population as it was not our intention to relate and ascertain the ancestry of the study population.

Diagnostic criteria for the experimental group were based on a) clinically enlarged tender liver with palpable abscess and associated systemic toxaemia, b) aspiration of anchovy sauce pus which was sterile on bacteriological examination, or the presence of *E. histolytica* trophozoites in the aspirated pus, c) high titers of anti-amoebic antibodies (titer of 1: 256 or more) as studied by an indirect haemagglutination assay, and d) may or not responded to specific anti-amoebic therapy, such as metronidazole or emetine.[7] The healthy control subjects were a) adult Malay (negative anti-amoebic antibody (titer less than 1:256), b) having no signs or symptoms of amoebiasis and c) no history of any underlying illness).

Disease severity in acute ALA is divided into two categories.[8] Category 1: Mild to moderate severity which is defined as those acute ALA patients with a) improvement and subsidence of symptoms and signs within 48 to 72 hours, c) abscess size of 10 cm or less, d) no compression lesion to inferior vena cava, e) single abscess, and f) no jaundice clinically. Category 2: Severe infection in acute ALA patients is characterised by a) a lack of improvement with subsidence of symptoms and signs within 48 to 72 hours, b) abscess size more than 10 cm c) compression lesion in a posterior located ALA may present with inferior vena cava obstruction d) multiple liver abscesses, e) presence of jaundice due to a large abscess or abscess at porta hepatic and/or f) complications such as ruptured an abscess.

The study was approved by human ethical committee of Universiti Sains Malaysia number: USMKK/PP/JEPeM [225.3(03)]. The serum specimens from consented patients and individuals were sent to the laboratory for HLA-DR/DQ typing and determination of specific type. The DNA extraction of the blood sample was obtained with Qiagen columns (QIAamp DNA minikit; Qiagen, Hilden, Germany). The extraction procedures were done according to manufacturer's instruction. HLA DR-DQ typing of the sample was obtained with polymerase chain reaction (PCR) amplification with sequence-specific primers (Olerup SSP low resolution typing kits, Stockholm, Sweden). Statistical analysis was done using SPSS software version 22. Fisher exact was used to test the association between two categorical variables.

RESULTS

HLA DR12 antigen was found to be the most common in the healthy control group (40%) and ALA group (55%). HLA DR8 and DR10 were only detected in the healthy group. The presence of HLA DR12, DR15 and DR16 were more frequent in the ALA group compared to the healthy control group at 55%, 45% and 15% respectively. HLA DR7, DR8, DR9, DR10, DR11, DR13, DR14 and DR17 were more common in the healthy control group than the ALA group. Neither HLA DR1 nor DR18 was detected in both groups.

In terms of HLA DQ antigen, HLA DQ7 and DQ8 were found to have the highest percentage in the ALA group at 65% respectively. In the healthy control group, HLA DQ8 had the highest percentage (57.5%). In comparing the two groups, HLA DQ2, DQ6 and DQ9 were found to be more frequent in the healthy control group. In the ALA group HLA DQ4 (10%), DQ7 (65%) and DQ8 (65%) were more frequently seen than in the healthy control group. No HLA DQ9 was detected in the ALA group. In this study it was found that all HLA DQ antigens were not significantly associated with any group ($p>0.05$) (Table I)

HLA DR12 had the highest frequency in the mild to moderate category. While in the severe category, HLA DR12 (50%) and DR15 (50%) had the highest frequency. When compared between categories, the presence of HLA DR9 (6.3%), DR11 (6.3%), DR12 (56.3%), DR14 (12.5%) and DR17 (6.3%) were more frequent in the mild to moderate category. In the severe category HLA DR4, DR7, DR13, DR15 and DR16 were more frequently seen compared to the mild to moderate category. HLA DR9, DR11, DR14 and DR17 were only seen in the mild to moderate category. However, all HLA DR antigens were not significantly associated with severity of the disease ($p>0.05$).

Commonly found antigens in the mild to moderate category were HLA DQ7 and DQ8 (68.8% respectively). These antigens were also higher in percentage when compared to the severe category. HLA DQ2 only seen in patients with mild to moderate category. A commonly found antigen in the severe category was HLA DQ5 (75%).These antigens were also higher in percentage when compared to the mild to moderate severity category (Table II).

It is mentioned here that in the HLA kit used for the study, HLA DR2, DR3, DR5, DR6, DQ1 and DQ3 were not included in the panel of HLA type. These markers were not part of the original HLA kit.

DISCUSSION

The HLA class II molecule is a heterodimeric membrane protein consisting of monomorphic α and highly polymorphic β chains, and is expressed on antigen APCs, B cells and activated T cells in humans. Genetic variability influences susceptibility to several diseases. A widespread range of diseases have been linked with different HLAs, like ankylosing spondylitis, rheumatoid arthritis, coeliac disease, insulin-dependent diabetes mellitus, multiple sclerosis, Goodpastures' syndrome, narcolepsy, Chagas disease, alopecia, tuberculosis, etc.[9]

Table I: Frequency of HLA-DR/DQ antigens in healthy control and ALA groups

Serotype	Healthy n(%)	ALA n(%)	p-value
HLA DR1	0 (0%)	0(0%)	-
HLA DR4	6(15%)	3(15%)	*1.000
HLA DR7	8(20%)	2(10%)	*0.471
HLA DR8	2(5%)	0(0%)	*0.548
HLA DR9	9(22.5%)	1(5%)	*0.142
HLA DR10	1(2.5%)	0(0%)	*1.000
HLA DR11	6(15%)	1(5%)	*0.407
HLA DR12	16(40%)	11(55%)	0.271
HLA DR13	6(15%)	2(10%)	*0.707
HLA DR14	6(15%)	2(10%)	*0.707
HLA DR15	15(37.5%)	9(45%)	0.576
HLA DR16	2(5%)	3(15%)	*0.322
HLA DR17	4(10%)	1(5%)	*0.656
HLA DR18	0(0%)	0(0%)	-
HLA DQ2	9 (22.5%)	2 (10%)	*0.307
HLA DQ4	1(2.5%)	2 (10%)	*0.255
HLA DQ5	18 (45%)	9(45%)	1.000
HLADQ6	12 (30%)	5(25%)	0.685
HLA DQ7	21 (52.5%)	13(65%)	0.357
HLA DQ8	23 (57.5%)	13 (65%)	0.576
HLA DQ9	2 (5%)	0 (0%)	*0.548

*Fisher exact test

Table II: Frequency of HLA-DR/DQ antigens in ALA group with severity of disease

Serotype	Mild-Moderate	Severe	p-value
HLA DR1	0 (0%)	0 (0%)	-
HLA DR4	2 (12.5%)	1 (25%)	*0.509
HLA DR7	1 (6.3%)	1 (25%)	*0.368
HLA DR8	0 (0%)	0 (0%)	-
HLA DR9	1 (6.3%)	0 (0%)	*1.000
HLA DR10	0 (0%)	0 (0%)	-
HLA DR11	1 (6.3%)	0 (0%)	*1.000
HLA DR12	9 (56.3%)	2 (50%)	*1.000
HLA DR13	1 (6.3%)	1 (25%)	*0.368
HLA DR14	2 (12.5%)	0 (0%)	*1.000
HLA DR15	7 (43.8%)	2 (50%)	*1.000
HLA DR16	2 (12.5%)	1 (25%)	*0.509
HLA DR17	1 (6.3%)	0 (0%)	*1.000
HLA DR18	0 (0%)	0 (0%)	-
HLA DQ2	2 (12.5%)	0 (0%)	*1.000
HLA DQ4	1 (6.3%)	1 (25%)	*0.368
HLA DQ5	6 (37.5%)	3 (75%)	*0.285
HLADQ6	4 (25.0%)	1 (25%)	*1.000
HLA DQ7	11(68.8%)	2 (50%)	*0.587
HLA DQ8	11 (68.8%)	2 (50%)	*0.587
HLA DQ9	0 (0%)	0 (0%)	-

*Fisher exact test

The associations of antibody responses with both innate and acquired immunity to amoebiasis indicate that CD4+ T cells play a role in the protection against *E. histolytica* infection. The invasion of the liver by *E. histolytica* appears to require a degree of down-regulated immune responses in the host.[10]

The association between human parasitic diseases and the MHC system (HLA) has not been extensively studied, even though the gap between infection and actual disease (i.e. amoebiasis) is wide enough to suggest the existence of host risk factors that could presumably be influenced by the MHC.[11, 12] In addition certain alleles in the MHC of the host that may contribute to peculiarity of immuno inflammatory prerequisites for successful amoebic invasion remains to be clarified.[13] It is theorised that the association of specific HLA alleles with susceptibility to infectious and autoimmune diseases is probably attributable to a direct involvement of the HLA molecules as an antigen presenter or possibly due to a close gene linkage.

HLA DR2, DR3, DR5 and DR6 are broad antigens serotype. DR15 and DR16 are the antigens split or variant of DR2. DR11 and DR12 are the antigens split or variant of DR5. DR13 and DR14 are the antigens split or variant of DR6. DR17 and DR18 are the antigens split or variant of DR3. Thus, these variants could explain the reason for no positivity or exclusion as one of the panel of HLA DR antigens for these broad antigens.

The high percentage of HLA DR12 and HLA DQ8 in both groups probably reflects the predominant genetic inheritant of HLA DR serotype in the Malay ethnic population. It is known that DRB1*12 allele is accountable in 15–36% of the Malay population in Malaysia. An earlier research identified that it is included as one of the most common HLA alleles in all Malay subethnic groups in Malaysia.[14] In addition, the presence of DQB1*03 alleles (HLA DQ8 is the antigen split for HLA DQ3) in 25–51% of all Malay subethic groups may support the increased frequency of HLA DQ8 in this present study.[14]

Expression of HLA DR3 may encode a risk for ALA development in previous study observed in the Mexican mestizo population.[13] Extended observation in a paediatric Mexican mestizo population with ALA, confirmed the increase in their expression of HLA DR3. However, increased frequency of HLA DR3 was not seen in this current study to support the previous study. It is suggested that the presence of HLA DR3 in patients with ALA may be related to the selected and/or induced immunodepression that reputedly accompanies the early stages of amoebic hepatic invasion in humans and experimental animals.[10]

Increased frequency of HLA DR12 and DR15 in this study may suggest that immunological attribution is due to the acquisition of invasive amoebiasis. In contrast with a previous study, it was found that HLA DR3 increased significantly in adults patients with ALA in a Mexican mestizo population when compared to a normal

population.[13] Comparing with paediatric population, increased frequency of HLA DR5 has been found but is absent in adults populations, showing some degree of pecularities in paediatrics compared to adults alleles.[15] In other non ALA related study, the role of HLA-DR15/HLA-DQ6 haplotype and its alleles have been reported to be associated with the development of choroidal neovascular lesions in Post Ocular Histoplasmosis Syndrome and susceptibility to the disease process.[16]

The possible association of HLA DR3 in ALA is that it may reflect a genetic marker for weak immune responses.[17] Expression of HLA DR3 may encode a risk for ALA development in the Mexican mestizo population.[18] Extended observation in a paediatric Mexican mestizo population with ALA, confirmed the increase in their expression of HLA DR3. However, increased frequency of HLA DR3 was not seen in this current study to support the previous study. It is suggested that the presence of HLA DR3 in patients with ALA may be related to the selected and/or induced immunodepression that reputedly accompanies the early stages of amoebic hepatic invasion in humans and experimental animals.[10]

In a previous study, potential protective association was observed between the HLA class II allele DQB1*0601 and the heterozygous haplotype DQB1*0601/DRB1*1501. This association may explain why amoebiasis does not occur in some children who are exposed to the parasite. This association also implicates HLA class II–restricted immune responses in protection against *E. histolytica* infection.[19]

This present study showed that HLA DR12 was the most frequent HLA DR serotype in the healthy control group and ALA experimental group. The HLA DR serotype found in the ALA group might have a protective role. Interestingly, HLA DR7, DR8, DR9, DR10, DR11, DR13, DR14 and DR17 were more frequently detected in the healthy control group when compared to the ALA group. Furthermore, HLA DR8 and DR10 were only detected in the healthy group.

Inheritance of these serotypes may possibly be involved in the protective role of acquiring ALA. However, the results of this study were statistically insignificant. Neither HLA DR1 or DR18 was detected in both groups. There is a possibility that the Kelantan population might genetically absence or low frequency of those HLA antigens as all the subjects were originated from Kelantan descendant. This particular finding is supported by a study on HLA polymorphism in subethnic groups in Malaysia. In that study, the Kelantan population evidenced low frequency of HLA DRB1*01 and DRB1*03 alleles at 4% respectively.[14]

Potential association of HLA DQ7 with invasiveness of the disease has been previously described in other diseases, especially in persistence of hepatitis B. However, while the detection of allele from the HLA DQ7 has been significantly associated with the clearance of hepatitis C instead of hepatitis B.[20] The higher percentage of HLA DQ2, DQ6 and DQ9 in the healthy control group may contribute to the protection against invasive disease. A potential protective role of HLA DQ*06 alleles have been described in a previous study.[19] HLA DQ3 has been described as a probable risk factor for CMV infection in high risk kidney transplant compared to patients negative for HLA DQ3. More symptomatic infections were found in patients with DQ3. However, the difference wasn't significant.[21] In our present study although HLA DQ3 was among the highest frequency in ALA group, the result still remains insignificant ($p>0.05$). A comparative study done in mexico city among patients with ALA and healthy inviduals has shown HLA-DRB1*08 and HLA-DQB1*04 were significantly higher in controls group compared to ALA group. Thus, based on the analyses, they have observed these alleles could have potential protective role in the development of ALA.[22]

Host genotype has been described to influence outcome of ALA as it involves in regulation of host-parasite relationship in disease pathogenesis.[23] HLA type may be useful as a predictor of outcome during the ALA onset and alerting the clinicians who manage the patients.

Currently, there is no standard classification of ALA severity in the literature. For the purpose of this study, it was decided that the level of the severity of ALA be based on clinical, laboratory, and complications assessments that reflected the aggressiveness of the disease.

It was difficult to predict whether HLA DR serotypes influenced the severity of ALA as HLA DR12 and DR15 were found in both groups. It was earlier speculated that those patients with HLA DR9, DR11, DR12, DR14 and DR17 would possibly develop a mild to moderate presentation of ALA. However, the present study showed an insignificant result for this speculation.

It was postulated that patients evidencing high levels of HLA DR9, DR11, DR14 and DR17 would manifest mild to moderate ALA. This study indicated that patients with these particular HLA DR antigens manifest mild to moderate ALA. Those with HLA DR4, DR7, DR13, DR15 and DR16 may tend to manifest as severe presentation. The presence of these antigens probably explains the more frequent proportion of those serotypes in the severe ALA category.

The present study showed HLA DR antigens were closely associated with the clinical diagnosis and disease severity. The inheritance of HLA DQ2, DQ7 and DQ8 may possibly lead to mild to moderate disease as well as better disease outcome. Genetically inherited high levels of HLA DQ5 may possibly lead to severe presentations and poorer outcome of ALA.

If broad antigens were to be considered, HLA DQ3 (68.8%) were included among the highest frequency of HLA antigens in patients with mild to moderate category while HLA DQ1 (75%) also was included as one of the most frequent HLA antigens in severe group. However, there was still no significant association between HLA DQ3 and HLA DQ1 with the severity of the disease ($p>0.05$) which was most likely due to the small sample size in this study.

CONCLUSION

In conclusion, immunological characteristic in terms of genetic predisposition in ALA provide a better understanding of its role in the disease acquisition, course and its outcome. This data will serve as an additional input to interested researchers and could be utilized in other research related to HLA and ALA. The authors would suggest for a more elaborate study to be conducted to provide more meaningful information regarding the association of HLA class II antigens and the disease process among Malaysian population.

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