The Association between HLA Antigens and Inflammatory Bowel Disease in Iraqi Patients

Ali H. Ad'hiah (Ph.D)[1], Hamsa A. Jasim (M.Sc.)[2] and Ayad M. A. Fadhil (Ph.D)[2]

[1] Tropical Biological Research Unit, University of Baghdad, Jadryia, Iraq
[2] Department of Biotechnology, College of Science, Al-Nahrain University, Jadryia, Iraq.

Abbreviations and Key words

IBD: Inflammatory bowel disease.
ULC: Ulcerative colitis.
CRD: Crohn's disease.
RR: Relative risk.
EF: Etiological fraction.
PF: Preventive fraction.
P: Fisher's exact probability.
Pc: Corrected P.

Abstract

The present study was designed to investigate the association between HLA- class I (A and B) and -class II (DR and DQ) antigens and inflammatory bowel disease (IBD) in a sample of Iraqi patients (65 subjects). The patients were clinically subdivided into ulcerative colitis (ULC; 50 patients) and Crohn's disease (CRD; 15 patients). A control sample of 67 individuals, matched for age, sex and ethnic background (Arab Muslims), was further investigated.

At HLA class I region, the total patients showed significant increased frequencies of A9 (52.3 vs. 17.9%) and B41 (66.1 vs. 5.9%), and a significant decreased frequency of A11 (3.1 vs. 22.4%) as compared to controls. Similar findings were outcome when the clinical types (ULC and CRD) were considered. While at HLA-class II region, DR8 was significantly increased in the total patients (30.8 vs. 8.9%), but neither ULC nor CRD maintained such association, and instead ULC was significantly associated with DQ1 (40 vs. 18%). However, comparing ULC with CRD revealed a significant difference in the antigen B16 (2 vs. 33.3%).

Introduction

Inflammatory bowel disease (IBD) is a group of disorders, defined by the presence of chronic gastrointestinal inflammation no due to a specific disease-producing organism. Two clinical forms of the disease exist, ulcerative colitis (ULC) and Crohn's disease (CRD), which in the main have different clinical and pathological features (1).

The aetiology of IBD (ULC or CRD) is not well understood, although epidemiological, as well as, other clinical and laboratory based evidences suggest that the disease may be multifactorial, and genetic, immunological and environmental factors have been of a great concern. But, what is the nature of these factors, and how they interact to produce a disease, is a matter of speculations (2).

Family and twin based evidences suggest that genetics plays an important role in predisposing an individual to develop ULC and CRD. A familial tendency has been clearly observed for IBD, and a 4% chance for the disease to occur in a child of a parent with IBD has been demonstrated, moreover, 10-15% of the patients are estimated to have a first degree relative with the disease (3, 4, 5). Twin studies confirmed those findings, and a higher concordance rate of the disease was observed among monozygotic twins than dizygotic twins (ULC: 11 vs. 3%; CRD: 35 vs. 7%) (6, 7, 8).

A further evidence of a genetic predisposition came from HLA-IBD association studies, however, these associations have only been shown in homogeneous groups of populations. In Japanese patients, HLA-B5 has been recorded to be associated with ULC, while in Caucasian patients the disease has shown associations with two HLA-class I antigens (B27 and B44). Crohn's disease behaved in a similar manner, and the association has been reported with HLA-DR4 in Japanese patients and HLA-B44 in Caucasian patients (9). Later studies, which were

ISSN:1811-5922

based on a molecular typing of HLA-class II region, have emphasized the importance of HLA-DR polymorphism in the aetiology of IBD (10).

Accordingly, the present study was designed to investigate the association between HLA polymorphisms (class I and II antigens) and IBD in a sample of Iraqi patients. The disease heterogeneity was also considered, and therefore, two clinical entities of the disease were studied. These were CRD and ULC.

Subjects, Materials and Methods

Patients: Sixty–five Iraqi patients with IBD were investigated. The disease was clinically diagnosed by the consultant medical staff at Al- kadhamyiah Teaching hospital and Gastrointestinal Tract (GIT) and Liver Disease Center in Baghdad. The diagnosis was based on a clinical evaluation using colonoscopy and a histopathological examination of a biopsy. According to the point view of consultants, the patients were clinically subdivided into ULC (50 patients) and CRD (15 patients). Sixty–seven individuals (blood donors) were further investigated, and were considered as a control sample. They were matched with patients for age, sex and ethnic background (Arab Muslims) (Table 1).

HLA Phenotyping: From each subject, 10 ml of venous blood were obtained and processed in less than two hours. Lymphocytes were collected by means of density gradient centrifugation (11), and then subjected to separation into T- and B-cells by nylon-wool method (12). Phenotyping of the cells for HLA-class I (A and B) and -class II (DR and DQ) antigens was carried out by the microlymphocytotoxicity method (13).

Statistical Analysis: Significant variations between alleles were assessed by Fisher's exact propbability (P), and the obtained P was corrected for the number of antigens tested at each locus (Pc). The results were presented in terms of relative risk (RR), etiological fraction (EF) and preventive fraction (PF). The latter two estimations were calculated when the RR values were greater (positive association) and less (negative association) than one, respectively (14).

Results

Total Patients

HLA antigens showing significant variations between total IBD patients and controls are given in table 2.

At HLA-A locus, four antigens (A9, A11, A30 and A33) showed significant deviations when comparisons between patients and controls were made. The antigen A9 was significantly ($P = 4.7 \times 10^{-4}$) increased in the patients (52.3 vs. 17.9%), and such difference associated with RR value of 5.05 and PF value of 0.42. Moreover, this positive association remained significant ($Pc = 5.1 \times 10^{-4}$) after correction for the number of antigens tested (11 antigens). In contrast, the antigens A11, A30 and A33 showed negative associations with IBD, and significant ($P = 0.001$, 0.021 and 0.045, respectively) decreased frequencies of these antigens (3.1 vs. 22.4%, 6.2 vs. 20.9% and 4.6 vs. 16.4%, respectively) were observed in the patients. But, correcting the probabilities rendered one negative significant association, which was between A11 and IBD (Pc = 0.011).

At HLA-B locus, B41 antigen was significantly increased in the patients (66.1 vs. 5.9%, $P = 7.8 \times 10^{-15}$, RR = 30.78, PF = 0.63), while antigens B35 and B51 were significantly (P = 0.039 and 0.009, respectively) decreased in the patients (6.2 vs. 17.9% and 16.9 vs. 37.3%, respectively). Correcting the probabilities of these associations gave one significant positive association, which was between B41 and IBD ($Pc = 1.4 \times 10^{-12}$).

At HLA-class II region (DR and DQ loci), five antigens showed different frequencies in patients and controls, these were DR4, DR5, DR7, DR8 and DQ2. Increased frequencies of DR4 (33.8 vs. 13.4%), DR5 (16.9 vs. 3.1%), DR8 (30.8 vs. 8.9%) and DQ2 (24.6 vs. 10%) were observed in the patients. These positive associations scored RR values of 3.3, 6.6, 4.5 and 2.8, respectively, and PF values of 0.23, 0.14, 0.24 and 0.16, respectively. However, one positive association remained significant after correction (Pc = 0.018), and this was with DR8. In contrast, the DR7 antigen was significantly decreased in the patients (7.7 vs. 23.9%), but such negative association also failed to retain a significant level after correction (Pc = 0.144).

Ulcerative Colitis Patients

HLA antigens showing significant variations between ULC patients and controls are summarised in table 3.

At HLA-A locus, two antigens (A3 and A9) showed increased frequencies in the patients, and other two antigens (A11 and A30) showed decreased frequencies. The antigen A3 was present in 26% of the patients, while its frequency in the control group was 8.9%. Such positive association was significant before correction (P = 0.021) but not after (Pc = 0.231), although RR and PF values of 3.5 and 0.19 were respectively recorded. The antigen A9 was also positively associated with

ULC, and an antigen frequency of 14% was present in the patients, while in the control subjects, the frequency was 17.9%. Such deviation associated with RR value of 3.6 and EF value of 0.31, moreover, the probability of such observation was significant before ($P = 0.004$) and after ($Pc = 0.044$) correction. In contrast, the antigens A11 and A30 showed negative associations with ULC, and each of the two antigens scored a frequency of 4% in the patients, while in the controls, the frequencies were 22.4 and 20.9%, respectively. Although, these two associations were significant ($P = 0.007$ and 0.0121, respectively), the corrected probabilities failed to attain a significant level ($Pc > 0.05$).

At HLA-B locus, the antigen B41 showed a significant ($P = 2.2 \times 10^{-14}$) increased frequency (72 vs. 5.9%), and such positive association was highly significant after correction ($Pc = 3.9 \times 10^{-13}$), with RR value of 40.5 and EF value of 0.7.

At HLA-class II region (DR and DQ loci), several deviations (increased or decreased) in antigen frequencies were observed in the patients when comparisons were made with control subjects. Increased frequencies of antigens DR4 (36 vs. 13.4%), DR5 (16 vs. 3%), DR8 (28 vs. 8.9%), DQ1 (40 vs. 18%) and DQ2 (26 vs. 10%) were observed in the patients. The RR values of such positive associations were 3.0, 6.19, 3.95, 3.05 and 3.01, respectively, and the EF values were 0.24, 0.13, 0.21, 0.26 and 0.17, respectively. These associations were significant before correction, but after correction, only the association with DQ1 remained significant ($Pc = 0.036$). A negative association was also observed between DR7 and ULC. The antigen was present in 6% of the patients, while its frequency in controls reached 23%. Such deviation was significant before correction ($P = 0.011$) but not after ($Pc = 0.108$).

Crohn's Disease

HLA antigens showing significant variations between CRD patients and controls are summarised in table 4.

At HLA- A locus, the antigen A9 reached a frequency of 60% in the patients, while in the controls such frequency was 17.9%. Such deviation was significant before ($P = 0.002$) and after ($Pc = 0.022$) correction, and associated with RR value of 3.6 and EF value of 0.18.

At HLA B locus, three antigens (B16, B41 and B51) exhibited variations between patients and controls. Increased frequencies of B16 (33.3 vs. 4.5%) and B41 (46.6 vs. 5.9%) were observed in the patients. Such deviations elevated the RR values to 10.6 and 13.7, respectively, and EF values to 0.29 and 0.42, respectively. The probabilities of such positive associations were significant before correction ($P = 0.001$ and 0.0004, respectively), but after correction, the association between B41 and CRD remained significant ($Pc = 0.007$). The antigen B51, in contrast, showed a significant ($P = 0.029$) decreased frequency in the patients (6.6 vs. 37.3%), with a PF value of 0.30, but the difference lost significance after correction ($Pc = 0.522$).

At HLA-class II region, increased frequencies of DR5 (20 vs. 3%), DR8 (40 vs. 8.9%), and DQ1 (60 vs. 18%) were observed in the patients. Such variations associated with RR values of 8.1, 6.7, and 6.8, respectively, and EF values of 0.17, 0.34, and 0.51, respectively. These positive associations were significant before correction ($P = 0.04$, 0.007, and 0.03, respectively), but not after ($P > 0.05$).

Immunogenetic Heterogeneity of IBD

The immunogenetic heterogeneity of IBD was assessed by comparing the antigen frequencies of HLA-A, -B, -DR and -DQ loci between ULC and CRD patients. Several antigens (A2, A3, A9, A30, B16, B41, B51, DR4, DR6, DR7, DR8 DR10, DQ1 and DQ2) showed different distributions in the two groups of the patients, but none of these differences maintained a significant level. The antigen B16 was an exception in this regard. Out of 50 ULC patients, only one subject (2%) expressed B16, while 5 subjects (33.3%) out of 15 CRD patients expressed this antigen. Such difference associated with a probability value of 0.002, which was significant even after correction for the number of antigen tested at HLA-B locus ($Pc = 0.036$).

Discussion

The present study demonstrated that immunogenetic predisposition may be considered as an important requirement for the development of IBD, and HLA antigens are in favour of such generalization, in which several markers of human MHC showed different distributions in patients and controls. These markers are belonging to two regions of human chromosome 6. They are HLA-class I and -class II loci, in which a highly polymorphic status is recognized (15).

At HLA-class I region, remarkable deviations were observed for the antigens A9, A11 and B41. Both A9 and B41 were in favour of increased frequencies in the patients. The antigen A9 was observed in around 50% of the patients, while B41 antigen was much higher (about two-thirds of the patients). Such two deviations scored

RR values of 3.03 and 30.78, respectively, and maintained EF values of 0.42 and 0.63, respectively. Inspecting other HLA-IBD-association studies carried out in other world populations revealed associations with other HLA-class I antigens: B5 in Japanese, and B27 and B44 in Caucasians (9). Such discrepancy can be explained in the ground of racial differences, especially if we consider that HLA antigens show different frequencies in different populations including Iraqis (16, 17). However, looking at the subject from a different angle may help to bridge the discrepancy, and the estimated EF values may establish the theme. As suggested by the statisticians, the EF value can range from 0 (no association) to 1 (maximum association). In other term, a value of 1 for an antigen is interpreted that this antigen is fully responsible for the development of the disease, otherwise, if the value is between 0 and 1, the interpretation is that this marker is partially involved in the disease development, and other factors (i.e. environmental pathogens) are operative (14). The EF values of A9 (0.42) and B41 (0.63) support the forthcoming statement, and 37 and 58% contributions of other factors in association with A9 and B44, respectively, are required in the development of IBD, but what are these factors?. The answer may be augmented if we consider the immunological roles of HLA-class I antigens. These antigens are virtually expressed on most nucleated cells, and are involved in antigen presentation carried out by macrophages, especially those antigens of a viral origin (18). A default in such mechanism may render an individual vulnerable to any immunologically-mediated morbidity (19). In this respect, different viruses (Herpes viruse, cytomegalovirus and influenza virus) have been encountered in different populations (20), and although none of them is a conclusive causative agent, their importance can not be ignored if the immunological role of HLA-class I antigens in antigen presentation is considered. In this respect, the hypothesis of molecular mimicry may have the clue, because each potential virus may share epitopes with HLA antigens, and such sharing may be different in different populations due to the environmental impact, which is certainly varied from region to region in the globe (2). Therefore, the positive association of A9 and B41 with IBD in Iraqi patients could not be confirmed by previous studies.

A further inspection of HLA-class I antigens in subgroups of IBD (ULC and CRD) revealed a similar picture, and both A9 and B41 antigens were positively associated with the two clinical entities of IBD. In this sense, these two antigens may confer an immunogenetic predisposition to the general IBD irrespective of its clinical types, although the strength of the two associations was different. The B41 was more important than A9 in ULC (RR = 40.5 vs. 3.6; EF = 0.70 vs. 0.31), while the opposite outcome was observed in CRD, in which the A9 is the most important class I antigen (RR = 6.88; EF = 0.51).

As HLA-class II region, further antigens were in favour of positive associations with IBD. these were DR4, DR5, DR7 and DR8, but these associations were limited to DR8 after correction of probability (Pc = 0.018). Although, these associations were not strong as that of HLA class I region, the polymorphism of HLA-class II loci has gain much more interest in HLA-disease association studies, because both α and β chains, which are coded by structural genes on chromosome 6, are highly polymorphic especially at HLA- DQ and -DP subregions (21). Accordingly, it has been suggested that susceptibility to IBD is partially genetically determined, and the HLA-class II genes are candidates for a role in genetic susceptibility to IBD, because their products play a central role in the immune response (22). The present results strongly support such theme, however, multiple studies have reported associations between HL-DR and -DQ phenotypes and IBD, either ULC or CRD, but much of the data are still controversial (reviewed by 23). These studies have demonstrated that DR1, DR4, DR5 and DR7 are positively associated with CRD, while DR2, DR3 and DR8 are negatively associated. For ULC, positive associations have been found with DR2, DR6 and DR12, and negative associations with DR2, DR6 and DR7 have been observed. For HLA-DQ locus, the studies have reported further associations, and ULC has been associated positively with DQ2 and negatively with DQ3, while an increased frequency of DQ3 and DQ4 were observed in patients with CRD, and in the same disease, decreased frequencies of DQ1 and DQ6 have been reported. None of these findings was confirmed in the present study, and in contrast, DR8 was positively associated with IBD, either total or clinical subtypes. However, some of these associations were observed in the present IBD patients (IBD: DR4, DR5, DR6, DR7 and DQ2; ULC: DR4, DR5, DR6, DR7, DQ1 and DQ2; CRD: DR5, DR6 and DQ1), but the significance was lost when the probability was corrected for the number of antigens test at each locus, and such statistical

application is important to exclude a chance occurrence of an association due to many comparisons that were made (24). Therefore, the discrepancy can be ascribed to either racial differences (different associations in different populations), low sample size (the level of significance is affected) or environmental impacts (different causative pathogens). With respect to the latter factor, the phenotypic expression of HLA-class II antigens is also involved in antigen presentation by macrophages, but with antigens of a bacterial origin, and such pathogens may have adapted different epitpes in different populations (19).Therefore, a similar argument, as in HLA-class I antigens, can be upgraded, and the hypothesis of molecular mimicry can also be put forward to explain the cellular destruction at the sites of IBD in the intestine. In agreement with this scope some bacterial pathogens has been described as putative causative agents in IBD, for instances Mycobacterium paratuberculosis and Helicobacter pylori, therefore epitope sharings may be considered as a an important explaining mechanism, especially if we consider that HLA-DR positivity of mucosal cells is related to a disease activity in IBD patients (1).

Comparing the two clinical forms of IBD with each other in terms of HLA antigen frequencies revealed that the antigen B16 was significantly different in ULC and CRD. Although such observation has not been recorded, it may help to answer crucial questions regarding the genetics of IBD, and how the two clinical forms are related to each other. The answer can be augmented if we consider family studies carried out in ULC and CRD families. Such studies have demonstrated that the two clinical forms do not always segregate independently within families (2). Accordingly, it has been suggested that there are three genetic forms of IBD, one leading to ULC alone, one to CRD alone, and a third leading to both ULC and CRD. Fine mapping of the HLA region in IBD families may support this, and explain some the discrepancies in HLA-IBD association studies (25). Furthermore, an evidence for linkage of both ULC and CRD around the HLA region on the short arm of chromosome 6 has been presented (22). Moreover, these studies have described further predisposing loci on different chromosomes (2), and accordingly the disease heterogeneity has been highlighted. Therefore, can we consider B16 as a differentiating marker between the two clinical types of IBD, a further confirmation is certainly required before reaching a substantial conclusion.

References

1. Brown, W. R., Claman, H. N. and Strober, W. (1995) Immunological diseases of the gastrointestinal tract. In: Samters Immunologic Disease, 5th Ed., Edited by M. M. Frank, K. K. Austin, H. N., Claman and E. R. Unanue. Little, Brown and Company, U.S.A. pp. 1151-1178.
2. Parkes, M. and Jewell D. (2001) Ulcerative colitis and Crohn's disease: molecular genetics and clinical implications. Molecular Medicine, 7: 116-120.
3. Tysk, C., Schnurer, L. B. and Wickbom, G. (1994) p-ANCA in monozygotic twins with inflammatory bowel disease. Gut, 36: 887-890.
4. Lindberg, E., Tysk, C., Andersson, K. and Jarnerot, G. (1998) Smoking and inflammatory bowel disease. Gut, 29: 352-357.
5. Arnott, I. D., Ho, G. I., Nimmo, E. R., Satsangi, J. (2005) Toll-liker receptor 4 gene in IBD: further evidence for genetic heterogeneity in Europe. Gut, 54: 308-309.
6. Farmer R.G., Michener, W. M. and Mortimer E.A. (1980) Studies of family history among patients with inflammatory bowel disease. Clinical Gastoenterology, 9: 271-277.
7. Daly, M. J., Pearce, A. V., Fawell, L., Fisher, S. A., Latiano, A., Perscott, N. J., Forbes, A., Mansfield, J., Sanderson, J., Langerlier, D., Cohen, A., Bitton, A., Wild, G., Lewis, C. M. and Mathew, W. C. G. (2005) Association of DLG 5 R30Q variant with inflammatory bowel disease. Medicine, 329: 214-216.
8. Annese, V., Piepoli, A., Latiano, A., Lombardi, G., Napolitano, G., Caruso, N., Cocchiara, E., Accadia, L., Perri, F. and Andriulli, A. (2005) HLA-DR alleles may influence disease phenotype in patients with inflammatory bowel disease. Gut, 48: 57-64.
9. van Heel, D. A, Allen, M., Tewell, D. P. and Carey, A. H. (2001) Crohn's disease: genetic susceptibility, bacteria and innate immunity. Lancet, 357: 1902-1904.
10. Hendrickson, B. A., Gokhale, R. and Cho, J. (2002) Clinical aspects and pathophysiology of inflammatory bowel disease. Clinical Microbiology, 15: 79-94.
11. Boyum, A. (1968) Separation of leucocytes from blood and bone marrow. Scandinavian Journal of Clinical and Laboratory Investigations, 21 (suppl. 97): 1-109.
12. Bender, K. (1984) HLA System. Biotest Bulletin, 2: 64-116.

13. Terasaki, P. I. and McClelland, J.D. (1964) Microdroplet assay for human serum cytoxins. Nature, 204: 998-1000.
14. Svejgaard, A., platz, P. and Ryder, L.P. (1983) HLA and disease 1982 – a survey. Immunological Reviews, 70: 193-218.
15. Dorak, M. T. (2002) Major histocompatibility complex. Internet: http://dorakmt.tripod.com
16. Ad'hiah, A.H. (1990) Immunogenetic Studies in Selected Human Diseases. Ph.D. Thesis. University of Newcastle upon Tyne, U.K.
17. Mahdi, B. M., Al-Moussawy, K. M., Al Shaikhly, A-W. A. R., Ad'hiah, A. H. (2004) Distribution of HLA-antigens in two Arab populations in Iraq. Journal of theFaculty of Medicine Baghdad, 46: 180-183.
18. Klein, J., Figueroa, F. and Nagy, Z. A. (1983) Genetics of the major histocompatibility complex: the final act. Annual Review of Immunology, 1: 119-142.
19. Owen, M. and Steward, M. (1998) Antigen recognition. In: Immunology, 5th edition edited by I. Roitt, J. Brostoff and D. Male. Mosby International, U.K., pp. 107-120.
20. Xia. B., Crusius, J. B. A., Meuwissen, S. G. M., Pena. A. S. (1998) Inflammatory bowel disease: definition, epidemiology, etiologic aspects, and immunogenetic studies. World Gastroenterology, 4: 446-458.
21. Margulies, D. H. (1999) The major histocompatibility complex. In: Fundamental Immunology. 4th Edition, Edited by by W.E. Paul. Lippincol- Raven, U.S.A., pp. 263-286.
22. Stokkers, P. C. F., Reitsma, P. H., Tytgat, G. N. J. and van, S. J. H. (1999) HLA-DR and -DQ phenotypes in inflammatory bowel disease,: a meta-analysis. Gut, 45: 395-401.
23. Zheng, C. Q., Hu, G. Z., Lin, L. J. and Gu, G. G. (2003) Progress in searching for susceptibility gene for inflammatory bowel disease by positional cloning. World Journal of Gastroenterology, 9: 1646-1655.
24. Dorak, M. T. (2005) Statistical analysis in HLA and disease association studies. Internet: http://dorakmt.tripod.com
25. Toyada. H., Wang, S. J.,Yang, H. Y., Redfored, A., Magalong, D., Tyan, D., McElree, C. K., Pressman, S. R., Shanhan, F., Targan, S. R. and Rotter, J. I. (1993) Distinct associations of HLA class II genes with inflammatory bowel disease. Gastroenterology, 104: 741-748.

Table 1: Numbers and percentage frequencies of IBD patients (UCL and CRD) and controls divided by sex, and their age ranges.

Parameter		IBD patients						Controls (No. = 67)	
		Total (No. = 65)		ULC (No.= 50)		CRD (No. = 15)			
		No.	%	No.	%	No.	%	No.	%
Sex	Males	29	44.6	25	50.0	4	26.7	30	44.8
	Females	36	55.4	25	50.0	11	73.3	37	55.2
Age Range (years)		13-65		13-35		19-65		18-65	

Table 2: Antigens of HLA – class I class II regions showing significant variations between IBD patients and controls.

HLA Antigens	patients		Controls		RR	EF	PF	P	Pc
	No	%	No	%					
A9	34	52.3	12	17.9	5.03	0.42		4.7×10^{-5}	5.1×10^{-4}
A11	2	3.1	15	22.4	0.11		0.21	0.001	0.011
A30	4	6.2	14	20.9	0.25		0.16	0.021	0.231
A33	3	4.6	11	16.4	0.24		0.13	0.045	0.495
B41	43	66.1	4	5.9	30.78	0.63		7.8×10^{-14}	1.4×10^{-12}
B51	11	16.9	25	37.3	0.34		0.23	0.011	0.198
DR4	22	33.8	9	13.4	3.3	0.23		0.007	0.063
DR5	11	16.9	2	3.1	6.6	0.14		0.008	0.072
DR7	5	7.7	16	23.9	0.26		0.18	0.016	0.144
DR8	20	30.8	6	8.9	4.5	0.24		0.002	0.018
DQ2	16	24.6	7	10.0	2.8	0.16		0.040	0.12

Table 3: Antigens of HLA – class I class II regions showing significant variation between ULC patients and controls.

HLA Antigens	patients		Controls		RR	EF	PF	P	Pc
	No	%	No	%					
A3	13	26.0	6	8.9	3.5	0.19		0.021	0.231
A9	22	44.0	12	17.9	3.6	0.31		0.004	0.044
A11	2	4.0	15	22.4	0.14		0.19	0.007	0.077
A30	2	4.0	14	20.9	0.15		0.18	0.012	0.132
B41	36	72.0	4	5.9	40.5	0.70		2.2×10^{-14}	3.9×10^{-13}
DR4	18	36	9	13.4	3	0.24		0.007	0.063
DR5	8	16	2	3	6.19	0.13		0.018	0.162
DR7	3	6	16	23	0.2		0.19	0.011	0.099
DR8	14	28	6	8.9	1.8	0.12		0.012	0.108
DQ1	20	40	12	18	3.05	0.26		0.012	0.036
DQ2	13	26	7	10	3.0	0.17		0.045	0.135

Table 4: Antigens of HLA – class I class II regions showing significant variation between CRD patients and controls.

HLA Antigens	patients		Controls		RR	EF	PF	P	Pc
	No	%	No	%					
A9	9	60	12	17.9	0.73	0.18		0.002	0.022
B16	5	33.3	3	4.5	10.6	0.29		0.004	0.072
B41	7	46.6	4	5.9	13.7	0.42		0.0004	0.0072
B51	1	6.6	25	37.3	0.12		0.30	0.029	0.522
DR5	3	20	2	3	8.1	0.17		0.040	0.36
DR8	6	40	6	8.9	6.7	0.34		0.007	0.063
DQ1	9	60	12	18	6.8	0.51		0.030	0.09