Original Article

HLA Class I and Class II Polymorphisms and Anti-nuclear Antibodies in Hyperprolactinaemic Iraqi Females with Primary Infertility

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Summary

Background: The study was conducted to investigate the association between hyperprolactinaemia and markers of human leukocyte antigen (HLA) system in a sample of Iraqi infertile females, together with the profile anti-nuclear antibodies (ANA).

Objectives: One hundred and seventy five female patients (age range: 20 -40 years) were recruited in this study. They were attending the Institute for Embryo Research and Infertility Treatment (Al-Nahrain University) during the period January 2005 - September 2006.

Results: After clinical and laboratry evaluations, it was found that 100 patients were hyperprolactinaemic, whereas the other 75 patients were euprolactinaemic, therefore, they were considered as a control group. Based on serum level of prolactin (22-29, 30-39 and ≥ 40 ng/ml), the total hyperprolactinaemic patients were divided into three groups; I (35 patients), II (40 patients) and III (25 patients), respectively. The HLA antigens showed significant variations between patients (total and groups) and controls. In total patients, B8 (25.0 *vs*. 9.3%), DR3 (48.0 *vs*. 17.3%) and DR4 (39.0 *vs*. 13.3%) showed significant increased frequencies, while B35 showed a significant decreased frequency (7.0 *vs*. 24%). The latter decrease was also observed (5.7 *vs*. 24.0%) in group I of patients, which also showed a significant increased frequency of DR3 (54.3 *vs*. 17.3%). In groups II and III of patients, only DR3 (45.0 and 56.0, respectively *vs*. 17.3%) and DR4 (37.5 and 56.0, respectively *vs*. 13.3%) showed significant increased frequencies. Autoantibody evaluation by ANA test revealed that 22% of the total patients was positive, while all control subjects were negative, and such positivity paralleled the increased level of serum prolactin.

Key words: Hyperprolactinaemia , human leukocyte antigen (HLA) .

Introduction:

Hyperprolactinaemia is defined as a consistently elevated serum prolactin level when physiological causes of prolactin hypersecretion are excluded. It is the most common disorder of the hypothalamic-pituitary axis, which is estimated to occur in around 30% of women that have menstrual irregularities (1).The clinical manifestations of hyperprolactinaemia include hypogonadl symptoms (oligoamenorrhea, decreased libido and infertility), galactorrhea and symptoms related to mass effect such as head ache and visual changes (2). The aetiology is not well understood, but immunogenetic predisposition to develop hyperprolactinaemia may be augmented, and such predisposition is presented by alleles of the major histocompatiblity complex (MHC), which is known in human beings as human leucocyte antigen (HLA) system (3).

The HLA alleles are controlled by genes located on a region of the short arm of chromosome 6, and the prolactin gene is also located in the proximity of such region, an observation that may suggest a genetic and/or functional relationship between the products of these genes (4, 5). Furthermore, the role of HLA system in the immune response is well documented, and the antigens of such system are involved in the immunological recognition of non-self antigens (6). Equally important, prolactin is known to regulate cellular functions including proliferation, differentiation, angiogenesis, and protection against apoptosis and inflammation (7). Thus, the profiles of immunity, humoral and cellular, are probably consequently affected by hyperprolactinaemia, and some autoimmune conditions may be pictured (8). Based on this presentation, the present study was designed to investigate the association between HLA polymorphisms and hyperprolacinaemia in a sample of Iraqi female patients with primary infertility, together with the profile anti-nuclear antibodies (ANA).

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Patients and Methods

One hundred and seventy five female patients (age range: 20 -40 years) were recruited in this study. They were attending the Institute for Embryo Research and Infertility Treatment (Al-Nahrain University) during the period January 2005 - September 2006. All patients underwent history and physical examinations. They were specifically questioned about irregular menses, galactorrhea, head ache, visual changes and infertility. Cases with physiological and pharmacological causes associated with hyperprolacinaemia such as lactation or drug use were excluded. Serum prolactin level was measured in all patients at the institute laboratory using a commercial kit (miniVIDAS, bioMerieux Laboratories, France), and a serum level of 1-22 ng/ml was considered as a normal range. After such evaluations, it was found that 100 patients were hyperprolactinaemic, whereas the other 75 patients were euprolactinaemic (normal prolactin level), and they came to the institute because of a male factor. Ultrasound examination and patent tubes were also normal in the latter group of patients, therefore, they were considered as a control group in the present study.

Ten milliliters of venous were drawn from each subject in heparinized tubes. The blood was subjected to a density-gradient centrifugation to collect lymphocytes, which were separated into T (HLA-class I typing) and B (HLA-class II typing) cells using the nylon wool method. The cells were phenotyped for HLA-class I (A, B and Cw) and HLA-class II (DR and DQ) alleles using commercially available antisera (Biotest, Germany), and following the principles of microlymphocytotoxity test (9). The sera were subjected to immunometric enzyme immunoassay for qualitative screening of anti-nuclear antibodies (ANA), using a commercially available kit (Biomaghreb Company, Tunisia). The instructions of the manufacturer were followed to detect these antibodies.

The results were presented in terms of numbers and percentage frequencies, while alleles showing positive or negative associations were further presented as odd's ratio (OR), etiological fraction (EF) and preventive fraction (PF). The significance of each association was assessed by Fisher's exact probability (P), which was corrected for the number alleles tested at each locus (A = 8; B = 16; Cw = 7; DR = 12; DQ = 4) (9). The statistical calculations were carried out using the computer programme PEPI version 4.0.

Results

Based on serum level of prolactin, the total patients (100 hyperprolactinaemic females) were divided into three main groups; I (35 patients), II (40 patients) and III (25 patients). Their serum prolacin levels were 22-29, 30-39 and $\geq$ 40 ng/ml, respectively.HLA-A, -B, -Cw, -DR and -DQ showing significant variations between patients (total and groups) and controls are summarized in table 1. The alleles B8, DR3 and DR4 showed significant (P = 0.012, 3.44 x 10^{-5} and 2.46 x 10^{-4}, respectively) increased frequencies in total patients (B8: 25.0 *vs*. 9.3%; DR3: 48.0 *vs*. 17.3%; DR4: 39.0 *vs*. 13.3%). Such deviations were associated with OR values of 3.24, 4.40 and 4.16, respectively, and EF values of 0.17, 0.37 and 0.30, respectively. In contrast, the allele B35 showed a significant (P = 0.003) decreased frequency in the patients (7.0 *vs*. 24.0%). The PF value of such decrease was 0.18. These differences remained significant after correction with the exception of B8, in which the Pc was not significant.

In group I of patients, the antigen B35 was significantly (P = 0.03) decreased (5.7 *vs*. 24.0), and such difference was associated with a PF value of 0.19, but the Pc was not significant. In contrast, a significant (P = 2.3 x 10^{-4}) increased frequency of DR3 antigen was observed in the patients (54.3 *vs*. 17.3%). Such increase scored OR and EF values of 5.66 and 0.45, respectively, and the difference remained significant after correction.The group II of patients showed increased frequencies of DR3 (45.0 *vs*. 17.3%) and DR4 (37.5 *vs*. 13.3%), and both differences were significant (P = 0.003 and 0.007, respectively). The OR and EF values of DR3 were 3.90 and 0.34, respectively, while such values for DR4 were 3.90 and 0.28, respectively, however, the DR4 failed to maintain a significant Pc.

Again, the DR3 and DR4 antigens showed a significant (P = 0.001 and 1.0 x 10^{-4}, respectively) increased frequency in group III of patients (56.0 for each *vs*. 17.3 and 13.3%, respectively. They scored OR values of 6.07 and 8.27, respectively, and EF values of 0.47 and 0.49, respectively. Both increases were associated with a significant Pc.

The sera of hyperprolactinemic patients (total and groups) and controls were evaluated for autoantibodies using ANA test. As shown in the table 2, 22% of the total patients showed a positive ANA test, while the sera of control subjects were negative for these autoantibodies. When the patient's groups were considered, a gradual increased percentage of sera positive for ANA (11.4, 15 and 48% for groups I, II and III, respectively) was observed, and such increase paralleled the increased level of prolactin.

Discussion

The results of HLA-hyperprolactinaemia association revealed that two antigens worth concern; they were DR3 and DR4. Both antigens were significantly increased in the patients, and the EF values of such positive associations had ranges of 0.34 - 0.47 and 0.28 - 0.49, respectively in total patients and their groups. From the statistical point of view, the

contribution of these two antigens in the aetiology of hyperprolactinaemia is between 30-50%, therefore, they can be considered as predisposing factors for such a disease entity, especially in females with the highest serum prolactin level (group III of patients). In this group of patients, the EF values of both antigens were nearly 0.50. However, the question is how these antigens contribute to the aetiology of hyperprolatinaemia. There is no direct evidence that suggests an association between HLA alleles and hyperprolactinaemia, but in humans the prolactin gene is located on the short arm of chromosome 6, close to the HLA-DRB1 region, and mutations in these genes could be associated with the pathogenesis of autoimmune diseases (4, 5). Linkage disequilibrium between HLA-DRB1 alleles and microsatellite marker alleles close to the prolactin gene was demonstrated in rheumatoid arthritis and systemic lupus erythematosus patients in comparison with healthy controls (10), suggesting the possibility of extended haplotypes encoding for HLA-DRB1 and high prolactin production, which contribute to susceptibility to the two autoimmune diseases (5, 8). Furthermore, Limas and colleagues (11) have explored the involvement of prolactin in neurohormonal adaptation to heart failure, and revealed that HLA-DQB1*0301 allele was significantly increased in patients with hyperprolactinaemia, moreover, histidine at position 30 of the HLA-DQB1 gene was found in 22% of the patients having a normal serum level of prolactin but in none of the hyperprolactinaemia patients, and accordingly, there was an inverse correlation between the presence of histidine at position 30 and the levels of serum prolactin. Based on these findings, HLA involvement in hyperprolactinaemia can be explained in the ground of linkage disequilibrium between DR3 and DR4 genes and prolactin encoding genes, as these genes are located on the short arm of chromosome 6 (6, 10, 12). Furthermore, such two positive associations may link autoimmunity and hyperprolatinaemia, because both antigens are positively associated with several autoimmune disorders; for instance, rheumatoid arthritis, type 1 diabetes mellitus, SLE, Grave's disease and Idiopathic Addison's disease (reviewed by 13). The results of antinuclear antibody (ANA) test may support such them, and 22% of the patients showed autoantibodies in their sera, moreover, the percentage of positive patients was increased as serum prolactin level was increased (11, 15 and 48% for groups I, II and III of patients, respectively). Therefore, increased level of prolactin may be involved in the initiation of autoimmunity, but the mechanism of such initiation is not known and further investigations are required to shed light on this subject. A further HLA association with hyperprolactinaemia was also observed in the present sample of patients, but this time it was a negative association. The antigen B35 showed a significant decreased percentage in the patients, and the PF value of such association was 0.18. Such observation may suggest that B35 is a protective factor against the development of hyperprolactinaemia. Such protective effect of B35 was questionable in an autoimmune disease (IgA nephropathy) (9), but it was clear in a disease with a suspected autoimmunity (schizophrenia) (14). Therefore, a further inspection of the antigen in hyperprolactinaemia is required. In conclusion, HLA alleles are important immunogenetic factors that confer susceptibility to the development of hyperprolactinaemia, and in this regard HLA-DR3 and -DR4 were important markers of such predisposition. Also, the results confirm the association of hyperprolactinaemia and autoimmunity, although the evidence was indirect, because the conclusion was based on the positivity of the patient's sera in the ANA test. Accordingly, further investigations are required to understand these correlations, and up to the best of our knowledge, the present findings in Iraqi infertile women were presented for the first time.

Table 1: HLA-class I (A, B and Cw) and class II (DR and DQ) antigens showing significant variations between hyperprolactinaemic patients (total and groups) and controls.

HLA Antigens	Patients		Controls		OR	EF or PF	P	Pc
	No.	%	No.	%				
	Total patients (No. = 100) vs. Controls (No. = 75)							
B8	25	25.0	7	9.3	3.24	0.17	0.01	N.S.
B35	7	7.0	18	24.0	0.24	0.18	0.003	0.05
DR3	48	48.0	13	17.3	4.40	0.37	3.4×10^{-5}	4.1×10^{-4}
DR4	39	39.0	10	13.3	4.16	0.30	2.5×10^{-4}	0.003
	Group I Patients (No. = 35) vs. Controls (No. = 75)							
B35	2	5.7	18	24.0	0.19	0.19	0.03	N.S.
DR3	19	54.3	13	17.3	5.66	0.45	2.3×10^{-4}	0.003
	Group II Patients (No. = 40) vs. Controls (No. = 75)							
DR3	18	45.0	13	17.3	3.90	0.34	0.003	0.04
DR4	15	37.5	10	13.3	3.90	0.28	0.007	N.S.
	Group III Patients (No. = 25) vs. Controls (No. = 75)							
DR3	14	56.0	13	17.3	6.07	0.47	0.001	0.01
DR4	14	56.0	10	13.3	8.27	0.49	1.0×10^{-4}	0.001

OR: Odd's ratio, EF: etiological fraction, PF: Preventive fraction, P: Probability, Pc: Corrected P, N.S.: Not significant.

Table 2: Observed numbers and percentage frequencies of ANA-positive sera obtained from hyperprolactinaemic patients (total and groups) and controls.

Groups		Number	ANA-Positive Sera	
			No.	%
Hyperprolactinemic Patients	Total	100	22	22.0
	Group I	35	4	11.4
	Group II	40	6	15.0
	Group III	25	12	48.0
Controls		75	0	0.00

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