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Short communication

Human leukocyte antigen (HLA) DQ2/DQ8 prevalence in recurrent pregnancy loss women

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ABSTRACT

Objective: Over the last few years, medical scholars have reported the significant association between recurrent pregnancy loss (RPL) and celiac disease (CD). Various pathogenic mechanisms underlying the pregnancy failure in CD have been suggested: among them the ability of anti-transglutaminase antibodies to impair the trophoblast invasiveness and endometrial endothelial cells differentiation and disrupt early placentation. CD shows a complex non-Mendelian pattern of inheritance, involving major histocompatibility complex (MHC) genes. The strongest effects are mapped to the classical human leukocyte antigen (HLA)-DQA1 and HLA-DQB1 genes. Specifically, the common haplotypes DQ2.5, DQ2.2, and DQ8 have been shown to increase CD risk by six-fold on average. MHC region contains genes with immunological functions and is responsible for the strongest association signals observed in most immune-mediated diseases. The aim of our study was to investigate the prevalence of the HLA-DQ2/DQ8 haplotypes in RPL, outside of CD.
Methods: The study population included women with history of RPL (≥3 spontaneous pregnancy losses) and women with at least two previous uncomplicated term pregnancies (control group, CTR). All women gave their informed consent to use their data for research purposes.
Results: 97 RPL women and 55 CTR were considered in the study. Mean age of the RPL sample was 37.7 (standard deviation, SD, 3.0; min 27; max 39). Mean age of the control group was 35.6 (SD 3.0; min 26 years; m, max 38). A significantly increased prevalence of HLA-DQ2/DQ8 haplotype positivity was found in RPL population compared to control women (52.6% vs 23.6%; $p < 0.01$).
Conclusions: Our observations show for the first time a higher proportion of individuals HLA DQ2/DQ8 positive in women with RPL as compared to controls (and to general population estimates). Further studies are needed to better understand (i) the possible pathogenic mechanism to this observation; (ii) the clinical and therapeutic implications of our observation in order to provide a new approach to RPL couples.

1. Introduction

Recurrent pregnancy loss (RPL) is historically defined as three or more subsequent spontaneous pregnancy losses [1]. In 2012, the American Society for Reproductive Medicine Practice Committee formalized the definition slightly differently—the Society defined RPL as "the subsequent occurrence of two or more clinically (i.e., pregnancies documented by ultrasonography or histopathological examination) failed pregnancies" [2]. This difference may have implications mainly for clinical research studies rather than for clinical practice. Indeed, the risk of another pregnancy loss after two miscarriages is only slightly lower than that of women with three or more spontaneous abortions (24%–29% vs 31%–33%); furthermore, no significant difference has been found when examining test results related to two or more prior losses [3,4].

RPL occurs in about 2–3% of clinically diagnosed pregnancies of reproductive-aged women. At present, accepted etiologies for RPL include parental chromosomal abnormalities, untreated hypothyroidism, uncontrolled diabetes mellitus, certain uterine anatomic abnormalities, antiphospholipid antibody syndrome, heritable and/or acquired thrombophilias, infections, and environmental factors [5–10]. In addition, an increased risk of auto- and cellular immune abnormalities, such as an increased positivity for anti-nuclear (ANA) and/or -thyroid antibodies

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have been observed in RPL women [5,11–13]. After evaluation for these causes, approximately 40% of all cases of RPL lack of an associated factor. This group of cases represents, to date, the challenge for researchers.

Over the last few years, medical scholars have examined the relationship between RPL and celiac disease (CD), one of the most common inflammatory disorders of the small intestine affecting up to 1% of individuals in Western populations [14–16]. In particular, our recent meta-analysis has reported an odds ratio (OR) value for CD of 5.82 (95% CI 2.30–14.74) for women experiencing recurrent miscarriage as well as a higher risk of miscarriage in celiac patients, bearing a relative risk (RR) of 1.39 (95% CI 1.15–1.67) [14]. The pathogenic mechanism underlying the pregnancy failure in CD is not well understood. Beyond the nutrient deficiency, which can be found in celiac patients and characterized by the lack of elements like zinc, selenium, and folic acid, the ability of anti-transglutaminase antibodies to impair trophoblast invasiveness and endometrial endothelial cells differentiation has been suggested as a possible disruptor of early placentation [15,16].

In celiac patients beyond the ingestion of gluten, which certainly represents the environmental trigger, the genetic predisposition strongly contributes to the development of the disease [17,18]. Studies on siblings confirmed a concordance of around 80% in monozygotic twins, less than 20% among dizygotic twins, and a risk for a patient's siblings to develop a CD around 20–60, as confirmed by familial aggregation studies [19–23]. The major histocompatibility complex (MHC) gene region is the main genetic factor in the disease development, with the strongest effects mapped to the classical human leukocyte antigen (HLA)-DQA1 and HLA-DQB1 genes. Specifically, the common haplotypes DQ2.5, DQ2.2, and DQ8 have been shown to increase disease risk by six-fold on average [24–26]. Approximately 25% of the general Caucasian population is HLA-DQ2/DQ8. From these genetically susceptible individuals, only 4% develop CD [16,27].

Given these premises, we aimed at studying the prevalence of HLA-DQ2/DQ8 positivity in RPL women, outside of CD. The observation of a possible correlation between HLA-DQ2/DQ8 haplotype positivity and RPL, if confirmed by further studies, including larger number of women, might suggest new diagnostic and therapeutical approaches for RPL women.

2. Materials and methods

2.1. Patients

The study population included women with history of RPL and women with previous uncomplicated term pregnancies (control group). RPL and control group women were recruited over a period of 6 months (from January to June 2015) at the Recurrent Pregnancy Loss Outpatient Clinic and the Gynecology Outpatient Clinic, respectively, at the Department of Obstetrics and Gynecology, A. Gemelli Universitary Hospital, Rome, Italy. RPL women have had three or more subsequent early (<10 weeks of gestation) spontaneous pregnancy losses clinically documented by ultrasonography and/or histopathology examination. Control group included women with at least two previous uncomplicated term pregnancies. The inclusion criteria for both groups were as follows: Caucasian, age < 39 years, healthy, regular ovulatory cycles (28–32 days), normal endocrine profile, normal serum levels of follicle-stimulating hormone (FSH < 10 mIU/ml), luteinizing hormone (LH < 10 mIU/ml), and anti-mullerian hormone (AMH > 2 ng/ml) on day 3 of the menstrual cycle, normal serum levels of total immunoglobulin (Ig) G and IgA, absence of gastrointestinal symptoms, absence of a prior diagnosis of CD [28]. In order to investigate the presence of a possible association between the HLA status and the presence of antibody positivity, the anti-thyroid (-thyroglobulin/-thyroid peroxidase), -cardiolipin, -β2 glicoprotein I (β2GPI), -prothrombin, -nuclear, -transglutaminase, -endomysium, and -gliadin antibody positivity was also performed. The autoantibody positivity was confirmed on two or more occasions at least 12 weeks apart. Screening for autoimmunity was performed after at least 8 weeks apart the last miscarriage.

The presence of thrombophilic defects including activated protein C resistance/factor V G1691A mutation, prothrombin G20210A mutation, protein C, protein S and/or antithrombin III deficiency, lupus anticoagulant, hyperomocisteinemia was recorded.

All women gave their informed consent to use, anonymously, their data for research purposes, and the protocol was approved by the ethics committee of A. Gemelli Universitary Hospital, Università Cattolica del Sacro Cuore, Rome, Italy.

2.2. Sample collection and analysis

Peripheral blood was collected in ethylene-diamino-tetra-acetic (EDTA) tubes from RPL and control women. HLA-DQ2/DQ8 analysis was performed, after amplification of human DNA isolated from peripheral blood, by real-time polymerase chain reaction following the manufacturer's instructions (XeliGen RT, Eurospital SpA, Italy). Briefly, PCR with sequence-specific primer tested for the following alleles: DQA1*01, DQA1*0201, DQA1*03, DQA1*05, DQA1*06, DQB1*02, DQB1*0301/03, DQB1*0301/04, DQB1*0302, DQB1*0305, and DQB1*04. The following DR alleles were typed in order to determine the presence of DQ/DR haplotypes: DRB1*03, DRB1*04, DRB1*07, DRB1*11. DQ2 positivity was defined as DQA1*05 in cohort with DQB2*02 (DQ2.5), or DQA1*0201 (DQ2.2)/DQA1*03 (DQ2.3) with B1*02. DQ8 positivity was defined as DQA1*03 with DQB1*0302.

Anti-thyroglobulin and -thyroperoxidase antibodies were tested by chemiluminescence immunoassay (CMIA method, ADVIA Centaur XP Siemens Healthcare, Italy). Anti-cardiolipin and -β2GPI antibodies were tested by using a specific chemiluminescence immunoassay (Zenit Autoimmunity, Menarini Diagnostics, Italy). Screening for ANA was performed by indirect immunofluorescence assay using a commercially available kit (Eurospital SpA, Italy).

2.3. Statistical analysis

Means and standard deviation (SD) were used to describe quantitative variables whereas absolute and relative frequencies were employed for categorical ones. Pearson chi-square and Fisher's exact tests were used to compare HLA DQ2/DQ8 positivity in patients and healthy controls and to evaluate the association between HLA DQ2/DQ8 and antibody positivity in patients with >3 recurrent pregnancy loss.

3. Results

One hundred four women with RPL history and fifty-five control women were recruited from the RPL Outpatient Clinic and the Gynecology Outpatient Clinic, A. Gemelli Universitary Hospital, respectively. Women showing a repeated positivity (on two or more occasions at least 4 weeks apart) for anti-transglutaminase, -endomisium, and -gliadin antibody were sent to gastroenterologist and subsequent biopsy: 7 RPL women (6.7%) were newly diagnosed with CD [17,18], excluded from the study and, after sending to gastroenterologist, treated with gluten-free diet. No newly diagnosed cases of CD were found in the control group. Mean age of RPL women (n = 97) was 37.7 years of age (minimum 27 years, maximum 39 years, SD = 3.0). Mean age of control group was 35.6 years of age (minimum 26 years, maximum 39 years, SD = 3.0; Table 1).

HLA DQ2/DQ8 positivity was found in 52.6% out of 97 RPL women and in 23.6% out of 55 controls ($p < 0.01$; Fig. 1). The odds ratio for HLA DQ2/DQ8 was 3.6 for patients in respect to controls (IC 95%; 1.71–7.65).

The percentage of patients having positivity for HLA DQ2/DQ8, anti –thyroglobulin/thyroid peroxidase (n = 27; 27.8%), -nuclear (n = 39; 40.2%), -cardiolipin (n = 9; 9.2%), -β2GPI (n = 9; 9.2%)