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Original Article

HLA sharing and maternal HLA expression in couples with recurrent pregnancy loss in Taiwan

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ABSTRACT

Objective: The aim of this study is to investigate the frequency and distribution of human HLA sharing and maternal HLA allele expression in couples with recurrent pregnancy loss in Taiwan.
Materials and methods: We retrospectively reviewed couples experienced two or more pregnancy loss before 20th weeks of gestation from March 2014 to November 2020 having HLA determination. Fertile individuals with one or more live-birth offspring receiving HLA allele determination during the same period were included as the control group. The distribution and frequency of HLA sharing were analyzed and presented by descriptive statistics. Fisher Exact Test were used to analyze specific maternal and paternal HLA allele comparing individuals with RPL to fertile group. P-value < 0.05 was thought to be statistically significant.
Results: 72 couples were enrolled from March 2014 to November 2020. Regarding the distribution of HLA sharing, HLA sharing between females and their male partners less and equal to 2 pairs were found in 40.3% of the couples. HLA sharing greater and equal to 3 pairs are found in 59.8% couples. HLA sharing was most frequently found in alleles HLA-A02, A11, DQ07, C07 and B60 in descending order. There was a significant lower expression of HLA-B13 in women with RPL compared to women who had successful pregnancy ($p = 0.0234$). Compared infertile men with fertile men cohort, the frequency of HLA-DR04 ($p = 0.0438$, OR 2.444, 95% CI 1.0251–5.8287), HLA-DR12 ($p = 0.001$, OR 30.85, 95% CI 4.0296–236.19) and HLA-15 ($p = 0.0357$, OR 9.354, 95% CI 1.1610–75.37) were found to be significantly higher in men with RPL. On the contrary, HLA-DR07 ($p = 0.0085$, OR 0.124, 95% CI 0.0264–0.587) and HLA-DR10 ($p = 0.0395$, OR 0.048, 95% CI 0.0027–0.8641) were found to be significantly lower in men with RPL.
Conclusion: We found a tendency to recurrent pregnancy loss in couples with more than 2 pairs of HLA sharing. The similarity of HLA sharing, the expression of maternal HLA-B13 allele and paternal HLA-DR alleles in Taiwanese couples might play a role in recurrent pregnancy loss.

Introduction

Recurrent pregnancy loss (RPL) is defined as loss of two or more consecutive pregnancies. RPL affects approximately 2% of couples in reproductive ages. Although different causes of RPL such as uterine anatomical anomaly, maternal immune problem, paternal or maternal chromosome abnormally, maternal endocrine or blood dysfunction, chronic endometrial infection have been identified, the etiology remains unknown in nearly 50% of the cases [1,2].

The genetic loci involved in the rejection of foreign organs are known as the major histocompatibility complex (MHC). The human MHC is called the Human Leukocyte Antigen (HLA) system because these antigens were first identified and characterized using alloantibodies against leukocytes. HLA are the biomarkers for maternal body to recognized fetus as a semi-allograft. Disparity between the HLA molecules of the mother and fetus is the genetic foundation associated with successful pregnancy. Increasing matching between fetus and maternal HLA allele patterns (class I and class II matching) is associated with adverse pregnancy outcomes, such as spontaneous abortion and preeclampsia [3,4]. Women who experienced RPL was

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reported to be unable to produce blocking antibodies reacting to paternally encoded alloantigens expressed at the trophoblast [3]. These relationship between HLA sharing and recurrent pregnancy loss have been reported in previous studies [5–7].

Until now, no universal conclusion has been made because of the heterogenicity and small size of the previous studies. It was also proposed that different ethnicity might have different HLA sharing in view of RPL [8,9]. The aim of this study is to find out the distribution and frequency of human HLA sharing in couples with RPL in Taiwan as well as the difference between different ethnic groups.

Materials and methods

Participants

This is a retrospective study, couples who experienced two or more consecutive pregnancy loss before 20th weeks of gestation from March 2014 to November 2020 were included in this study. Women with identified causes of recurrent pregnancy loss such as uterine factors, immune problems, fetal congenital or chromosomal abnormality, and endocrine disorders were excluded. Couples with paternal or maternal chromosome anomaly were also excluded. Fertile individuals with one or more live-birth offspring who received HLA allele determination for other medical reasons during the period were included as the control group.

Laboratory examination and statistical analysis

HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ were examined in each couple by collecting blood samples through the method of PCR-sequence specific priming (SSP) conducted by the immunological laboratory department in Chi Mei Medical Center. HLA sharing between women and their male partner were checked manually. The distribution and frequency of HLA sharing were analyzed and presented by descriptive statistics. Fisher Exact Test were used to analyze specific maternal HLA allele comparing women with RPL to fertile women who had at least one live-birth offspring. P-value < 0.05 was thought to be statistically significant.

Ethics approval

This study has been reviewed and approved by the Institutional Review Board of Chi Mei Medical Center (IRB No. 11003-004).

Results

Frequency of HLA sharing in couples with RPL

From March 2014 to November 2020, a total of 136 couples received HLA typing examination in reproductive center of Chi-Mei Medical Center. After screening, seventy-two couples fulfilled the inclusion criteria of this study and were enrolled for final statistical analysis. Fifty-one fertile women and 59 fertile men with one or more live-birth offspring who received HLA allele determination for other medical reasons during the period were included as the control group. The mean age of infertile females was 36.8 ± 4.5. The mean age of infertile male was 38.9 ± 5.5. The mean BMI of females was 23.4 ± 3.7 kg/m^2. The mean BMI of male was 25.2 ± 2.9 kg/m^2. Thirty-eight out of 72 couples had history of 2 consecutive pregnancy losses; 24 out of 72 had 3 pregnancy losses; 7 out of 72 had 4 times of pregnancy losses; 1 out of 72 had 5 times, 6 times and 8 times of pregnancy losses respectively (Table 1). In regard to the distribution of HLA sharing, 11.1% (8/72) couples had no HLA allele sharing, 16.7% (12/72) had 1 pair of HLA sharing, 12.5% (9/72) had 2 pairs of HLA sharing, 27.8% (20/72) had 3 pairs of HLA sharing, 23.6% (17/72) had 4 pairs of HLA sharing, 2.8% (2/72) had 5 pairs of HLA sharing, 4.2% (3/72) had 6-pair HLA sharing and 1.4% (1/72) had 7-pair HLA sharing (Table 1). HLA sharing between females and their male partners less and equal to 2 pairs were found in 40.3% of the couples. HLA sharing greater and equal to 3 pairs are found in 59.8% couples (Table 2).

Table 1
Demographic characteristics.

Characteristics	N = 72 (%)
Female age (y/o)	36.8 ± 4.5
Male Age (y/o)	38.9 ± 5.5
Female BMI (kg/m^2)	23.4 ± 3.7
Male BMI (kg/m^2)	25.2 ± 2.9
Times of miscarriage	
2	38 (52.8%)
3	24 (33.3%)
4	7 (9.7%)
5	1 (1.4%)
6	1 (1.4%)

Mean ± SD, Number (%).

Distribution of HLA sharing and maternal HLA alleles in couples with RPL

The shared HLA allele in this population were more predominantly expressed in HLA-A02 (29.2%, 21 couples), HLA-A11 (29.2%, 21 couples), HLA-DQ07 (25.0%, 18 couples), HLA-C07 (22.2%, 16 couples), HLA-B60 (20.8%, 15 couples), HLA-C10 (18.1%, 13 couples), HLA-DQ06 (13.9%, 10 couples), respectively (Table 3). Compared infertile women to fertile women cohort, among all the HLA alleles investigated in our population, HLA-B13 was found to be significantly lower in women with RPL ($p = 0.0234$, OR 0.2714, 95% CI 0.0879–0.8379). Surprisingly, compared infertile men with fertile men cohort, HLA-DR04 ($p = 0.0438$, OR 2.444, 95% CI 1.0251–5.8287), HLA-DR12 ($p = 0.001$, OR 30.85, 95% CI 4.0296–236.19) and HLA-15 ($p = 0.0357$, OR 9.354, 95% CI 1.1610–75.37) were found to be significantly higher in men with RPL. On the contrary, HLA-DR07 ($p = 0.0085$, OR 0.124, 95% CI 0.0264–0.587) and HLA-DR10 ($p = 0.0395$, OR 0.048, 95% CI 0.0027–0.8641) were found to be significantly lower in men with RPL.

Discussion

A successful pregnancy begins with a normal implantation. It is believed that during the process of implantation, fetal–maternal interface was formed. Fetus, as a semi-allograft, thrive under maternally immune-tolerated status [8]. In the process of pregnancy, complex interaction occurs between fetal trophoblasts and maternal decidual immune cells. Rather than direct contact, it is a hub buffer between fetus and maternal uterus that allows successful implantation and appropriate placental growth, to ensure immune tolerance of the fetus. Also, this hub provides protection

Table 2
Frequency of HLA sharing.

HLA sharing of couples (pairs)	Number of couples (%)
0	8 (11.1%)
1	12 (16.7%)
2	9 (12.5%)
3	20 (27.8%)
4	17 (23.6%)
5	2 (2.8%)
6	3 (4.2%)
7	1 (1.4%)

Mean ± SD, Number (%), Total number of couples: 72.

Table 3
HLA-sharing allele with higher frequency.

HLA allele	Number of couples (%)
A02	21 (29.2%)
A11	21 (29.2%)
DQ07	18 (25.0%)
C07	16 (22.2%)
B60	15 (20.8%)
C10	13 (18.1%)
DQ06	10 (13.9%)
DR04	8 (11/1%)
C01	7 (9.7%)

Number (%), Total number of couples: 72.

for the mother from immune response that may be induced by the fetus [9]. Recent studies have suggested that the subsidence of certain autoimmune diseases during pregnancy and flare up again after delivery might indicate the immunomodulation in the maternal body for rebalancing its immune and inflammation status [10]. Various kinds of cells were found to participate in this immunomodulatory process, such as decidual natural killer cell, macrophage, dendritic cells, helper T cell, regulatory T cell and effector T cell, etc. Several molecules, such as HLA molecules, cytokines, transforming growth factor β and fetal DNA have also been reported. All kinds of signaling pathways, types of cells and molecules orchestrated were reported to create a state of feto-maternal microchimerism participating in the immunomodulatory mechanism when pregnancy occurs [11].

HLA alleles are DNA sequences that code with HLA molecules. The HLA region comprises several genetic loci located on chromosome 6p21.31 which containing the most polymorphic genes known in humans. Some HLA molecules are antigens that were identified and characterized using alloantibodies against leukocytes. They are the biomarkers for the maternal body to recognize the fetus as a semi-allograft. There are currently 32,330 HLA and related alleles described and included in the IPD-IMGT/HLA Database worldwide [12]. Until 2020, 1012 individuals underwent whole-genome SNP array and NGS-based HLA typing. Their data of HLA alleles were used to establish Taiwanese HLA imputation references in the Taiwan Biobank (TWB). It was reported that HLA class II genes found to be associated with rheumatoid arthritis (RA) in Taiwan population [13]. Classical HLA molecules are expressed on the surface of somatic cells and placental trophoblast. The physiological function of HLA class I molecules, HLA-A, HLA-B and HLA-C, is to present peptides to T cells and to inhibit the activity of natural killer cells. HLA-C are also reported to express in trophoblast and may play a role in placentation [14]. Nonclassical MHC class Ib include HLA-E and HLA-G molecules. HLA-G was found to be expressed on the extravillous trophoblast and seems to have immunomodulatory functions during pregnancy [15,16]. The expression of HLA-G is largely limited to the placental trophoblast, where it might mediate protection of the fetus from rejection by the mother [17]. Serum level of soluble HLA-G was reported significantly lower in women with preeclampsia compared to healthy pregnant women in the third trimester [18,19].

In this study, we investigated two aspects of HLA expression in our population. One is the pattern of HLA sharing in couples, another is specific maternal and paternal HLA allele expression compared to their fertile control. Association between HLA sharing in couples with RPL was found. In our patients with RPL, 59.8% of couples shared more than two pairs of the same HLA alleles; 27.8% of these patients with RPL have shared three pairs of HLA alleles and 23.6% have shared four pairs of HLA alleles. It seems that a decrease in the disparity between the HLA molecules of the couple might increase the risk of pregnancy loss from our study. The pathogenetic mechanism may be the incapability of the maternal body to recognize the fetus as semi-allograft so to interfere with the induction of the immunomodulatory process for tolerating the fetus in the environment of the uterus. In our study, maternal HLA-B13 was found to be significantly lower in women with RPL compared to the fertile group. With respect to larger database, the reported frequency of HLA-B13 allele was 9.7% in Taiwan Chinese immigrants from South China and 5.3% in Taiwan Tzu Chi Cord Blood Bank [20]. Several specific HLA alleles were reported to be associated with recurrent miscarriage. Previous meta-analysis has reported the increased risk of recurrent miscarriage in women having HLA-DRB1*4, HLA-DRB1*15 or HLA-E*01:01 allele expression. The same study also reported decreased risk of recurrent miscarriage in women with HLA-DRB1*13 or HLA-DRB1*14. These data may indicate the role of HLA molecules in the pathogenesis of recurrent pregnancy loss. The differences found in HLA alleles may be related to different populations [21]. In previous studies, the HLA-DRB1*03 allele was found with increased prevalence in RPL patients. However, with high resolution technique, it was later reported that it is HLA-DRB1*07 that associated with RPL [22]. We found significant difference in frequency of HLA-DR04, HLA-DR12, HLA-DR15, HLA-DR07, HLA-DR10 in paternal HLA allele compared to fertile men. This evidence indicated that HLA-DR polymorphism in Taiwanese men may related to the etiology of RPL. The mechanism of regulating implantation or pregnancy among these alleles is still unknown and may be further discovered on the proteomics basis.

The shared HLA alleles were found to predominantly distribute in certain alleles. The top five alleles shared in couples with RPL in our population were HLA-A02 (29.2%, 21 couples), HLA-A11 (29.2%, 21 couples), HLA-DQ07 (25.0%, 18 couples), HLA-C07 (22.2%, 16 couples), HLA-B60 (20.8%, 15 couples). HLA-A sharing was the most predominantly found HLA molecule in this population. Previous studies have reported dominant HLA sharing alleles in PRL couples in different ethnicities. In the population with RPL in Iran, the prevalence of HLA-DRB1 sharing between the couples with unsuccessful pregnancy outcomes was significantly higher compared to those with successful outcomes (63.3% vs. 23.3%, $p < 0.004$). In particular, HLA-DRB1*07:01 allelic group was significantly more frequent in the patients with unsuccessful outcomes compared to the controls (18.3% vs. 8%, $p < 0.04$) [4]. Meuleman et al. have systematically reviewed data associated with HLA sharing and HLA alleles in patients with recurrent miscarriages. They reported increased risk of recurrent miscarriage in couples sharing HLA-B allele (OR 1.39, 95% CI 1.11–1.75) and HLA-DR allele (OR 1.57, 95% CI 1.10–1.25). They also reported increased risk of recurrent miscarriage in women expressing HLA-DRB1*4 (OR 1.41, 95% CI 1.05–1.90), HLA-DRB1*15 (OR 1.57, 95% CI 1.15–2.14) and HLA-E*01:01 allele (OR 1.47, 95% CI 0.20–1.81). On the other hand, decreased risk was found in women expressing HLA-DRB1*13 (OR 0.63, 95% CI 0.45–0.89) and HLA-DRB1*14 (OR 0.54,95% CI 0.31–0.94) [21].

It seems that the distribution of HLA sharing in Taiwanese population with RPL is non-consistent with previous reports. Our study reconfirmed that different ethnicity might have different HLA sharing in view of RPL. In our study, shared HLA allele were more predominantly expressed in HLA-A02 (29.2%, 21 couples), HLA-A11 (29.2%, 21 couples), HLA-DQ07 (25.0%, 18 couples), HLA-C07 (22.2%, 16 couples), HLA-B60 (20.8%, 15 couples), HLA-C10 (18.1%, 13 couples), HLA-DQ06 (13.9%, 10 couples). Among these alleles, HLA-C are expressed on trophoblast cell which may participate directly in the process of cell recognition and immunoregulation. Other alleles were thought to express mostly on somatic cells which may play a preluding role in this immunomodulation process.

Until now, the relationship and HLA sharing and RPL have not reached a universal conclusion. Nevertheless, we found the

similarity of HLA gene expression between women and their partners may play a role in recurrent pregnancy loss. There was a propensity of three or more pairs of HLA sharing in our patients with history of RPL. Higher frequency of HLA sharing was found in HLA-A02, HLA-A11, HLA-DQ07, HLA-C07, HLA-B60, HLA-C10 and HLA-DQ06. Furthering investigates this issue may provide the basis of immunological treatment in patients experiencing recurrent pregnancy loss.

Our study reported the first data on HLA allele expression in the Taiwanese RPL population. Also, we demonstrated the possible correlation between the number of shared HLA alleles and couples with RPL. We reported the several significantly different frequency of HLA allele in Taiwanese women and men with RPL, indicating the role of HLA determination when evaluating women having RPL. However, there were limitations to this study. We descriptively analyzed the distribution and frequency of HLA sharing in the Taiwanese population because of difficulty in collecting HLA sharing data of control group. All the couples having HLA typing for determining HLA sharing, meaning women matched with her husband, had the diagnosis of infertility or RPL. Therefore, we were incapable of collecting fertile control group of HLA sharing retrospectively. Lacking a broadly representative fertile control group for HLA sharing made it difficult to elucidate the meaning of the distribution of HLA sharing in this population compared with the fertile population. Also, the small sample size must be considered in the interpretation of these findings. These showed the need to establish HLA mapping in our population, not only among patients with RPL but also in the general population. In this study, we determined low-resolution HLA typing by PCR-SSP method providing DNA-based typing result at the level of the digits composing the first field in the DNA-based nomenclature. A larger number of participants and high-resolution HLA typing methods, such as sequencing-based typing or next generation sequencing, could provide more evidence in exploring the etiology of recurrent pregnancy loss. Several future directions could be considered. First, well-designed prospective studies with high resolution technique were needed to investigate the underlying pathogenesis of unexplained recurrent pregnancy loss and to elucidate the significance of the data. Second, further investigation into HLA expression in trophoblast may provide more evidence about its role in RPL on a biological basis.

Author contributions

Dr. YC Tsai designed this study, provided data and revised this article. Dr. TW Hsiao wrote this article and collected data; Ms. LY Lin conducted statistics analysis; the other authors also provided data and joined the discussion.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influence the work reported in this research.

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