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Cancer diagnosis among women with recurrent pregnancy loss: A retrospective cohort study

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ABSTRACT

Research Question: To examine the relationship between unexplained recurrent pregnancy loss (RPL) and the risk for cancer morbidity.

Design: We conducted a retrospective observational cohort study, based on data from a tertiary medical center. RPL cases (exposed) were defined as women presenting with 3 or more unexplained confirmed pregnancy losses at 5 -24 weeks, whose first visit to the RPL clinic was between 1990 and 2010. Unexposed group included women giving birth and who were not RPL patients matched by both age and the year of giving birth/admission (1:5 ratio). Data from the RPL registry and the live birth registry were cross-linked to the Israeli national cancer registry according to the unique ID number and merged into one database.

Results: The study group comprised 937 RPL patients who were matched by maternal age (*P*=1.0) and admission date (*P*=0.84) to 4685 women who achieved a live birth. We found no difference in overall cancer incidence between groups (adjusted odds ratio [OR] 0.76, 95% confidence interval [CI] 0.55-1.03; *P*=0.08). Secondary RPL group had a trend toward decreased cancer morbidity incidence as compared to primary RPL (adjusted OR 0.65, 95% CI 0.41-1.03; *P*=0.07). Analysis by cancer type showed a similar risk for breast cancer among women with RPL as compared to live birth, but a significantly lower risk for gynecological cancers among women with RPL (adjusted OR 0.25, 95% CI 0.08-0.79; *P*=0.018)

Conclusions**:** Unexplained RPL may be related to a lower risk for gynecological cancers, possibly explained by hyper-responsive immunological mechanisms involving uterine NK cells.

Keywords: Recurrent pregnancy loss, Breast cancer, Gynecological cancer

INTRODUCTION

Recurrent pregnancy loss (RPL) involves 2 or more losses and affects approximately 1-5% of women of reproductive age ("Evaluation and Treatment of Recurrent Pregnancy Loss: A Committee Opinion," 2012; Khalife et al., 2019; Papas & Kutteh, 2020). The factors leading to RPL include advanced maternal age, environmental exposures, uterine structural anomalies, endocrine imbalances, anti-phospholipid syndrome, parental chromosomal aberrations, and various genetic causes (Colley et al., 2019; "Evaluation and Treatment of Recurrent Pregnancy Loss: A Committee Opinion," 2012; Khalife et al., 2019; Papas & Kutteh, 2020). Endometrial receptivity and the implantation milieu have also been suggested as underlying causes (Ewington et al., 2019; Ticconi et al., 2019). The etiology for RPL is identified in only ~50% of women ("Evaluation and Treatment of Recurrent Pregnancy Loss: A Committee Opinion," 2012; Khalife et al., 2019; Papas & Kutteh, 2020).

The mother's immune response plays a critical role in maintaining pregnancy. Antigens expressed on the surface of fetal or placental tissues may induce alloimmune responses, and immunologic mechanisms maintaining the continuation of normal pregnancy (Saito et al., 2010; Trowsdale & Betz, 2006). Various models and theories involving disrupted immune functions of T helper populations and natural killer (NK) cells may be involved in RPL (Dosiou & Giudice, 2005; Guerrero et al., 2020; Piccinni, 2006). Uterine NK cells produce a variety of cytokines and immune-modulatory proteins capable of influencing trophoblast growth, implantation and vascularization of the deciduas (Lash et al., 2011). Increased cytotoxic NK activity was observed in the uterus of women with unexplained RPL(Kuon et al., 2017), and an increased number of toxic NK cells in the blood and the decidua (El-Badawy et al., 2020; Zhu et al., 2017). In addition, women with unexplained RPL have higher

numbers of circulating activated CD4+ and CD8+ T cells (Kuon et al., 2015)and lower levels of circulating IL10 + CD56bright NK cells (Zhu et al., 2017)compared to healthy controls.

NK cells are also involved in tumor recognition: cancer cells express neoantigens, which can present peptides bound to molecules on the surface of cancer cells, distinguishing them from their normal counterparts (Chen & Mellman, 2013). Inhibitory receptors on NK cells target cancer cells lacking major histocompatibility class I (MHC-I), marking them for programmed cell death(Chen & Mellman, 2013; Marcus et al., 2014); therefore, immune mechanisms that may be involved in unexplained RPL may also be involved in the responses to tumor development later in life. Here, we examined the relationship between unexplained RPL and the risk for cancer morbidity in a large cohort of Israeli women.

MATERIALS AND METHODS

Design and setting

We conducted a retrospective analysis of medical records of patients who attended the RPL clinic in a tertiary referral medical center and the registry of women who delivered at the same medical center. The study was approved by the institutional ethics committee (6584-19-SMC).

Patients

The medical records of patients presenting with 3 or more confirmed pregnancy losses between 5 and 24 weeks of pregnancy, whose first visit to the RPL clinic was between 1990 and 2010, were included in the study. This time period was chosen to enable sufficient follow-up for subsequent cancer detection. Only patients with unexplained RPL were included in the study, i.e., the patients' endocrine profile, antiphospholipid antibodies levels, uterine cavity and parental karyotypes were all normal.

The unexposed (control) group consisted of women who gave birth during the same period and did not have present RPL. The control group was matched by maternal age and the year of giving birth. The first consultation at the RPL clinic was considered as the start of follow-up, and as the matching point for selecting the control patients (e.g., a woman who attended the clinic in 1995 after 3 pregnancy losses was matched to a control woman who gave birth in 1995). Each RPL case was paired with five controls. Controls were classified by the highest of the two criteria (difference in the date of admission or difference in maternal age, in days) - from lowest to highest. Matching was capped at 3-year intervals for maternal age and 8-year intervals for date of admission. Follow-up ceased on the date of cancer diagnosis, or by 2018.

Data collection

RPL was defined as ‘primary’ if no live birth preceded the pregnancy loss, or as ‘secondary’ if the pregnancy loss followed one or more live births. Due to the limited number of variables available for analysis, only maternal age, the number of pregnancy losses and follicular stimulating hormone (FSH) values were assessed. Data from three databases: the RPL registry, the live birth registry of the medical center’s delivery room and the Israel national cancer registry, were crosslinked according to the women’s ID number and merged into one database. At the time of the analysis, the databases were updated until 2018. Since 1982 all Israeli medical institutions are obliged to report any cancer diagnosis to the National Cancer Registry. The report includes all records of diagnosis and pathology reports, providing detailed information regarding the type of cancer and the time of diagnosis. Cancers are classified using the International Statistical Classification of Diseases and Related Health Problems (ICD-9), as shown in Supplemental Table 1.
The incidence of cancer was analyzed by 2 timepoints: 1) any time up to 2018, that means developing cancer early in life before admission to the RPL clinic (lifetime risk), and 2) cancer diagnosis only after admission to the RPL clinic/matched timepoint for controls. These timepoints were chosen to discriminate between possible different underlying mechanisms: an inherited immune abnormality vs. an acquired immune abnormality.

Outcome measures

The primary outcome measure was the incidence of any cancer in RPL patients compared to controls. The incidence of breast and gynecological cancers (i.e. endometrial, cervical, ovarian and vulvar cancers) in these two populations were also analyzed.

Statistical analysis

Statistical analysis was performed using Python 3.7.6, Pandas 1.0.1, Numpy 1.18.1, Scipy 1.4.1, Statsmodels 0.11.0 and Lifelines 0.25.5. Quantitative variables were summarized as mean and standard deviation (SD). Quantitative variables with non-normal distribution were compared using the Mann-Whitney test. Categorical variables were summarized as number and percentages and compared by chi-squared test. Logistic regression models were constructed to study the association and independent impact of RPL on cancer, while controlling for maternal age (for validation of the matching results). Kaplan-Meier survival curves were used to compare the cumulative incidence of cancer during the follow-up period. Statistical significance was assumed at a level of <0.05.

RESULTS

The study group comprised 937 RPL cases who were matched by maternal age (*P*=1.0) and admission date (*P*=0.84) to 4685 women who had a live birth and comprised the unexposed (control group ,1:5 ratio). Mean follow-up was 16.3 ± 5.3 years for RPL cases and 15.9 ± 4.9 years for the control group. RPL patients' characteristics are summarized in Table 1. The RPL and control groups were compared for lifetime cancer risk and post-admission cancer risk. The lifetime risk for cancer was 5.3% (49/937) among RPL patients and 6.8% (317/4685) among the control group (*P*=0.08). After adjusting for maternal age, the odds ratio (OR) for cancer morbidity in the RPL group relative to the control group was 0.76 (95% confidence interval [CI], 0.55-1.03; *P*=0.081). A similar trend was seen for cancer risk post-inclusion in the study (adjusted OR 0.76, 95% CI 0.5-1.07; *P*=0.117). In addition, while comparing the cumulative incidence of cancer morbidity, a trend for lower lifetime and post-admission cancer morbidity among RPL patients was observed (*P*=0.06 and 0.08 by log-rank test, respectively).

To further characterize the biological mechanism underlying this observation, the RPL group was stratified according to primary or secondary losses. Women with secondary RPL showed a trend towards lower cancer morbidity compared to controls (Figure 1 a,b). This was not observed in primary RPL (Figure 1 c,d). Stratification of patients by high-order pregnancy loss (more than 3 losses) did not show cancer risk differences between RPL patients and control patients.

Figure 2 shows the distribution of cancers by type in the RPL and control groups. Breast cancer was the most common tumor in both groups with a similar incidence. However, the incidence of gynecological cancers was significantly lower in the RPL group compared to the control (3/937, 0.3% vs. 60/4685, 1.3%; *P*=0.01). After

adjustment for maternal age, the OR was 0.25 (95% CI 0.08-0.79; *P*= 0.018) and was also seen in the survival analysis (Figure 3b).

DISCUSSION

In this study we analyzed cancer morbidity among patients suffering unexplained RPL compared to age and time-matched control patients who gave birth to a live child. We found no difference in overall cancer incidence between groups, however a trend toward decreased incidence in the secondary RPL group as compared to primary RPL. The distinction between primary to secondary RPL implies that hyper-responsive immunological mechanisms in addition to previous successful pregnancy, may be a protective factor for malignancy. Analysis by cancer type also showed a similar risk for breast cancer among women with RPL and controls, but significantly lower risk for gynecological cancers among women with RPL.

Two previous studies have addressed the association between RPL and the development of subsequent tumors. Charach et al. (2018) reported a significantly higher incidence of breast and uterine cancers among RPL patients with two or more consecutive losses who were seen between 1988-2013 and followed-up until 2013. However, the search system was not detailed, and it is not clear whether terminations of undesired pregnancies were excluded from the study. The comparison group included patients who were selected from a random pregnancy registry during 1988-2013 and were not diagnosed as RPL. Cancer diagnosis was based on the local hospital registry, as identified by a medical secretary (Charach et al., 2018). The second study was a retrospective cohort study among women born in The Netherlands between 1957 and 1972 who had invasive cancers after the age of 40 and were followed until 2017. The study did not report an association between pregnancy loss and later development of 11 site-specific types of cancer or cancer overall. The analysis was based on rates rather than on survival analysis. Pregnancy losses were not categorized by their cause (unexplained vs. explained) and

pregnancy terminations were also included. The comparison group was chosen by year of maternal birth and not for achieving a live birth (Mikkelsen et al., 2019). The different study designs, different time periods and the different comparison groups could explain the different conclusions between the current and previous studies. Additionally, the women's reproductive history may have been different in the previous studies. Nulliparity is associated with an increased risk of breast cancer, while multiparity is protective regardless of maternal age at first birth(Kelsey et al., 1993). The relationship between miscarriages and breast and ovarian tumors has also been previously investigated: The European Prospective Investigation into Cancer and Nutrition reported that incomplete pregnancies (miscarriages and induced abortions) were related to an increased risk of epithelial ovarian cancer (Braem et al., 2012), but this specific association was not confirmed by other reports. Indeed, two meta-analyses have refuted the association and found no significant association between the number of incomplete pregnancies and incidence of breast or ovarian cancers(Dick et al., 2009). In general, incomplete pregnancies seem to confer some protection from epithelial ovarian and borderline ovarian tumors (Marcus et al., 2014), although this protective effect is weaker than that provided by full-term pregnancies. The current study showed a similar trend, as we found no association between RPL and breast cancer, and a reduced rate of gynecological cancers among women with RPL. Interestingly, RPL was not increased among women harboring BRCA1 and 2 mutations (Gal et al., 2004), hence the diagnosis of RPL in our clinic does not seem to involve a bias related to BRCA-1.

The present findings are biologically plausible, as only women with unexplained RPL were included in the study, while patients with uterine structural anomalies, endocrine imbalances, antiphospholipid antibodies and parental chromosomal

aberrations were excluded. It is possible that women with RPL, who may harbor an activated immune system, that cannot be efficiently downregulated(Zhu et al., 2019), and NK cells stimulated into hyper-toxicity (Dick et al., 2009), show a greater response to cancer cells in the uterine environment and related organs with subsequent elimination.

The study has several limitations. Immune function, NK cell level or activity, or cytokine responses were not evaluated and products of conception were not analyzed. Large scale prospective studies are needed to study the pathogenesis and mechanism of NK cell and malignancy. Unexplained RPL was diagnosed by excluding other factors, rather than by a positive immune response. In addition, we could not assess other risk factors, specifically BMI and smoking status. As women may have ceased smoking after being diagnosed with unexplained RPL, smoking status cannot explain the protective effect observed in gynecological cancers but not in breast cancer. We can acknowledge that both exposed and unexposed groups originated from the same center and therefore generally share the same access to medical care, same race/ethnicity and similar educational profile. Lastly, patients were followed-up for a mean period of 16 years; therefore, no conclusions can be drawn concerning cancer that may present later in life.

The strengths of this study emanate from its large patient cohort, its design and appropriate control group (same center, careful age matching and follow-up period) and the strict criteria used for classification of unexplained RPL and cancer diagnosis.

In conclusion, this study showed that unexplained RPL may reduce the risk for gynecological cancers. Our findings provide indirect support that a hyper-responsive immunological mechanisms, possibly related to uterine NK cells, may provide some

protection from cancer to women with unexplained RPL. Further research should include additional risk factors such as BMI, exposures, NK numbers and activity and embryonic aneuploidy.

Key message

Women with unexplained repeated pregnancy loss have no differences in overall cancer incidence but lower incidence of gynecological cancer as compared to their age and time matched control women who had a live birth. The mechanism may be an increased immunological response to cancer cells mediated by uterine NK cells.

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FIFURE LEGENDS

Figure 1. Kaplan-Meier survival analysis comparing lifetime (a,c) and post-admission (b,d) cancer morbidity among women with primary vs. secondary RPL and controls (live birth).

Figure 2. Cancer type distribution (%) in RPL and control patients.

Figure 3. Kaplan-Meier survival analysis comparing breast cancer morbidity (a) vs. gynecological cancer morbidity (b) among women with RPL and controls (live birth).