Journal of Reproductive Immunology 106 (2014) 34–40

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

Do uterine natural killer cell numbers in peri-implantation endometrium predict hypertensive disorder in pregnancy in women with a history of reproductive failure?

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ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 28 April 2014
Accepted 30 April 2014

Keywords:
Uterine natural killer cell
Recurrent miscarriage
Recurrent implantation failure
Gestational hypertension
Pre-eclampsia

ABSTRACT

The aim of this study was to investigate whether or not increased uterine natural killer (uNK) cell numbers in the peri-implantation endometrium are associated with an increased risk of hypertensive disorders in a subsequent pregnancy. This is a retrospective study including 80 women with a history of unexplained recurrent miscarriage or recurrent implantation failure. Precisely timed endometrial biopsies were obtained from women 7–9 days after the luteinising hormone surge. uNK cells were immunostained for CD56+ and expressed as a percentage of total stromal cells. Patients were defined as having a high uNK cell count if the percentage of total stromal cells was more than 13.9%. Five out of 29 (17.2%) women in the high uNK cell count group and 5 out of 51 (9.8%) women in the normal uNK cell count group developed gestational hypertension. Pre-eclampsia was diagnosed in 2 (6.9%) patients in the high uNK cell count group and 1 (2.0%) patient from the normal uNK cell count group. There was no significant difference in the incidence of either gestational hypertension ($P = 0.483$) and pre-eclampsia ($P = 0.296$) between groups. The overall incidence of hypertensive disease in women with high uNK cell count (24.1%) was two times higher than women with normal uNK cell count (11.8%), but it was not statistically significant ($P = 0.208$). An increased uNK cells count in the peri-implantation period in a cycle prior to conception did not appear to significantly increase the likelihood of hypertensive disease of pregnancy.

1. Introduction

Hypertensive disorder in pregnancy is a major clinical problem occurring in about 5–10% of all pregnancies (Lo et al., 2013). Gestational hypertension and pre-eclampsia are both disorders of pregnancy that are considered to share the same underlying aetiology (Villar et al., 2006). In patients with mild gestational hypertension remote from term, a significant proportion will ultimately develop pre-eclampsia (Barton et al., 2001). Thus, it is considered an early or mild stage of pre-eclampsia, perhaps preceding renal involvement with proteinuria. Hypertensive disorder in pregnancy is the leading cause of maternal and perinatal mortality and morbidity worldwide (Sibai et al., 2005). Currently, the only proven treatment for this condition is

Abbreviations: IVF, in vitro fertilization; LH, luteinising hormone; RIF, recurrent implantation failure; RM, recurrent miscarriage; uNK, uterine natural killer.

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http://dx.doi.org/10.1016/j.jri.2014.04.005
0165-0378/

delivery with removal of the placenta. Hypertensive disease in pregnancy has been reported to be responsible for 42.5% of indicated preterm births and perinatal outcome is compromised owing to prematurity (Meis et al., 1998). An understanding of the underlying pathophysiology may help to identify women at risk of this problem.

There is some evidence to suggest that immunological incompatibilities between the mother and the developing foetus, resulting in abnormal placental formation, may play a significant role in pre-eclampsia (Redman and Sargent, 2005). The endometrial leucocyte population consists mainly of uterine natural killer (uNK) cells. The uNK cells are very different from peripheral blood natural killer cells; in particular, they express large amounts of CD56 and are referred to as $CD56^{bright}$ cells, compared with peripheral blood natural killer cells, which are predominantly $CD56^{dim}$. They also produce a number of cytokines rather than acting as a cytotoxic leucocyte (Tabiasco et al., 2006).

During the first trimester of pregnancy, uNK cells are the main type of leucocyte present at the maternal–foetal interface and are thought to play an important role in spiral artery remodelling. During the early weeks of gestation, trophoblast cells migrate into the decidua and invade maternal spiral arteries in order to form hybrid vessels lined with both maternal and foetal cells. These physiological changes create a low-resistance arteriolar system without maternal vasomotor control, which allows the substantial increase in blood supply to the growing foetus (Mandala and Osol, 2012). The uNK cells aggregate around spiral arteries in the first three months of pregnancy, during the active phase of blood vessel development (Bulmer et al., 1991). They secrete various angiogenic factors that affect angiogenesis and vascular stability, including vascular endothelial growth factor, placental growth factor, angiopoietin-2, matrix metalloproteinases and interferon γ (Hanna et al., 2006; Lash et al., 2010).

A higher density of uNK cells is also found in decidual tissue in close proximity to the invasive trophoblast, compared with the average density of cells in tissue away from the trophoblast, which indicates a migration of the uNK cells towards the invading trophoblast tissue (Helige et al., 2014). uNK may therefore also affect placental function by interacting with trophoblast cells directly via the binding of their inhibitory and activating receptors (KIRs and KARs) to the MHC class 1 molecules expressed by the trophoblast cells (Hiby et al., 1997).

It has been suggested that the increased uNK cell number during the implantation process interferes with trophoblastic invasion of the vessels and predisposes to pre-eclampsia (Bachmayer et al., 2006). However, it is not known if an increase in uNK cell number in the endometrium prior to pregnancy, which is associated with recurrent miscarriage and recurrent implantation failure (Tuckerman et al., 2007, 2010), is also associated with an increased risk of development of hypertensive disease in pregnancy. The aim of this study was to investigate whether or not increased uNK cell numbers in the peri-implantation endometrium are associated with an increased risk of hypertensive disorders in a subsequent pregnancy.

2. Materials and methods

2.1. Subjects

Women included in this study were recruited from the recurrent miscarriage clinic and assisted conception unit in Jessop Wing, Sheffield Teaching Hospitals, UK. All women included in the study had uNK cell numbers measured in a precisely timed endometrial biopsy obtained in the peri-implantation period no more than 6 months prior to conceiving. They all had a singleton intra-uterine pregnancy confirmed by ultrasound in the first trimester that progressed to beyond 24 weeks' gestation.

The recurrent miscarriage (RM) group consisted of 71 women with a history of three or more consecutive miscarriages in the first trimester. All women with RM had completed investigations according to our established protocol (Li, 1998) with normal results: parental karyotypes, thyroid function test, follicle-stimulating hormone, luteinising hormone (LH), prolactin, progesterone, free androgen index, lupus anticoagulant, anticardiolipin antibodies, anti-beta 2 glycoprotein I antibodies, thrombophilia screening, pelvic ultrasound and hysterosalpingography.

The recurrent implantation failure (RIF) group consisted of 9 women who had failed to achieve a clinical pregnancy after three or more in vitro fertilisation (IVF) treatment cycles in which at least four or more good quality embryos had been placed during three or more embryo transfer cycles. None of them had received embryos conceived with eggs or sperm from donors. Women with RIF underwent investigation including hysteroscopy, parental karyotypes, follicle-stimulating hormone, LH, lupus anticoagulant, anticardiolipin antibodies, anti-beta 2 glycoprotein I antibodies and thrombophilia screening.

Women with medical diseases including pre-existing hypertension, diabetes mellitus, thyroid disease, renal disease and auto-immune disorders were excluded. None of the women received any treatment during the pregnancy, irrespective of the uNK cell count.

2.2. Endometrial biopsy and uNK cell count

Daily measurement of LH from the mid-follicular phase in either urine or serum was used to identify the LH surge. Women were advised not to have unprotected sexual intercourse during the biopsy cycle to avoid undergoing a biopsy in the conception cycle. Endometrial biopsies were collected using a Pipelle sampler (Prodimed, Neuilly en Thelle, France) in the luteal phase of the menstrual cycle between day 7 and day 9 after the LH surge. Samples were then immediately fixed in formalin overnight and automatically wax embedded for immunocytochemistry study. Histological examination showed that all endometrial biopsies showed evidence of secretory transformation.

2.3. Immunocytochemistry

Five-micrometre sections of endometrial tissue were cut and dewaxed in xylene, rehydrated through the alcohols to Tris-buffered saline pH 7.6 (TBS), and quenched in 0.3% hydrogen peroxide in methanol for 20 min.