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ORIGINAL PAPER

CD4+ and CD8+ preimplantation endometrial population in women with unexplained recurrent miscarriage

Ahmed Abdelrahim[1], Eman Alkholy[2], Ahmed Gamal Badawie[3]

[1]Department of Obstetrics and Gynaecology, Ain Shams University, Cairo, Egypt

[2]Department of Obstetrics and Gynaecology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt

[3]Department of Obstetrics and Gynaecology, October 6 University, Cairo, Egypt

Abstract

Introduction: The aim of this study is to assess the secretory-phase endometrial leucocytes in women with 2 or more unexplained abortions and in healthy controls.

Material and methods: This cross-sectional study was performed in 3 tertiary centres: Ain Shams University, Al-Azhar, and October 6 University Maternity Hospitals. The study included 50 women who consented to participate in this study. Women were divided in 2 groups; the first group consisted of 25 non-pregnant women with unexplained recurrent pregnancy loss, while the second group (n = 25) included non-pregnant women as a control group who had no history of recurrent pregnancy loss. Endometrial biopsies were taken from all participants around the expected time of implantation (one week after induction of ovulation by human chorionic gonadotrophins) to elucidate the T lymphocyte population, CD4+ (helper-T) and CD8+ (suppressor-T) markers.

Results: Women with 2 or more unexplained abortions had significantly less endometrial CD8+ ($p < 0.05$), and consequently their endometrial CD4/CD8 ratio was higher in relation to the controls. There was no significant difference in endometrial CD4+ in relation to controls ($p > 0.05$).

Conclusions: From the results we can conclude that CD8 is more valuable than CD4 in women with recurrent spontaneous miscarriage. CD8 is better positive than negative in such patients.

Key words: endometrial leucocytes, CD4+, CD8+, unexplained recurrent miscarriage.

Introduction

Recurrent early pregnancy loss (RPL) means the loss of ≥ 3 consecutive first-trimester gestations. Unfortunately, it is not uncommon in obstetrics and affects up to 5% of females [1, 2]. Many obstetricians recommend starting the management after only 2 abortions, especially in those over 30 years old [3, 4]. The main issue in such pregnancy failures is usually a disturbed maternal-embryonic interaction, genetic examinations of the abortuses are usually normal [5–9]. Other factors include chromosomal abnormalities as balanced translocations, endocrinopathies, acquired or hereditary thrombophilias, and uterine cavity abnormalities [2, 10–12]. In more than 50% of cases, no cause is identified, and the management will be empirical [13–15]. Abnormal maternal embryonic interaction includes a rejection of the semi-allogenic embryo during "window of implantation" [16–21]. The pre-implantation leukocyte population, which represent > 20% of endometrial cells, was studied to detect the differences between RPL women and healthy controls, and even to predict the outcome.

Endometrial immune cells differ to a large extent throughout the phases of the menstrual cycle [20, 21]. So, timing of endometrial biopsy is crucial to achieve valuable results. Early in the pregnancy, just before the start of a subsequent abortion, would logically be the ideal time. However, it is still vague if variants in endometrial cells populations are the underlying mystery of miscarriage. As early gestation, endometrial biopsies in patients with recurrent abortions are likely to have a negative effect on the baby and are thus avoided.

Material and methods

The study includes 50 women enrolled from 3 tertiary centres: Ain Shams University, Al-Azhar, and October 6 University Maternity Hospitals. The samples were gathered from January 2019 to January 2022. The study was approved by the Ethics Committee, and written consent was obtained. A mid-luteal phase endometrial biopsy was taken 7 days after induction of ovulation by human chorionic gonadotrophins.

Corresponding author:
Eman Alkholy, Department of Obstetrics and Gynaecology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, e-mail: nero_kamal_90@hotmail.com

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The women were divided into 2 groups:

- the first group consisted of 25 women as cases (n = 25), who had a history of unexplained recurrent early miscarriage, recruited from a recurrent miscarriage clinic,
- the second group consisted of 25 women as controls (n = 25), who had no history of recurrent miscarriage; they were seeking medical advice for using a contraceptive method in an outpatient clinic.

Inclusion criteria were as follows:

- the age of the patients and control group were between 18 and 40 years old,
- the parity of the patients was 3 or more recurrent miscarriage below 12 weeks.

Exclusion criteria were as follows:

- known or definitive causes explaining miscarriage, such as history of maternal endocrinopathies, hyperprolactinaemia, luteal insufficiency (detected due to repeatedly decreased luteal progesterone level), hyper- and hypothyroidism, hyperandrogenism, polycystic ovary syndrome, or hypersecretion of luteinizing hormone and insulin resistance,
- acquired (antiphospholipid syndrome) or hereditary thrombophilic disorders,
- different forms of uterine malformation ruled out by ultrasound and hysteroscopy,
- chromosomal abnormalities (when available in a previous pregnancy).

Both groups were subjected to the following procedures:

- full history, detailed examination (general, abdominal, and local),
- investigations were reviewed from cases that include mainly glucose tolerance curve, lupus anticoagulant antibodies, karyotyping, anticardiolipin antibodies, semen analysis from their husbands, pelvic ultrasonography, and hysterosalpingography,
- histological examination: endometrial biopsies were collected around the expected time of implantation (HCG + 7 days) using a small biopsy catheter (Pipelle catheter),
- then the biopsy is fixed in 10% buffered formalin for preparation of paraffin block and then a 5-um-thick section was cut from the paraffin block on positively charged slides stained with haematoxylin and eosin to demonstrate T-lymphocyte subsets using CD4 (T-helper) and CD8 (T-suppressor) markers by immunohistochemical study,
- immunohistochemical study: 5-um-thick sections were cut from the paraffin blocks on positively charged slides and used for immunohistochemical study.

The antibodies used were as follows:

the CD4 marker used was CD4- Ab-8 (clone 4 B12) (mouse monoclonal antibody) manufactured by NeoMarkers for the Lab Vision Corporation used for the detection of T-helper cells. Use Ab at 1: 10–1: 20 for 60 min at room temperature using UltraVision LP systems to detect Ab:

- the CD8 marker used was CD8 (clone SP16) (rabbit-monoclonal antibody) manufactured by NeoMarkers For Lab Vision Corporation used for the detection of suppressor T-cells. Use Ab at 1–50 for 30 min at room temperature using UltraVision LP systems to detect Ab,
- immunohistochemistry was performed according to Hsu *et al.* [22] and applying the supersensitive ABC universal kit (Biogenix, USA).

Table 1. The demographic differences between the study and control groups

Parameters	Study group (n = 25)	Control group (n = 25)	*P*
Age (years)			
Mean ±SD	27.29 ±3.2	28.31 ±3.7	> 0.05* NS
Body mass index			
Mean ±SD	28.1±3.8	27.9 ±3.2	> 0.05* NS
Gravidity			
Mean ±SD	5 ±1.2	4 ±1.3	< 0.05** S
Parity			
Mean ±SD	0.7 ±0.2	5 ±1.3	< 0.05** S
CD4			
Mean ±SD	3.4 ±1.3	3.6 ±1.1	> 0.001 HS
CD8			
Mean ±SD	2.6 ±1.5	8.2 ±2.4	< 0.001 HS
CD4/CD8			
Mean ±SD	1.2 ±0.91	0.54 ±0.7	< 0.001 HS

HS – highly significant, NS – non significant, S – significant, SD – standard deviation

Sample size calculation

An online statistical calculator was used for sample size calculation provided that the power of the significance tests was 80%, confidence level 95%, alfa error 5%, the catchment area population was included, and the total number of candidates was 50, to fulfil these criteria and divided into 2 equal groups.

Results

Table 1 shows that there was no statistically significant difference between the 2 groups as regards age and body mass index (BMI), but there was a significant difference between the 2 groups as regards parity and gravidity. The CD4 level was the same in both groups. CD4/CD8 were higher in cases compared to controls while the CD8 level was lower in cases compared to controls.

Table 2 shows no significant statistical inverse correlation between CD4 and parity, and significant statistical positive correlation between CD8 and parity by Spearman correlation. On the other hand, there was

Table 2. Correlation between CD4 and CD8 vs. different variables among cases

Parameters	CD4		CD8	
	R	*p*	R	*p*
Age	0.07	> 0.05	0.05	> 0.05
BMI	0.04	> 0.05	0.06	> 0.05
Gravidity	–0.06	> 0.05	0.04	> 0.05
Parity	0.3	> 0.05	0.4	< 0.05 S
CD8	0.2	> 0.05		

BMI – body mass index

Table 3. Validity of CD4 and CD8 in prediction of implantation failure

Parameters	CD4	CD8	Both	CD4/CD8
Best cut-off	3.4	5.1	–	–
Sensitivity (%)	61	82	84	72
Specificity (%)	81	75	89	77
PPV (%)	34	68	71	62
NPV (%)	52	71	74	78
Accuracy (%)	44	67	75	64

NPV – negative predictive value, PPV – positive predictive value

no significant correlation of CD4 or CD8 vs. other variables among cases.

Table 3 shows that CD8 is more sensitive with positive predictive value and negative predictive value but less specific than CD4. The best cut-off value of CD4 was 3.4, and of CD8 it was 5.1.

Discussion

To see if there were any differences between women who had RPL and healthy controls – or even to forecast the success of upcoming pregnancies – a number of studies examined leukocyte concentrations in individuals with repeated miscarriages [19, 23–26].

However, the majority of studies did not exclude enough potential causes of abortion to categorise the patients as idiopathic with no known causes of miscarriage in either spouse. Therefore, it is still unknown how endometrial immune cells contribute to the emergence of recurrent idiopathic abortion and how hypothetical allo-immunologically induced miscarriage functions.

After thoroughly ruling out any other known causes for recurrent miscarriages, we looked at the concentration of CD4, CD8, and CD4/CD8 ratio in precisely dated secretory endometrial tissue from healthy controls and patients with repeated consecutive abortions to address the question of potentially altered endometrial immune cell concentration.

In the current clinical trial, we discovered that there was no correlation between maternal age and the likelihood of experiencing recurrent miscarriage ($p > 0.05$). This goes against the findings of earlier research [2–4] that suggested that the probability of recurrence rises with maternal age. In recent years, however, patients older than 30 years with 2 consecutive miscarriages are urged to be included in screening tests for couples with recurrent abortions as well.

In the current study, we discovered that BMI had no significant relationship to the percentage of endometrial CD4 or endometrial CD8; this is consistent with the study by Metwally *et al.*, which found that an elevated BMI did not appear to have an effect on endometrium leukocyte concentrations [5].

Contrary to the findings of Lasher *et al.*, who claimed that obesity is significantly related with an elevated risk of first-trimester recurrent miscarriage, our current investigation demonstrated no significant relationship between BMI and the frequency of recurrent miscarriage ($p > 0.05$) [27].

The current study's findings, which support Jauniaux *et al.* in that the probability of recurrence rises with the number of prior, sequential miscarriages, showed that the number of abortions had a strong and significant relationship with the likelihood of recurrent miscarriage ($p = 0.001$) [4].

Contrary to research by Klentzeris *et al.*, which revealed that women with unexplained infertility had considerably larger numbers of (CD4+) T cells, the current investigation observed no significant differences in the amount of endometrial (CD4+) T cells compared to controls ($p > 0.005$) [28].

Additionally, the current research refutes claims made in other research that immunological effects, particularly those involving T-helper cells (CD4) and natural killer cells, may contribute to the preservation of pregnancy [29–32].

In this investigation, we discovered that women with unexplained recurrent abortion had significantly fewer endometrial (CD8+) T cells than controls ($p = 0.05$), which is in acoordance with a study by Klentzeris *et al.* that identified fewer (CD8+) T cells in this population [28]. Similarly to this, Lachapelle *et al.* in 1996 reported that recent research has found that the immune cell distribution in the endometrium of non-pregnant individuals with histories of recurrent abortions is changed [33].

This result differs from Maruyama *et al.* [34] and Michimata *et al.* [19], who found no differences in endometrial leukocyte concentrations in patients with a history of RPL and healthy controls by immunohistochemistry, and this agrees with other studies.

In the current study, we found that there was a statistically significant difference in the endometrial CD4/CD8 ratio, being higher in women with recurrent spontaneous abortion than in normal, fertile, non-pregnant controls [18, 33, 35].

The biopsies were accurately scheduled, and the timing was confirmed by histological testing.

In addition, all other pertinent documented reasons for recurrent miscarriages were ruled out, restricting our patients to those who were considered to be definitively idiopathic. A significant difference from other studies that only used the antiphospholipid syndrome, maternal oligomenorrhoea as a potential symptom of endocrinopathies, and parental chromosomal anomalies as exclusion criteria is this study's systematic exclusion of other potential causes for repeated miscarriages [18].

Clifford *et al*. also lacked rigorous exclusion criteria as they only used parental chromosomal defects, an antiphospholipid syndrome, and uterine anomalies discovered sonographically as exclusion factors [35].

When evaluating the significance of our findings, it is important to take into account another limitation of our study: the small sample size may have led to some false-negative findings because it was very challenging to use an invasive technique on patients who had experienced recurrent spontaneous abortions.

This backs up the conclusions of research by *et al*. claiming that because invasive examinations of intact foetuses in individuals with a history of repeated abortions, Strowitzki cannot be carried out [36]. In recurrent abortions, it is still speculative to believe that assumed immunological changes in the decidua occur before a future loss.

Comparing decidual leukocytes after such a subsequent miscarriage to those found in patients who underwent elective pregnancy terminations at the same gestational age may also produce ambiguous results because fictitious immunological variations may be more of a result than a cause of miscarriage.

Conclusions

Our data did not demonstrate differences of concentrations of endometrial CD4+ between healthy controls and patients with recurrent miscarriage following certain elimination of all other documented reasons for recurrent miscarriage. However, compared to healthy controls, we found that women with recurrent miscarriage had significantly fewer endometrial CD8 cells, and their CD4+/CD8+ ratio was higher. As a result, CD8+ is more useful in predicting pregnancy failures because it is a positive rather than a negative predictor.

Disclosure

The authors report no conflict of interest.

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