CONTRIBUTION OF EMBRYONIC CHROMOSOMAL ABNORMALITY TO THE ETIOLOGY OF ECTOPIC PREGNANCY

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Introduction and Study Objectives

Ectopic pregnancy (EP) is reported to be composed of 0.5% of all pregnancies in Japan. Epidemiological studies have provided evidence that many factors may be involved in the etiology of EP. It is well acknowledged that almost all these identified etiological factors are maternal: previous EP, previous tubal surgery, previous pelvic inflammatory diseases, sexually transmitted diseases (mainly Chlamydia trachomatis infection), induced conception cycle (controlled ovarian hyperstimulation with/without ART procedure), pregnancy after tubal sterilization, pregnancy with an intrauterine contraceptive device (IUD) in situ, endometriosis, maternal smoking at the time of conception, and so on. These risk factors together were reported to explain about 70% of all EP, which means that one-third of EP occurs in patients with no identified risk factors.

Maternal risk factors have been studied extensively, but little research has focused on fetal (i.e., embryo-related) factors. In theory, chromosomally abnormal embryos could be predisposed for ectopic implantation as a result of delayed intratubal migration.

Today EP is diagnosed at a rather early stage of gestation and the medical management characterized by methotrexate treatment is being applied as the first-line therapy. Under these circumstances, a chance to analyze the cytogenetic information of EP has become less and less. Fortunately we could have a certain study period (2007-2014), during which we managed all EP cases by surgical procedures and resected chorionic villi were all analyzed for the cytogenetic study.

The purpose of this study is to compare the frequencies of chromosomal abnormality of EP conceptus with those of intrauterine pregnancies, to identify variables that might affect the incidence of EP, and to assess their roles on the etiology of EP.

Materials and Methods

We have had 90 consecutive operation-treated EP for 8 years since 2007. Only cases with assessable specimens were included in this study (n=88). All chorionic villous samples obtained during surgical treatment could be successfully analyzed for cytogenetic study using direct and culture method (Group A).

As controls, we chose 753 consecutive singleton intrauterine pregnancies in the first half of 2014 (Group B). All cases of miscarriage (n=103), induced abortion (n=11), intrauterine fetal death (n=2), and fetal/neonatal anomaly (n=2) could be examined by karyotype analysis of chorionic villi or placenta. Intrauterine pregnancies delivering a healthy neonate with normal appearance were assumed to be normal karyotyped subjects (n=635). Our laboratory did not apply PGD/PGS to ART cases. As our clinic is one of the general obstetric facilities in a provincial city, high-risk pregnancies (e.g., higher-order birth, pre-term labor, severe FGR, severe PIH, and pregnancies complicated by uncontrolled medical disease) are not managed in our clinic and those cases are usually transferred to a highly-specialized perinatal center in advance. In this context, intrauterine high-risk pregnancies were not included in Group B.

Demographic information defining our study population were all collected from medical records, including maternal age, parity, risk factors for EP, method of conception, and neonatal sex. Risk factors for EP included in this study are as follows: previous EP, previous pelvic surgery, prior pelvic inflammation, prior sexually transmitted diseases, endometriosis, and infertility treatment other than ART. Smoking was excluded as a risk factor for EP, because we could not get sufficient information on the habit of smoking tobacco. Between-group differences were assessed using the t test or chi-square test for continuous or categorical data, respectively. The odds ratios of variables of interest (with 95% confidence interval and P value) for EP were calculated using multivariate logistic regression.

Table 1: Clinical Baseline Characteristics of EP Cases (Group A: N=88)

Mean Maternal Age (yrs ±SD)	31.8 ± 4.3 (range: 24 - 43)
Age Distribution	
20 - 24 :	4
25 - 29 :	20
30 - 34 :	46
35 - 39 :	16
40 - :	2
Mean Gestational Weeks at Dx (wks ±SD)	6.8 ± 1.0 (range: 5 – 9)
Parity	
primipara	70
multipara	18
Prior Spontaneous or Artificial Abortion	
none	57
1	14
2 or more	17
Fetal Heart Beat (ultrasound findings at Dx)	
positive	25
negative	63
Risk Factors for EP	
none	40
one or more	48
Operative Method	
Laparotomy	
Salpingotomy	4
Salpingectomy	10
Resection of EP site	2
Laparoscopy	
Salpingotomy	23
Salpingectomy	43
Resection of EP site	5
Others	1
Site of EP	
tubal ampulla	72
tubal isthmus	8
interstitial portion	2
uterine cervix	1
ovary	3
peritoneum	2
Method of Conception	
natural	50
ovulation induction (without IUI / ART)	13
IUI	11
ART (early cleavage ET / blastocyst ET)	14 (10 / 4)

Table 2: Clinical Baseline Characteristics of Intrauterine Pregnancy Cases (Group B: N=753)

Mean Maternal Age (yrs ± SD)	31.7 ± 4.4 (range: 19 – 43)
Parity	
primipara	406
multipara	347
Pregnancy Outcome	
delivery (beyond 22 wks of gestation)	639
live term or post-term (37 wks~)	624
live preterm (22-36 wks)	13
intrauterine fetal death	2 (34 wks, 40 wks)
miscarriage	103
induced abortion (including fetal anomaly)	11
Method of Conception	
non ART	692
ART (early cleavage ET / blastocyst ET)	61 (15 / 46)
Risk Factors for EP	
None	636
One or more	117

Table 3: Cytogenetic Study in EP Cases (Group A: N = 88)

Normal karyotype:	85 (96.6%)
46,XX	*40*
46,XY	*45*
Abnormal chromosomal complement:	3 (3.4%)
47,XX, +15 *(26 y.o., nullipara, chlamydial infection, Dx at 7 wks)*	
47,XX, +16 *(38 y.o., nullipara, no risk factor, Dx at 6 wks)*	
47,XY, +22 *(30 y.o., multipara, prior myomectomy, Dx at 6 wks)*	

Table 4: Cytogenetic Study in Intrauterine Pregnancy Cases (Group B: N=753)

Normal karyotype:	677 (89.9%)
46, XX	*334*
46, XY	*343*
Abnormal chromosomal complement:	76 (10.1%)
Trisomy 16	*15*
22	*11*
15	*6*
21	*6*
Other Trisomy	*16*
Monosomy X	*9*
Tetrasomy	*3*
Polyploidy	*5*
Others	*5*

Table 5: Characteristics of the Study Groups Comparison between EP and Intrauterine Pregnancy

characteristic	Group A (EP: N=88)	Group B (intrauterine pregnancy: N=753)	P value
Maternal age(yrs)	*31.8 ± 4.3*	*31.7 ± 4.4*	*0.816*
Parity			
multipara	*18* (15%)	*347* (46%)	*< 0.001 **
primipara	*70* (85%)	*406* (54%)	
Chromosomal abnormality			
yes	*3* (3%)	*76* (10%)	*0.042 **
no	*85* (97%)	*677* (90%)	
Risk factors for EP			
yes	*48* (55%)	*117* (16%)	*< 0.001 **
no	*40* (45%)	*636* (84%)	
ART pregnancy			
yes	*14* (16%)	*61* (8%)	*0.015 **
no	*74* (84%)	*692* (92%)	
Sex chromosome			
male	*46* (52%)	*368* (49%)	*0.546*
female	*42* (48%)	*385* (51%)	

Table 6: Odds Ratios for EP according to Patient Characteristics

variable	crude OR (95% CI, P value)	aOR* (95% CI, P value)
Parity		
multipara	*0.30 (0.17 - 0.50, <0.001)*	*0.27 (0.15 - 0.48, < 0.001**)*
primipara	*1.00 (reference)*	*1.00 (reference)*
Chromosomal abnormality		
yes	*0.31 (0.08 - 0.87, 0.05)*	*0.18 (0.04 - 0.55, < 0.01**)*
no	*1.00*	*1.00*
Risk factors for EP		
yes	*6.52 (4.11 - 10.41, <0.001)*	*6.93 (4.27 - 11.36, < 0.001**)*
no	*1.00*	*1.00*
ART pregnancy		
yes	*2.15 (1.11 - 3.92, 0.02)*	*1.46 (0.67 - 3.04, 0.32)*
no	*1.00*	*1.00*
Sex chromosome		
male	*1.15 (0.74 - 1.79, 0.55)*	*1.08 (0.67 - 1.75, 0.76)*
female	*1.00*	*1.00*

* Values of aOR (adjusted odd ratio) were all adjusted for maternal age and parity.
** P<0.05 was considered statistically significant.

Results

Table 1 and 2 summarize the clinical baseline characteristics of Group A and B. Table 3 and 4 show the result of cytogenetic analysis of Group A and B. In Group A, only 3 cases (3.4%: 3/88) showed abnormal chromosomal composition. On the other hand, 76 cases in Group B were identified as having chromosomal abnormalities (10.1%: 76/753). The rate of abnormal karyotype in Group A was statistically lower than in Group B (P<0.05) (Table 5). One or more risk factors for EP were identified in 48 cases of Group A (54.5%: 48/88), and 117 cases of Group B (15.5%, 117/753), also indicating a statistically significant difference (P<0.001) (Table 5).

Odds ratios for EP according to variables of interest are shown in the Table 6. Adjusted odds ratios for multipara, cases with chromosomal abnormality, and cases with EP risk factors, were 0.27 (95% CI: 0.15-0.48, P<0.001), 0.18 (95% CI: 0.04-0.55, P<0.01), and 6.93(95% CI: 4.27-11.36, P<0.001), respectively. These results indicated that multipara and embryos with chromosomal abnormality were significantly associated with less likelihood of developing EP. EP risk factors were reconfirmed to have strong relation to the genesis of EP. Maternal age, ART pregnancy, and sex of embryo did not affect the incidence of EP.

CONCLUSIONS

1) Embryos with abnormal karyotype and multipara might possess some defensive mechanisms against EP.

2) Maternal age, ART pregnancy, and sex of conceptus did not affect the occurrence of EP.

3) Widely known risk factors for EP were confirmed to be a more plausible explanation for the etiology of EP than embryo-associated factors.