The assessment of causes of recurrent pregnancy loss in material of the Division of Reproduction at Poznan University of Medical Sciences

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Abstract

Introduction: Recurrent miscarriages affect 1-2% of fertile couples. Basic examinations in couples experiencing recurrent miscarriage are karyotyping of the parents, the evaluation of uterine cavity and examination for the presence of antiphospholipid antibodies. The aim of this study was evaluation of the causes of recurrent miscarriages considered in three categories – genetic, anatomic and coagulation disorders (antiphospholipid syndrome and inherited thrombophilia) using the correct nomenclature in our own material. Material and methods: The study included 81 patients who experienced at least three miscarriages up to and including 21 weeks of gestation. All patients were hospitalized in the Division of Reproduction of Poznan University of Medical Sciences to evaluate the reason of pregnancy loss. Every woman had karyotype analysis with genetic consultation, antiphospholipid antibodies examination, inherited thrombophilia examination, assessment of uterine cavity as well as basic examinations. Results: Fifty five percent of patients had known the reason of pregnancy loss. Analysis of all miscarriages, showed that the most common reason for pregnancy loss was uterine cavity anomalies (16%), followed by antiphospholipid syndrome (12.3%) and inherited thrombophilia (8.6%) as well as abnormal karyotype (3.7%). Conclusions: Couples with recurrent pregnancy loss should be referred to specialist centers where basic tests can be performed to determine the cause of miscarriage. All applicable tests should be done in each couple with recurrent pregnancy loss, because in the ethiopathogenesis of recurrent miscarriage two or three factors may be involved. In appropriate situations, basic tests should be expanded to cytogenetic analysis of the chorion.

Key words: recurrent miscarriage, recurrent pregnancy loss, etiology

Introduction

Recurrent miscarriages affect 1-2% of fertile couples. Classic definition of miscarriage is pregnancy loss before 20 weeks of gestation while recurrent miscarriages involve three or more consecutive pregnancy loss.

There are different definitions of recurrent miscarriages in literature. Special Interest Group Early Pregnancy (SIGEP) of European Society for Human Reproduction and Embryology (ESHRE) defines recurrent miscarriages as three early (before 10 weeks of gestation) or two late (between 10 and 20 weeks of gestation) pregnancy loss.

There is other definition of recurrent miscarriages in North America [1]. Polish definition of recurrent miscarriages is also different than accepted by SIGEP because miscarriage in Poland is assumed as pregnancy loss up to 22 weeks of gestation.

Homogenous and clear definition of recurrent miscarriages is extremely important to establish a plan of examinations aimed to determine the cause and to compare the results of these examinations between various early pregnancy units [2].

Several recommendations have been published regarding the evaluation and management of recurrent miscarriage. Basic examinations in couples experiencing recurrent miscarriage, according to these recommendations, are karyotyping of the parents, the evaluation of uterine cavity and examination for the presence of antiphospholipid antibodies. There is evidence that abnormalities in these examinations have a causal relationship with pregnancy loss.

Karyotype abnormalities in couples with recurrent pregnancy loss are reciprocal and rarely Robertsonian translocations, wherein women are carriers twice as often as men. The course of subsequent pregnancy depends on the type of rearrangement, however the chance for healthy childbirth reaches 70% [3].

A lot of attention is being paid to genetic examination of aborted material, although these examinations are not routinely performed in specialized centers mainly because of the high cost [4].

Anomalies of the uterus are traditionally viewed as the cause of pregnancy loss in the second trimester, however they may also be implicated in recurrent early miscarriage [4]. The most common congenital uterine malformation associated with pregnancy loss is uterine septum, followed by bicornuate uterus.

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These defects can lead to impaired decidualisation, implantation and placental development.

Antiphospholipid syndrome (APS) is closely linked with recurrent early and late pregnancy loss as well as third trimester complications of pregnancy such as placental insufficiency and as consequence intrauterine growth restriction of fetus, or even intrauterine fetal death [5]. Classification with APS is based on documented clinical events such as vascular thrombosis and/or adverse obstetric event as well as laboratory results such as the presence of lupus anticoagulant in plasma and/or anti-cardiolipin, and/or anti-β2 glycoprotein I antibodies in medium or high titre. The Sapporo APS classification criteria (1998) were replaced by the Sydney criteria in 2006. Based on the most recent criteria, classification with APS requires one clinical and one laboratory manifestation. Impaired placentation and thrombotic changes in the vessels of the decidua and chorion are regarded as a cause of pregnancy loss in women with APS.

Inherited thrombophilia is recently seen as cause of recurrent pregnancy loss, however it is not a basic examination in this field. It has been hypothesized that factor V Leiden mutation, prothrombin gene mutation, antithrombin deficiency, protein S deficiency and protein C deficiency may be associated with recurrent miscarriage as a result of decreased uterine perfusion [6, 7].

Other investigation as infective screen, endocrinological and immunologic investigations are controversial because of limited and poorly designed studies.

The aim of this study was evaluation of the causes of recurrent miscarriages considered in three categories – genetic, anatomic and coagulation disorders (antiphospholipid syndrome and inherited thrombophilia) using the correct nomenclature in our own material.

Material and methods

The study included 81 patients who experienced at least three miscarriages up to and including 21 weeks of gestation.

We have adopted following terms in this study:

- early recurrent miscarriage (ERM) – three or more consecutive miscarriages up to 10 weeks of gestation,
- late recurrent miscarriage (LRM) – two or more consecutive miscarriages between 10 and 21+6 weeks of gestation,
- primary recurrent pregnancy loss (pRPL) – three or more consecutive miscarriages up to 22 weeks of gestation,
- secondary recurrent pregnancy loss (sRPL) – three or more miscarriages up to 22 weeks of gestation interrupted by pregnancy which last more than 22 weeks of gestation and finished with the birth of a child or intrauterine fetal death.

According to preceding terminology patients were divided into 4 groups:

- Group I – consisted of 63 patients with ERM,
- Group II – consisted of 18 patients with LRM,
- Group III – this group included 57 patients with pRPL,
- Group IV – consisted of 24 patients with sRPL.

All patients were hospitalized in the Division of Reproduction of Poznan University of Medical Sciences to evaluate the reason of pregnancy loss. Every woman had karyotype analysis with genetic consultation, antiphospholipid antibodies examination, inherited thrombophilia examination, assessment of uterine cavity as well as basic examinations. Patient’s partner also had karyotype analysis. Some women had also extra examinations (such as hormonal analysis), that will not be analyzed in this study, because they were not significant cause of miscarriages.

Classical cytogenetic examination was used to evaluate karyotype of both partners. Karyotype was determined on the basis of peripheral blood lymphocyte culture and chromosome-banding techniques. Karyotype analysis determined the number of chromosomes in metaphase plates and analysis of banded chromosomes. The analysis was conducted in the Cytogenetic Laboratory Genesis.

Antiphospholipid antibodies were analyzed three month after last miscarriage.

All 81 patients were tested for presence of lupus anticoagulant – LA, anticardiolipin antibodies – ACA and anti-β2-glycoprotein-I antibodies – aβ2GPI in three different laboratories in Poznan between 2002-2012 year. Some patients had recognized APS according to Sapporo criteria. From 2006, when antiphospholipid antibodies were present, tests were repeated after 12 weeks (according to Sydney’s criteria, 2006). Patients with positive antibodies for a second time were classified as antiphospholipid syndrome (APS).

Cardiolipine antibodies were tested in human plasma using enzyme immunoassay (ELISA) for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin/beta-2-glycoprotein I.

Anti-β2-glycoprotein-I antibodies were tested using enzyme immunoassay (ELISA), for the quantitative mea-
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Measurement of IgG class autoantibodies against beta-2-glycoprotein I in human plasma.

Lupus anticoagulant test was performed using activated partial thromboplastin time (APTT)-based assay and dilute Russell’s viper venom time (dRVVT). Both tests were composed of three steps: screening, mixing and confirmation test.

Testing for inherited thrombophilia were aimed to estimate carrier of factor V Leiden mutation, prothrombin G20210A mutation, protein C deficiency and protein S deficiency.

Blood for genetic tests was drawn regardless of the time interval since the pregnancy’s end. Purification of total DNA was performed using QIAamp DNA Blood Mini Kit (Qiagen Inc. Germany). Factor V G1691A mutation and prothrombin G20210A variant was diagnosed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Free protein S was tested using The Monoclonal Free Protein S Antigen assay (ELISA method). Diluted patient plasma was incubated in the wells coated with antibodies specific for human protein S. Bound free protein S was quantified using a horseradish peroxidase. A chromogenic substrate of tetramethylbenzidine and hydrogen peroxide were added to develop a colored reaction. The intensity of the color was measured spectrophotometrically at 450 nm in optical density units.

Protein C was measured using chromogenic assay method. Protein C was activated by a specific fraction from the Agkistrodon contortrix snake venom. The amount of activated protein C was determined by monitoring the rate of hydrolysis of a specific chromogenic substrate. The release of pNA (p-nitroaniline) was measured at 405 nm in optical density units.

All tests were done in three laboratories in Poznan up to 2006 year. Since then, all test were done in the Hemostasis Laboratory of J. Strusia Hospital in Poznan. This laboratory has a TUV Rheinland ISO9001.

Uterine cavity was assessed in the hysterosalpingography or the sonohysterography. While hysterosalpingography is injection of contrast (Uropolin) to uterine cavity by cervical canal followed by X-ray imagination, the sonohysterography is injection of isotonic fluid to uterine cavity during ultrasound examination.

Congenital as well as acquired uterine anomalies were detected by mentioned methods because they can be responsible for pregnancy loss. Uterine malformation can be septum, bicornuate uterus, intrauterine adhesions, endometrial polyps, fibromas or hypoplastic uterus.

Statistical analysis

The statistical analysis was based on the Shapiro-Wilk, Mann Whitney, two-sided Fisher and Pearson tests. The analysis was performed using STATISTICA v.10 software. The level of statistical significance was \( p < 0.05 \).

Results

The study included 81 patients with recurrent pregnancy loss that had a total of 290 miscarriages. Mean number of pregnancy loss per patient was 3.58 (min. 3 – max. 8) and mean time of gestational age was 9.9 weeks (SD 3.4; min. 6 max. 21). The youngest patient was 24, the oldest one 41, the mean age was 30.9.

Fifty five percent of patients had known the reason of pregnancy loss. The remaining 45% of patients had no abnormalities in the karyotype of the couple, uterine cavity, as well as there were no positive antiphospholipid antibodies and inherited thrombophilia. Analysis of all miscarriages, without assignment to particular groups, showed that the most common reason for pregnancy loss was uterine cavity anomalies (16%), followed by antiphospholipid syndrome (12.3%) and inherited thrombophilia (8.6%) as well as abnormal karyotype (3.7%).

Among anomalies of uterus that was recognized in 13 women with recurrent miscarriages, the most common abnormalities were septum \( (n = 5) \) and bicornuate uterus \( (n = 4) \).

Antiphospholipid antibodies were positive in 10 patients: 4 of them had positive lupus anticoagulant, 3 of them had anticardiolipin antibodies and 3 of them had antibodies against \( \beta_2 \) glicoprotein I.

On the other hand, inherited thrombophilia was diagnosed in 7 patients. Four women had factor V Leiden mutation, 2 were protrombin gene mutation carriers and 1 had protein S insufficiency.

Genetic abnormalities applied only to 3 patients that were diagnosed with Robertsonian translocation \( (n = 1) \), balanced \( (n = 1) \) and mosaics \( (n = 1) \). All male karyotypes were normal.

Seven patients (8.6%) had multiple cause of pregnancy loss.

The causes of miscarriage in particular groups are shown in the Table 1.

According to them, the most common reason for early recurrent miscarriage is antiphospholipid syndrome (14.3%), followed by uterine anomalies (12.7%) and inherited thrombophilia (7.9%). Genetic abnormalities such as balanced and Robertsonian translocations was diagnosed in 2 patients (3.17%). It is worth noting that...
in 6 out of 63 patients (9.5%) multiple causes of miscarriages were found (Tab. 2). One woman had three abnormalities – antiphospholipid syndrome, uterine septa and mosaics. There were totally 219 miscarriages in ERM group, that finished at mean 8.5 weeks of gestations (SD 1.5).

Among causes of late recurrent miscarriage the uterine anomalies were in the top place (27.7%), inherited thrombophilia in the second place (11.1%) followed by antiphospholipid syndrome and mother genetic abnormalities (mosaics) equally. There was only one patient with multiple cause of miscarriage in this group – abnormal mother karyotype was accompanied by uterine septa. There were a total of 71 miscarriages in LRM group, that finished at mean 12.3 weeks of gestations (SD 4.2).

Table 1. Causes of recurrent pregnancy loss

<table>
<thead>
<tr>
<th>Group</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal karyotype</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Early recurrent miscarriage</td>
<td>2/63-3.17%</td>
</tr>
<tr>
<td>Late recurrent miscarriage</td>
<td>1/18-5.5%</td>
</tr>
<tr>
<td>Primary recurrent pregnancy</td>
<td>3/57-5.26%</td>
</tr>
<tr>
<td>Secondary recurrent pregnancy</td>
<td>0/24</td>
</tr>
</tbody>
</table>

* There were no significant statistical differences between ERM and LRM groups as well as between pRPL and sRPL groups

Table 2. Multiple causes of recurrent pregnancy loss

<table>
<thead>
<tr>
<th>Number of patients with multiple causes in each group</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (ERM) 6/63-9.5%</td>
<td>1. Bicornuate uterus + balanced translocation</td>
</tr>
<tr>
<td></td>
<td>2. Bicornuate uterus + APS (β2 GPI)</td>
</tr>
<tr>
<td></td>
<td>3. Hypoplastic uterus + APS (aCL)</td>
</tr>
<tr>
<td></td>
<td>4. Uterine septum + APS (aCL) + Robertsonian translocation</td>
</tr>
<tr>
<td></td>
<td>5. Intrauterine adhesions + APS (aCL)</td>
</tr>
<tr>
<td></td>
<td>6. Factor V Leiden mutation + APS (LA)</td>
</tr>
<tr>
<td>Group II (LRM) 1/18-5.5%</td>
<td>1. Uterine septum + mosaics</td>
</tr>
<tr>
<td>Group III (pRPL) 6/57-10.5%</td>
<td>1. Bicornuate uterus + balanced translocation</td>
</tr>
<tr>
<td></td>
<td>2. Bicornuate uterus + APS (β2 GPI)</td>
</tr>
<tr>
<td></td>
<td>3. Hypoplastic uterus + APS (aCL)</td>
</tr>
<tr>
<td></td>
<td>4. Uterine septum + mosaics</td>
</tr>
<tr>
<td></td>
<td>5. Uterine septum + APS (aCL) + Robertsonian translocation</td>
</tr>
<tr>
<td></td>
<td>6. Factor V Leiden mutation + APS (LA)</td>
</tr>
<tr>
<td>Group IV (sRPL) 1/24-4.2%</td>
<td>1. Intrauterine adhesions + APS (aCL)</td>
</tr>
</tbody>
</table>

* There were no significant statistical differences between ERM and LRM groups as well as between pRPL and sRPL groups

Distribution of the primary recurrent pregnancy loss causes was similar to the distribution of the early pregnancy miscarriage causes. The incidence of complex causes was also comparable. The dominant cause of secondary recurrent pregnancy loss was uterine malformation (twice uterine septum and twice intrauterine adhesions). Only one woman, like in the late recurrent miscarriage group, had multiple causes of pregnancy loss. Mean time of pregnancy loss was 9.6 (SD 2.9) and 10.8 (SD 4.1) weeks of gestation in the pRPL and the sRPL groups respectively (p = NS).
Discussion

Examinations offered to couples with recurrent pregnancy loss (RPL) should reveal not only the cause of miscarriages but also lead to live birth of healthy child.

However, the heterogeneities of RPL cause high percentage of couples with undetermined cause of consecutive miscarriages [8]. Our results show that it is as high as in other publications ~ 45%. There is no doubt that making chorion karyotyping a standard examination may change this situation. Sugiuara-Ogasawara et al. analyzed 482 couples with recurrent miscarriages and show that the most common cause of recurrent miscarriages is abnormal embryo karyotype (41%), followed by abnormal parents karyotype (10%), uterine abnormalities (5%), endocrine diseases (6%) and APS (3%). According to these data, percentage of unexplained RPL was reduced to 25% [9]. The chances of healthy child birth by the couple with unexplained cause of RPL depends on woman age and the number of previous pregnancies. Statistical actuarial analysis revealed probability of healthy child birth in 35 old woman with 4 unexplained pregnancy loss to as high as 68% [10].

This study shows a significant role of uterine abnormalities in the ethiopathogenesis of RPL. They were a main cause of all miscarriages, as well as LRM, pRPL and sRPL. The most common uterine anomalies were uterine septum and bicornuate uterus like in other publications [11-13]. High percentage of uterine anomalies (12.7%) in the ERM group i.e. miscarriages before 10 weeks of gestation was surprising to us.

Pregnancy loss in women with bicornuate uterus or uterine septum is explained by the decrease in uterine cavity as well as cervical incompetence. Another theory assumes the presence of fibrous tissue in uterine septum with insufficient vascularization, which has negative impact on implantation. Impaired local expression of VEGF receptor (KDR and Flt-1) in the covering septum endometrium may be another factor responsible for recurrent pregnancy loss [14].

Although there are no randomized controlled trials evaluating the effect of surgical correction of the uterus for pregnancy development, it is emphasized that the percentage of pregnancy loss in women with recurrent miscarriages who have undergone hysteroscopic resection of the uterine septum decreased from 87.5% to 44.4%. However, there is no research that compare live birth rate among women who underwent metroplastic surgery and who did not.

A study published by Sugiuara-Ogasawara et al. in 2011 showed that 59.5% of women with a bicornuate uterus or uterine septum finished first pregnancy with a success and cumulative live birth rate did not differ significantly between women with abnormal and normal uterus (75.0% vs. 85.5%) [15].

The range of antiphospholipid antibodies in women with recurrent pregnancy loss varies between 8% and 42% [16-18]. Such a large discrepancy in the incidence of APS is mainly connected with the lack of standardized sera for testing and single determination of antiphospholipid antibodies, as well as compliance with different APS criteria.

The APS rate was 12.3% in present analyzed material and in the group of ERM even 14.3%. In previously published work from our center but on a different material, the frequency of APS in women with ERM was 1.33% [19]. How to explain such differences? In this retrospective study we present the patients who were diagnosed on the basis of different criteria (from Sapporo and Sydney), the interval between first and second test was in some patients less than 12 weeks, as a aPL positive were regarded patients with titers < 40 GPL and sometimes also after single assay, as well as the laboratory test were carried out in three different laboratories. We have obtained a significant reduction in the incidence of APS in women with RPL since testing in the one authorized laboratory, in strict compliance with Sydney criteria. Therefore, we believe that efforts should be made to create a central laboratory, which will be specialized in the determination of antiphospholipid antibodies and seek to verify the APS criteria.

Another important cause of RPL in our study was inherited thrombophilia. Its prevalence in different types of pregnancy loss was comparable (7.9-11.1%). The meta-analysis of 31 retrospective studies has shown that the relationship of thrombophilia with late pregnancy loss is stronger than early miscarriages [20]. Another meta-analysis showed that factor V Leiden and prothrombin gene mutation carriers have double the risk of experiencing recurrent miscarriage compared with women without these thrombophilic mutation [21].

In light of these and our own results it seems advisable to perform screening for at least factor V Leiden mutation in women with pregnancy loss despite of current American College of Chest Physicians recommendations that do not recommend screening for thrombophilia in women with pregnancy loss, as well as thromboprophylaxis in patients with inherited thrombophilia and poor obstetric history [22].

Evaluation of partners karyotype is one of the basic tests performed in the diagnosis of recurrent pregnancy
loss. The most common result is normal karyotype but in rare cases one of the partners is a chromosome aberration carrier. It is estimated that there are from 3.0 to 6.0% chromosome aberration carriers among couples with RPL [23, 24]. In our study, only 3 out of 81 women were diagnosed with karyotype abnormalities. Chance of having a healthy child by parents that one is carrier of chromosome aberration depends on the gender of carrier, mother age, number of previous miscarriages and localization of chromosomal cracks involved in aberrations. Goddijn et al. on the basis of their research have calculated the chances of 70%, as Stephenson and Sierra (71%) (3.25). Therefore, on the basis of the cited publications, it can be assumed that the chance of having healthy child by the couple with chromosomal aberration after an average 3.7 miscarriage is 47.5%. In our material, successful pregnancy outcome affected 72.7% of couples with balanced translocation after average 2.34 miscarriages and genetic testing [26].

In our study in 7 (8.6%) women with RPL multiple causes of pregnancy loss were detected. Most was a combination of uterine anomalies and the presence of antiphospholipid antibodies (n = 4). There observations emphasize the need to perform all basic tests in couples with recurrent miscarriage. Identifying a single cause such as abnormal karyotype in a partner, does not exempt from other tests, as well as the diagnosis of uterine septum followed by surgical correction does not rule out other causes such as factor V Leiden mutation carrier.

Therefore, the couples with RPL should be referred to specialist centers where all mentioned test could be done, now expanded by cytogenetic analysis of the chorion.

Finally, we present our opinion on the nomenclature. The term primary recurrent pregnancy loss is a broader definition than recurrent early or late miscarriage although their causes in our material are more in line with the causes of early recurrent miscarriage. However, it seems that in clinical practice term recurrent pregnancy loss is more useful to qualify the couple to tests that may determine the cause of miscarriage, whereas, distinction between early and late recurrent miscarriage should be reserved for scientific research.

Conclusions

1) Couples with recurrent pregnancy loss should be referred to specialist centers where basic tests can be performed to determine the cause of miscarriage.

2) All applicable tests should be done in each couple with recurrent pregnancy loss, because in the ethiopathogenesis of recurrent miscarriage two or three factors may be involved.

3) In appropriate situations, basic tests should be expanded to cytogenetic analysis of the chorion.

References

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