Prevalence and Causes of Recurrent Abortion among Women in Beni-Suef Governorate

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Abstract: Objective: The current study searched for prevalence, causes or risk factors and how to prevent recurrent abortion among women in Beni-Suef Governorate. In women, progesterone is a hormone produced mainly in the ovaries. After an egg is released by the ovaries (ovulation), progesterone helps make the uterus ready for implantation of a fertilized egg. Peri-implantation pregnancy loss contributes to more than 20% of unexplained infertility. Deficient hormone levels result in aberrant growth and support of the uterine lining making it un-ideal for implantation. Polymorphisms within the progesterone receptor (PGR) gene, allele and genotype frequencies of patients with repeated abortions were compared also to a control group. **Design:** Risk factors of abortion like age, consanguinity, drug abuse, hormonal disturbances, and exposure to fever, X-rays or smoke during their pregnancies in a group of 40 women with repeated abortions compared with controls matched in number and whom their pregnancies were completely normal. Serum progesterone and the locus rs590688of their PGR were screened for single nucleotide polymorphisms (SNP). **Results:** There was a significant difference between patients and control groups in risk factors and in the serum progesterone levels (p<0.001). The SNP was detected (locus rs590688). The more frequent wild type (*1) allele and the rarer (*2) allele were found in the control group and in the study group at different frequencies (control group: *1/*1: 70%, *1/*2: 27.5%, *2/*2: 2.5%; patient group: *1/*1: 35%, *1/*2: 47.5%, *2/*2: 17.5%). The genotypes distributions differed significantly from each other (P<0.001). **Conclusions:** The data suggest that the rarer PGR allele may be associated with an increased likelihood of repeated abortions to its multi-factorial causes. Couples who experience recurrent pregnancy loss may benefit from a medical evaluation and psychological support.

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1. Introduction:

Recurrent Spontaneous Abortions (RSA) is defined as repeated occurrence of 3 or more miscarriages before 24th week of gestation. The modern definition, however, is the spontaneous loss of 2 or more consecutive pregnancies before 20 weeks of gestation [(1)]. Miscarriage is the most common complication of early pregnancy. The occurrence of repeated pregnancy loss (RPL) affects about 1–5% of couples [(2)]. RPL is a multi-factorial disorder and various factors have been implicated, including uterine anomaly, chromosomal abnormalities, endocrine dysfunction, thrombophilia, immune disorders, lifestyle factors and maternal infections. However, the exact underlying causes remain undetermined in up to 50% of cases [(3)]. Clinical miscarriages (those occurring after the sixth week of gestation) occur in 8% of pregnancies [(4)]. Most clinically apparent miscarriages (two thirds to three-quarters in various studies) occur during the first trimester [(5)].

Implantation of the embryo is a critical event in pregnancy. In humans, peri-implantation pregnancy loss contributes to more than 20% of unexplained infertility [(1)]. .Progesterone, a 21 carbon steroid hormone, mainly produced in the ovaries, placenta, brain and the adrenal glands, is required for the maintenance of pregnancy and treatment with progesterone supplementation was observed to prevent abortions. It is mainly involved in the female menstrual cycle, pregnancy and embryogenesis in most mammalian species. It stimulates and regulates various functions - i) help in preparing the body for conception and pregnancy (implantation of the embryo, promoting uterine growth and suppressing myometrial contractility) ii) acts as an anti-inflammatory agent and regulates the immune response and iii) regulates estrogen levels and thus prevents endometrial cancer. Progesterone receptor (PGR) mediates the physiologic effects of progesterone [(1)].

Certain infections, including *Listeria monocytogenes, Toxoplasma gondii*, rubella, herpes

simplex virus (HSV), measles, cytomegalovirus, and coxsackie viruses, are known or suspected to play a role in sporadic spontaneous pregnancy loss. However, the role of infectious agents in recurrent loss is less clear, with a proposed incidence of 0.5%2 to 5%.8 The proposed mechanisms for infectious causes of pregnancy loss include: (1) direct infection of the uterus, fetus, or placenta, (2) placental insufficiency, (3) chronic endometritis or endocervicitis, (4) amnionitis, or (5) infected intrauterine device [(6)].Human Cytomegalovirus (HCMV) are speherical approximately 120-200 nm in diameter, The virus is considered as the largest member of the herpesviruses family and it's genome is the largest genome of all herpesviruses so the number of the virus encoded proteins and complexity of their functions in the life cycle of the virus reflect the size of its genome . HCMV can be transmitted from the mother to the fetus transplacently during pregnancy in about 40% of cases then HCMV can impairs Cytotrophoblasts differentiation a matter that may explain early abortion occurring in women with primary infection [(7)] .

It seems logical that cigarette smoking could increase the risk of spontaneous abortion based on the ingestion of nicotine, a strong vasoconstrictor that is known to reduce uterine and placental blood flow. However, the link between smoking and pregnancy loss remains controversial, as some, but not all, studies have found an association [(8&9)].

The antiphospholipid syndrome (aPL) is a generally accepted cause of recurrent pregnancy loss [(10)]. High levels of antiphospholipid antibodies may account for 3–15% of recurrent miscarriages. In these cases, pregnancy outcomes are improved by the use of aspirin or heparin ([11& 12)].

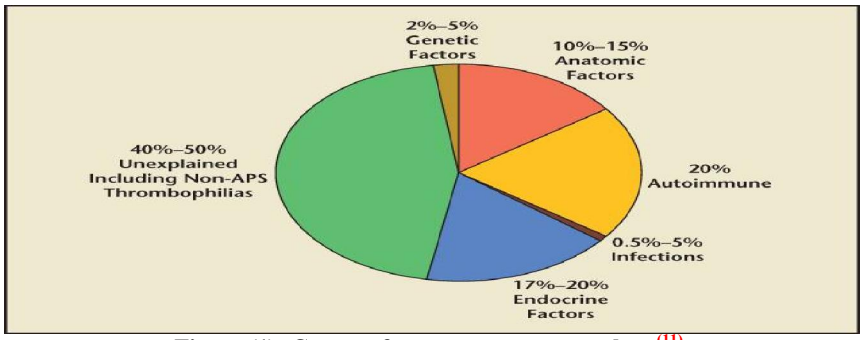


Figure (1): Causes of recurrent pregnancy loss [(11)].

2. Subjects and Methods:

The aborted women in Beni-Suef Governorate were so depressed and some refused to be in the study. Among one thousand married women, approximately 20% of all recognized pregnancies result in miscarriage, 3% of women experienced two consecutive miscarriages, and only 1% experience three or more. The case control study included a group of only 40 women who had experienced at least two consecutive spontaneous abortions (twice or more) from big hospitals in Beni-Suef Governorate after taking their consent and another control group matched with number and age with applying a well prepared questionnaire about the possible risk factors like exposure to smoke or X-rays early in pregnancy, consanguinity, drug abuse during first weeks of pregnancy, Rh negativity, exposure to fever during pregnancy, direct contact with animal and malnourishment. All subjects had undergone a comprehensive examination, including a detailed history, a physical examination, and ultrasound or hysterosalpingography (to detect uterine anomalies and endometrial defects). Routine laboratory investigations were done for all subjects. They were screened also for Rh group, Toxoplasma (IgM/IgG), HCMV (IgM/IgG), antiphospholipidsIgM/IgG (aPL), serum progesterone level, PR gene for single nucleotide polymorphisms (SNP), one SNP at locus (rs590688).

Specific laboratory tests:

- Toxoplasma screenIgG/IgM using one step advanced quality [TM] , In Tech products, INC, USA[(13)].
- HCMV screenIgG/IgM using one site rapid test CTK Biotech. Inc , San Diego CA 92121, USA[(14)].
- Antiphospholipid screen IgG/IgM using ELISA technique (Orgentec, ORG Diagnostika GmbH Mainz, Germany kit) [(15)].
- Serum progesterone using ELISA technique [(16).]
- For detection of PR gene polymorphism, one step SNP at locus (rs590688) [(17)] using Step one Applied Biosystem, USA and Qiagen kits, Genomic DNA was extracted from the peripheral blood lymphocytes using a DNA isolation kit; QIAamp genomic DNA blood kits; QIAGEN group. 1067590 03/2011; we explored on the site: www.qiagen.com/gDNA.

Statistical analysis:

Statistical analysis of the collected data was run on Statistical Package for Social Science Software (SPSS version 20.0 Inc., Chicago, III, USA). Quantitative data were summarized as mean ± SD and comparison between groups was done using t-test. Qualitative data was presented as number (percent) and compared using Chi-Square (X^2). Two-tailed analyses were performed. Tests were considered statistically significant at a P-value <0.05.

3. Results:

It was found 20% of all recognized pregnancies resulted in miscarriage, 3% of women experienced two consecutive miscarriages and only 1% experience three or more which agreed approximately with other researchers.

As regards the patients group, the age ranged from16 years to 48 years while ranged from17 years to 47 years in the control group. Percent risk of RSA was significantly increased in over 40 years subgroup of patients group compared with controls (p<0.001). There was also significant difference in under 20 yrs subgroup of patients group compared with controls (p<0.001). Normal pregnancy was apparent significantly in 20-40 yrs subgroup of patients group compared with controls (p<0.001), as shown in table (1).

Table (1): Percent risk of RSA at various maternal ages:

Age (yrs)	No. of controls	No. of patients	P- value	% risk of RSA
Under 20	4	11	P<0.001	27.5
20-40	26	13	P<0.001	32.5
Over 40	10	16	P<0.001	40

There was no significant difference between patients and controls in mean age (p > 0.05) but the significant differences were at various maternal ages as shown in table (2).Risk factors as shown in table (2) and figure (1) show significant difference where p<0.001.

Table (2): Demographic and epidemiological data of patients and controls.

Parameter	Patients (n=40)	Controls (n-40)	*P*-value
Mean age±SD (yrs)	33.13 ± 10.341	33.05 ± 8.941	P>0.05
Locality			
Rural	17 (42.5%)	10 (25%)	*P*<0.001
Urban	23 (57.5 %)	30 (75%)	*P*<0.001
Smoking exposure	37 (92.5%)	5 (12.5%)	
X-ray exposure	5 (12.5%)	0 (0%)	
Drug abuse during preg.	8 (20%)	2 (5%)	
Positive consanguinity	24 (60%)	6 (15%)	
Fever during pregnancy	10 (25%)	3 (7.5%)	*P*<0.001
Chronic diseases	8 (20%)	0 (0%)	
D&C after abortion	30 (75%)	0 (0%)	
Socioeconomic status	Low	Good	
Direct contact with animals	Apparent	Rare	

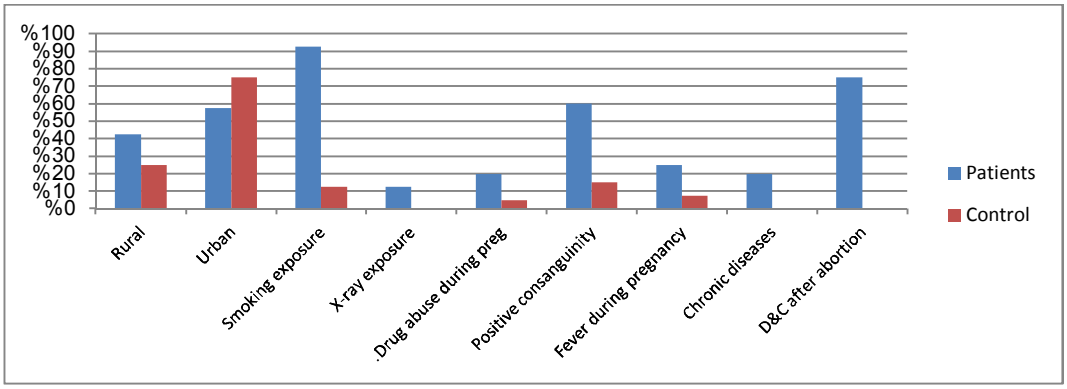


Figure (2): Comparative study between patients and controls as regards some risk factors of abortion.

As regards maternal Rh blood group negativity, toxoplasmosis, HCMV infection and aPL, there were significant differences between patients and control groups where p<0.001 as shown in table (3).

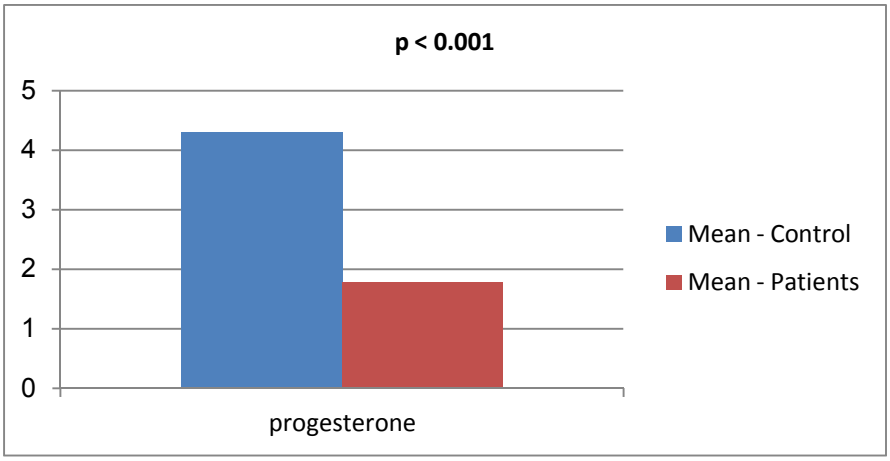
Table (3): Comparative laboratory data between patients and controls.

Parameter	Patients (n=40)	Controls (n-40)	*P*-value
Negative RH group	3 (7.5%)	0 (0%)	*P*<0.001
Positive toxoplasma (IgM/IgG)	5 (12.5%)	0 (0%)	*P*<0.001
Positive HCMV (IgM/IgG)	4 (10%)	0 (0%)	*P*<0.001
Positive aPL (IgM/IgG)	5 (12.5%)	0 (0%)	*P*<0.001

There was a significant difference between patients and control groups in the serum progesterone levels as shown in table (4) and figure (3).

Table (4): Serum progesterone levels in both patients and controls.

	Patients (Mean ± SD)	Controls (Mean ± SD)	t-test	*P*-value
S. progesterone (ng/ml)	1.80± 2.74	4.31± 2.75	5.16526	*p*< 0.001



(Figure 3): Comparative study of serum progesterone levels (ng/ml) **in patients and control groups.**

The SNP was detected (locus rs590688). The more frequent wild type (*1) allele and the rarer (*2) allele were found in the control group and in the study group at different frequencies (control group: *1/*1: 70%, *1/*2: 27.5%, *2/*2: 2.5%; patient group: *1/*1: 35%, *1/*2: 47.5%, *2/*2: 17.5%). The genotypes distributions differed significantly from each other (*P*<0.001) as shown in table 5.

Table (5): Allele and genotype frequencies of PGR polymorphisms

SNP	Allele frequency				Genotype frequency			
	Allele	Case (n=40)	Control (n=40)	*P* value	Genotype	Case (n=40)	Control (n=40)	*P* value
rs590688	1*	47 (58.7%)	67 (83.8%)	< 0.001	1*/1*	14 (35%)	28 (70%)	< 0.001
	2*	33 (41.3%)	13 (16.2. %)		1*/2*	19 (47.5%)	11 (27.5%)	
					2*/2*	7 (17.5%)	1 (2.5%)	

4. Discussion:

Miscarriage, the most common complication of pregnancy, is the spontaneous loss of a pregnancy before the fetus has reached viability. No underlying cause is found in many of these women [18]. It was found 20% of all recognized pregnancies resulted in miscarriage, 3% of women experienced two consecutive miscarriages and only 1% experience three or more which agreed approximately with other researchers [11]. After a miscarriage, making the decision to go in for another pregnancy is difficult. Therefore, these patients experience psychological conditions including anxiety and depression [19 & 20]. Collection of much information as possible should be done to try to find out the possible causes of the pregnancy loss and whether they might influence a future pregnancy. Several factors have been correlated with higher miscarriage rates, but whether they cause miscarriages is debated. Increasing maternal age affects ovarian function and increases

rates of aneuploidy in association with older oocytes[21]. All women with recurrent first-trimester miscarriage and all women with one or more second-trimester miscarriages should have pelvic ultrasound to assess uterine anatomy [18]. Chromosomal problems due to a parent's genes are, however, a possibility. This is more likely to have been the cause in the case of repeated miscarriages, or if one of the parents has a child or other relatives with birth defects [22].

Progesterone plays a crucial role in the regulation of female reproduction, including regulating the menstrual cycle, maintaining implantation, promoting uterine growth and suppressing uterine contraction [23 & 24] .Another cause of early miscarriage may be progesterone deficiency [22] which was apparent in this study. The major physiological effect of progesterone is mediated by PGR or by changing the isoforms ratio and/or the expression level of the PGR [25]. During the past two decades, mifepristone (RU486, progesterone antagonist) has been widely used to terminate early pregnancy by competitively blocking the PGR [26 & 27]. Currently there is no known way to prevent an impending miscarriage. However, fertility experts believe that identifying the cause of the miscarriage may help prevent it from happening again in a future pregnancy. In a case control study, researchers found that use of the supplement dehydroepiandrosterone before and during pregnancy reduced the risk of pregnancy loss[28].

Patients with antiphospholipid syndrome and RSA have higher levels of natural killer (NK) cells than those with APS and no RSA. NK cells have long been suspected to cause RSA since Aoki's original report[29] showing that increased numbers of NK cells in the peripheral blood of women with RSA predict the likelihood of another miscarriage. NK cells are the only lymphocytes found in the endometrium in pregnancy, and are able to induce apoptosis in the trophoblast if cytokine activated. There have even been attempts [30 & 31] to reduce NK cell levels with intravenous immunoglobulin (IVIg). Anti-Phospholipid Screen IgG/IgM assay is a quantitative enzyme immunoassay (EIA) intended to screen for the presence of IgG and IgM class autoantibodies against cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and beta-2-glycoprotein I in human serum or plasma as an aid in the diagnosis of an increased risk of thrombosis in patients with systemic lupus erythematosus (SLE) or lupus-like disorders [15]. Patients having antiphospholipid syndrome as the only aetiological factor for RSA are at increased risk of thrombosis later in life. A history of RSA associated with aPL is a risk factor for subsequent thrombosis in the long term [32]. Autoimmune disease occurs when the body's own immune system acts against itself. Therefore, in the case of an autoimmune-induced miscarriage the woman's body attacks the growing fetus or prevents normal pregnancy progression[33]. Further research has also suggested that autoimmune disease can cause genetic abnormalities in embryos which in turn can lead to miscarriage[34].

Viral infection by rubella, toxoplasmosis, parvovirus, herpes simplex, chlamydia and mycoplasma can lead to miscarriage. Acute infectious fever may lead to abortion. It has been found that the inflammatory reactions induced in the placenta by T. gondii are capable of stimulating synthesis and release of prostaglandin F_{2a} which has a luteolytic action leading to decreased progesterone level and subsequent abortion [35]. The risk of intrauterine cytomegalovirus infection and disease in the fetus or newborn largely depends on time of primary maternal infection during pregnancy. The highest risk of severe symptoms in the fetus exists around conception and in the first trimester of pregnancy[36].

Socioeconomic status: Risk of pregnancy loss is higher in poor group probably due to malnutrition and protein deficiency which was agreed in this study. Maternal nutrient depletion may contribute to the increased incidence of preterm births and fetal growth retardation among these women as well as the increased risk of maternal mortality and morbidity[37]. In the past, it was assumed that the fetus functioned as a parasite and withdrew its nutritional needs from maternal tissues [38].

While lifestyle factors have been associated with increased risk for miscarriage in general, and are usually not listed as specific causes for RPL, every effort should be made to address these issues in patients with RPL. Of specific concern are chronic exposures to toxins including smoking, alcohol, and drugs[10].Caffeine consumption has also been correlated to miscarriage rates, at least at higher levels of intake [39]. The role of nurses in abortion services and support has developed. Although a woman physically recovers from a miscarriage quickly, psychological recovery from depression for parents in general can take a long time (another risk factor). People differ greatly in this regard: some are able to move on after a few months, but others take more than a year. Still others may feel relief or other less negative emotions [40].

Conclusions:

The data suggest that the rarer PR allele may be associated with an increased likelihood of repeated abortions to its multi-factorial causes, and multiple factors are implicated in the pathogenesis of recurrent miscarriage. Couples who experience recurrent pregnancy loss may benefit from a medical evaluation and psychological support.

Recommendations:

Preconceptional care should be encouraged when the patient decide to become pregnant again. Health education should be done for these women to avoid the risk factors. The study needs to find more risk factors and to be done in a larger scale and in all governorates of Egypt to reduce the risk of abortion as possible.

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