Original article

Evaluation of etiology and pregnancy outcome in recurrent miscarriage patients

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ABSTRACT

The purpose of this study was to evaluate etiology and pregnancy outcome of recurrent miscarriage women. The enrolled patients (280) were evaluated for Triiodothyronine, Thyroxine, Thyroid stimulating hormone, prolactin, chromosomal analysis, Haemoglobin A1C, blood sugar, Magnetic resonance imaging, 3D-ultrasound, auto-antibodies profile (antiphospholipid antibodies, anticardiolipin antibodies, lupus anticoagulant, antinuclear antibodies, anti-thyroid antibodies and β2 glycoprotein1), torch profile (Toxoplasmo gondii, rubella, cytomegalo virus and herpes simplex virus), blood vitamin D3 levels, psychological factors, Body mass index and thrombotic factors (protein S and C deficiency, Prothrombin G20210A mutation, anti-thrombin III, Factor V Leiden and Methylenetetrahydrofolate reductase mutation), uterosalpingography (hysteronsalpingography) and hysteroscopy. The therapeutic regimens either singly or combined were employed for the treatment of recurrent miscarriage patients on the basis of etiology (single or multiple) and include intravenous immunoglobulin, low molecular weight heparin, low dose aspirin, levothyroxine, progesterone, folic acid, human chorionic gonadotrophin, vitamin D3, psychotherapy, genetic counselling. However, patients with idiopathic recurrent miscarriage were treated with progesterone supplementation, anticoagulation and/or immune modulatory agents. The incidence of primary recurrent miscarriage was highest and most of the women experienced recurrent miscarriage during first trimester. Endocrinological disorders (39%) were found as the major pathological factor for recurrent miscarriage. Other factors include uterine abnormalities (5.7%), vitamin D3 deficiency (3.5%), psychological factors (3.2%) infection (3.6%), autoimmune abnormalities (1.8%) and protein S deficiency (1.8%). However, 40% cases were idiopathic. The overall live birth rate achieved after the management of recurrent miscarriage patients was 75.7%. Enocrinopathy was the major cause of recurrent miscarriage. The overall live birth rate achieved was 75.7% with highest pregnancy outcome in secondary recurrent miscarriage patients after the management.

1. Introduction

The process of reproduction in human beings is characterized by inefficiency. Early loss of pregnancy is perhaps the most widespread obstetric problem that occurs in over two thirds of human conceptions (Silver and Warren, 2006). Clinically recognized pregnancy loss is widespread that influences about 15–25% of pregnancies (ASRM, 2012, D'Ippolito et al., 2020). Miscarriage is the loss of foetus earlier than the 23rd week of gestation (Kruger and Botha, 2007). Usually recurrent miscarriage (RM) is defined as the failure of 3 or more successive clinically documented conceptions prior to 20 weeks of development. However according to American Society

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for Reproductive Medicine (ASRM) RM is defined as two or more consecutive pregnancy losses recognized by ultrasound or histopathology (ASRM, 2008, 2012). Nevertheless, less than 5% of conceived human females undergo 2 consecutive miscarriages and merely 1% experiences 3 or more (ASRM, 2008, Stephenson and Kutteh, 2007, Branch et al., 2010, ASRM, 2013, Grimstad and Krieg, 2016). Clinically apparent RM among Indian women is observed to be 7.46% (Patki and Chauhan, 2016). RM is considered as a significant reproductive health matter since it affects a large number of pregnancies. The frequency of RM differs broadly among research reports due to variations in the meaning and decisive factors used, in addition to the distinctiveness of populations. The risk of pregnancy loss subsequent to first two, three and four successive miscarriage is 30%, 33% and 40% respectively in patients with no previous live birth (Ford and Schust, 2009). Primary RM stands for the collapse of several pregnancies in a woman without any previous live birth while secondary RM means multiple pregnancy failures in a woman after at least one successful pregnancy (Silver et al., 2011, Kolte et al., 2015, El Hachem et al., 2017). The various known set of causes associated with RM include parental chromosomal aberrations, uterine malformations, infectious diseases, endocrine problems, and autoimmune defects; nevertheless, the causes remain idiopathic in around 50% of RM cases (Ford and Schust, 2009, Fritz and Speroff, 2012, Jeve and Davies, 2014, Arias-Sosa et al., 2018, Ali et al., 2020) (Fig. 1). Many chromosomal anomalies have been reported to be connected with unexplained RM and these anomalies include skewed X inactivation, sperm DNA fragmentation, length of telomeres and micro deletions in the Y chromosome that are not identified by conventional cytogenetic techniques. However, the association of these abnormalities with idiopathic RM is still controversial due to variations in results among different studies, populations as well as definitions of the disease (Arias-Sosa et al., 2018). Conversely, immunological rejection may account for most of these unexplained cases of pregnancy loss (Ghaebi et al., 2017). RM is an extremely heterogeneous condition (Ali et al., 2020). The success of pregnancy depends on proper and balanced communiqué between the mother and fetus by means of placental and decidual tissue. Any interruption or deviation in the signaling may bring about the pregnancy failure (Rull et al., 2012, Grimstad and Krieg, 2016). RM is a challenging reproductive condition that leads to psychological stress in affected couple, their families and physicians. Different treatment regimens have been employed for the management of this critical reproductive problem such as correction of uterine alterations by surgery, treatment of antiphospholipid syndrome with aspirin

and heparin and administration of progesterone, anticoagulation and/or immune modulatory agents in patients with unexplained RM. Even if these treatment protocols over the years have been found to improve the outcome of pregnancy in RM patients (Kolte et al., 2015) but have not completely solved the problem. This necessitates further investigation in the etiology and management of RM. The study was undertaken due to the lack of research data on the etiology and management of RM in the present population and to determine major contributing factors as well as to reduce the ambiguity of idiopathic RM that causes mental trauma in affected couples and clinicians. The present study may serve as an assisting guide for the clinicians during study and treatment of RM.

2. Materials and methods

2.1. Study design

This was a prospective outpatient clinic-based cohort study carried out in 280 RM patients enrolled for the study. Majority of the women (196) experienced two consecutive miscarriages and the remaining 84 had undergone more than two successive miscarriages. The patients who visited the antenatal clinics for regular health check were screened for eligibility criteria and data availability. The clinical details were obtained from RM patients via keen observation of their diagnostic investigations and prescription cards as well as interview as per the pre-structured questionnaire for the study to properly record the details as per hospital protocol. The patients were tested for T3 (Triiodothyronine), T4 (Thyroxine), TSH (Thyroid Stimulating Hormone), prolactin, chromosomal analysis, HbA1C (Hemoglobin A1C), blood sugar, MRI (Magnetic Resonance Imaging), 3D-ultrasound, auto antibodies profile (antiphospholipid antibodies, anticardiolipin antibodies, lupus anticoagulant, antinuclear antibodies, anti-thyroid antibodies and β2 glycoprotein1), torch profile (Toxoplasmo gondii, rubella, cytomegalo virus and herpes simplex virus), blood VD3 (Vitamin D3) levels, psychological factors, BMI (body mass index) and thrombotic factors (protein S and C, Prothrombin G20210A mutation, antithrombin III, factor V Leiden and MTHRF (Methylenetetrahydrofolate reductase) mutation), uterosalpingography (hysterosalpingography) and hysteroscopy. All the tests were performed in accordance with relevant guidelines and regulations by following the guidelines of ESHRE (European Society of Human Reproduction and Embryology), November 2017. Psychological assessment for stress was performed using validated scales such as Fertility Problem Inventory (FPI), Hospital Anxiety and Depression Scale (HADS), and Perceived Stress Scale (PSS). Polycystic ovarian syndrome (PCOS) was diagnosed on the basis of criteria established by ESHRE and ASRM. According to these scientific societies PCOS diagnosis requires at least two of the three criteria: oligo-ovulation or an-ovulation, biochemical and/or clinical hyper-androgenism, and polycystic ovaries as visible on ultrasound.

All the patients were given different RM management therapies during their pregnancy period for carrying the gestation successfully to full term. The therapies include levothyroxine, progesterone, folic acid, hCG (human chorionic gonadotrophin), LMWH (low molecular weight heparin), LDA (low-dose aspirin), VD3 (vitamin D3), intravenous immunoglobulin (IVg), psychotherapy, genetic counselling. The therapeutic regimens were given either singly or combined on the basis of etiology (single or multiple). However, patients with idiopathic recurrent miscarriage were treated with progesterone supplementation, anticoagulation and immune modulatory agents (Table 1). Informed consent in both English and vernacular was taken from the participants. This study
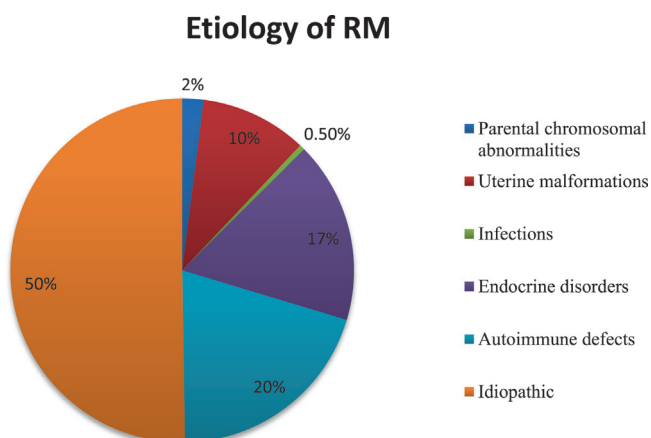

Fig. 1. Etiology of RM. Showing the proportion of various known and unknown causes of RM (Ford and Schust, 2009, Jeve and Davies, 2014).

Table 1
Therapeutic interventions for preventing recurrent miscarriage and increasing pregnancy outcome.

S. NO	Etiology of RM		Therapeutic and preventive interventions
1.	Uterine abnormalities	Bicornuate uterus Fibroids/ myometrial fibroids Cervical polyps Cervical weakness	Metroplasty Myomectomy Polypectomy Cerclage
2.	Endocrinological disorders	Hypothyroidism Hyperprolactinemia Diabetes mellitus Polycystic ovarian syndrome Single ovarian cysts	Levothyroxine Bromocriptine/cabergoline Insulin Metformin Surgical intervention
3.	Genetic abnormalities	Maternal Paternal Embryonic	Pre-conceptional genetic counselling
4.	Autoimmune defects	Antiphospholipid antibodies (APA) Lupus anticoagulant (LAC) Anti thyroid antibodies (ATA) Antinuclear antibodies (ANA)	Low molecular weight Heparin, Low-dose aspirin, Intravenous immunoglobulin Levothyroxine supplementation
5.	Infections	Toxoplasma gonodii Cytomegalovirus Herpes simplex virus	Antiviral drugs
6.	VD3 deficiency		VD3 supplementation
7.	Psychological disorders	Social trauma, Fear related to Pregnancy	Psychotherapies
8.	Obesity		Life style interventions/ Pharmacotherpy
9.	Thrombophilic factors	Protein S deficiency	Anticoagulants
10	Idiopathic		Progesterone supplementation, Aspirin, and immune modulatory agents

was approved by Institutional Ethical Committee of Government Medical College, Srinagar, Jammu and Kashmir, India under the reference No.121/ETH/GMC.

2.2. Study site

Study was carried out at outpatient antenatal clinics at the Department of Obstetrics and Gynecology in Government Medical College associated Lalla Ded Hospital Srinagar, Jammu and Kashmir, India, during last three years where RM patients come across from Kashmir for regular checkup. The study site was approval by the Institutional Ethics Committee of Government Medical College, Srinagar, Jammu and Kashmir, India.

2.3. Study participants

The study included only those antenatal RM cases who fulfilled the below given inclusion and exclusion criteria.

3. Inclusion criteria for patients:

- Gravida 3 women or more with at least two consecutive miscarriages, primary or secondary ≤ 24 weeks of gestation.
- All patients were Kashmiri women population.
- The age of patients ranged between 18 and 45 years.
- Patients who willingly signed the consent form.

4. Exclusion criteria for patients:

- Patients with a history of only one miscarriage.
- Patients with a history of two or more induced abortions.

5. Statistical analyses

The data collected was statistically analyzed using SPSS version 20.0 (Chicago, IL, USA). The groups were compared using Chi-square test and t-test. Results were assumed statistically significant at $p < 0.05$.

6. Results

The mean age of enrolled RM patients was 30.5 (±5) years. Mean height, weight and parity of these patients are given in Table 2. Majority of the women experienced RM during the first trimester, some women had second trimester RM and a large percentage of women undergo RM in either trimesters (Fig. 2). It shows that early gestational months are the most unsafe period for women that suffer from RM. Additionally, we observed that most of the first trimester miscarriages remained unexplained (idiopathic). In this study the RM patients with known etiology were 60%. Among these known causes endocrinological disorders were found as the major pathological factor for RM. They were statistically significant ($p = 0.01$) and account for 38.9% cases. Subsequently uterine abnormalities accounted for 5.7% of cases and were highly significant ($p = 0.001$). The genetic variances that bring about the first trimester pregnancy losses were found responsible for RM in 0.7% of cases. Autoimmune abnormalities and Protein S deficiency each accounted for 1.8%. The auto antibodies have been associated with late first and second trimester abortions. Vitamin D3 deficiency and psychological factors each accounted for 3.5% and 3.2% cases respectively. Obesity was found to affect 0.7% RM patients. In addition, infections ($p = 0.01$) distressed 3.6% cases of RM. However, 40% cases in our study were idiopathic (Table 3). Single defect was found in 39.3% (110/280) RM women and multiple defects (two, three or more) were observed in 60.7% (170/280) cases.

6.1. Comparison between primary and secondary RM patients

The women that experienced primary RM had lesser mean age (30 ± 5) as compared to secondary RM women (31.6 ± 4.7). Similarly the mean parity was lesser in primary RM, however, the mean height and weight was lesser in secondary RM women (Table 4). Most of the women suffered from primary RM. The incidence of primary vs. secondary RM found is shown in Fig. 3. Uterine abnormalities were seen more prevailing in secondary RM (7%) compared to primary RM (5.2%). Endocrine defects, chromosomal disorders were equally prevalent in both categories. VD3 deficiency was higher in primary RM group (4.3%) as compared to secondary RM group (1.4%). However, autoimmune defects, infections ($p = 0.04$), psychological disorders, obesity and thrombophilic factors were present only in primary RM cases. Additionally, higher proportion of cases was idiopathic in secondary RM group compared to primary RM group (Table 5).

Table 2
Basic demopo; graphic and anthropometric characteristics of RM patients.

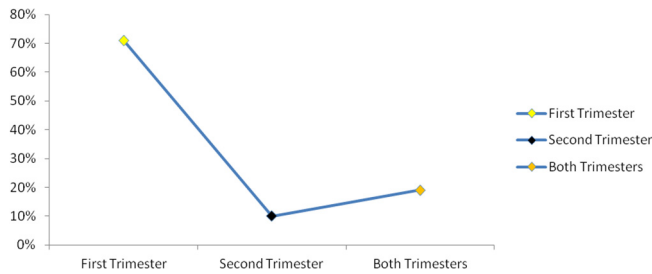| Age	Height(cm)	Weight (kg)	Parity
30.5 ± 5	144 ± 14.6	72 ± 11.8	0.23 ± 0.5

Values are presented as mean ± SD

Fig. 2. RM in different trimesters. Shows the incidence of first trimester, second trimester and both trimester RMs.

6.2. Comparison between patients with two and more than two consecutive miscarriages

The mean age of women with two consecutive miscarriages and more than two miscarriages was similar. Similarly the mean parity was higher in the group with two miscarriages compared to the group with more than two miscarriages. The mean height was almost similar in both groups. However, mean weight was higher in the group with two miscarriages. The rate of miscarriage was higher in women with two miscarriages compared to those with more than two miscarriages (70% vs. 30%) (Table 6).

Table 3
Different causes of RM. Illustrating the etiology of RM along with their contributing percentage as well as the proportion of idiopathic RM cases.

Etiology/causes of RM	Sub-causes of RM	Present	Absent	Cases in each etiology (N)	Mean ± SD	p value	Screening Techniques	Percentage of each cause (n %)
Uterine abnormalities	Bicornuate uterus	2	14	16	8 ± 8.48	0.001	MRI ,3D-ultrasound, Hysterosalpingography, Hysteroscopy	(16/280) 5.7%
	Fibroids/ myometrial fibroids	6	10		8 ± 2.82			
	Cervical polyps	5	11		8 ± 2.82			
	Cervical weakness	2	14		8 ± 4.24			
	Utero-placental insufficiency	1	15		8 ± 9.89			
Endocrinological disorders	Hypothyroidism (TSH ≥ 4.0μIU/mL)	84	25	109	54.5 ± 41.71	0.01	T3, T4, TSH, Hb A1C, Blood sugar, Ultrasound, Prolactin	(109/280) 38.9%
	Hyperprolactinemia (Prolactin ≥ 17.9 ng/mL)	2	107		54.5 ± 74.24			
	Diabetes mellitus	11	98		54.5 ± 60.10			
	Polycystic ovarian syndrome	5	104		54.5 ± 70.00			
	Single ovarian cysts	7	102		54.5 ± 65.76			
Genetic abnormalities	Maternal	2	0	2	1 ± 1.41	0.5	Karyotyping	(2/280) 0.7%
	Paternal	0	2					
	Embryonic	0	2					
Autoimmune defects	Antiphospholipid antibodies (APA)	1	4	5	2.5 ± 2.12	1.7	Auto antibodies profile test (APA, ACA, ATA,ANA, LAC, β2 glycoprotein 1)	(5/280) 1.8%
	Anticardiolipin antibodies (ACA)	0	5		2.5 ± 3.53			
	Anti thyroid antibodies (ATA)	1	4		2.5 ± 2.12			
	Antinuclear antibodies (ANA)	2	3		2.5 ± 0.70			
	Lupus anticoagulant (LAC)	1	4		2.5 ± 2.12			
	β2 glycoprotein1	0	5		2.5 ± 3.53			
Infections	Toxoplasma gonodii	4	6	10	5 ± 1.41	0.01	Torch profile test (TG,CGV,HSV, Rubella)	(10/280) 3.6%
	Cytomegalovirus	2	8		5 ± 4.24			
	Herpes simplex virus	4	6		5 ± 1.41			
	Rubella	0	10		5 ± 7.07			
VD3 deficiency	≤ 10 ng/dl	3	7	10	5 ± 2.82	1.00	Blood VD3 Levels	(10/280) 3.6%
	≤ 20 ng/dl	7	3					
Psychological disorders	Social trauma	6	3	9	4.5 ± 2.12	1.00	Psychometric tests	(9/280) 3.2%
	Fear related to Pregnancy	3	6					
Obesity	≥25 kg/m²	2	0	2	1 ± 1.41	1.00	BMI (body mass index)	(2/280) 0.7%
	≥30 kg/m²	0	2					
Thrombophilic factors	Protein S deficiency	5	0	5	2.5 ± 3.53	0.2	Thrombotic tests (Factor V Leiden, Prothrombin G20210A mutation, Protein S activity, Antithrombin activity, Protein C activity)	(5/280) 1.8%
	Protein C deficiency	0	5					
	Factor V Leiden	0	5					
	Prothrombin G20210A mutation	0	5					
	MTFHR mutation	0	5					
Idiopathic		112	–	168			No Test positive	(112/280) 40%

Table 4
Basic demographic and anthropometric characteristics of primary and secondary RM patients.

Primary RM patients (n = 209)				Secondary RM patients (n = 71)			
Age	Height	Weight	Parity	Age	Height	Weight	Parity
30 ± 5	145.3 ± 14.4	73 ± 12	0.11 ± 0.4	31.6 ± 4.7	140 ± 14.6	69 ± 10.7	0.66 ± 0.99

Values are presented as mean ± SD.

Uterine deformities were seen widespread in women with ≥3miscarriages (7%) compared to women with two miscarriages (5%). Endocrine defects were more prevalent in women with ≥3 miscarriages (45.4%) compared to women with two miscarriages (23.8%). Infections and VD3 deficiency was equally prevalent in both categories but psychological disorders were found slightly higher in the category of women with ≥3 miscarriages (3.5%) compared to the

category with two miscarriages (3%). Autoimmune defects were higher in women with ≥3 miscarriages (2.3%) as compared to women with two miscarriages (1.5%). However, genetic disorders, obesity and thrombophilic factors were observed only in women with ≥3 miscarriages. Additionally, higher proportion of cases was idiopathic in the group with ≥3 miscarriages (50%) compared to the group with two consecutive miscarriages (34.7%) (Table 7).

6.3. Pregnancy outcome index of RM women

All the women enrolled in the study were pregnant. After the management of these women the overall rate of live birth was 76% with mean period of gestation equal to 37.78 ± 3.61. Miscarriages took place in 68 women. The live birth rate in women with primary RM was 73.7% (154/209) and those of secondary RM women was 81.6% (58/71) with almost similar mean period of gestation in both groups. Live birth rate was higher in case of secondary RM as compared to primary RM (81.6 vs. 73.7). However, the rate of live birth was almost similar in 2 RM and ≥3 RM women (73.9% vs. 79.7%). The gestational weeks were similar in either group (Table 8).
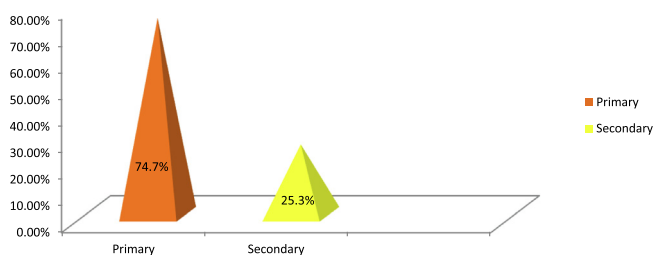

Fig. 3. Different types of RM. Illustrates the respective incidence of primary vs. secondary RM among women of reproductive age group.

Table 5
Comparison between the etiologic factors of primary RM and secondary RM.

Etiology/ causes of RM	Sub-causes of RM	Primary RM (n = 209)	N%	Secondary RM (n = 71)	N%	p value
Uterine abnormalities	Bicornuate uterus	2	11/209 (5.2%)	0	5/71 (7%)	0.22
	Fibroids/ myometrial fibroids	2		4		
	Cervical polyps	4		1		
	Cervical weakness	2		0		
	Utero-placental insufficiency	1		0		
Endocrinological disorders	Hypothyroidism (TSH ≥ 4.0μIU/mL)	59	81/209 (38.7)	25	28/71 (39.4%)	0.39
	Hyperprolactinemia (Prolactin ≥ 17.9 ng/mL)	2		0		
	Diabetes mellitus	8		3		
	Polycystic ovarian syndrome	5		0		
	Single ovarian cysts	7		0		
Genetic abnormalities	Maternal	1	1/71 (1.4%)	1	1/71 (1.4%)	1.00
	Paternal	0		0		
	Embryonic	0		0		
Autoimmune defects	Anti phospholipid antibodies (APA)	1	5/209 (2.4%)	0	–	0.06
	Anticardiolipin antibodies (ACA)	0		0		
	Anti thyroid antibodies (ATA)	1		0		
	Antinuclear antibodies (ANA)	2		0		
	Lupus anticoagulant (LAC)	1		0		
	β2 glycoprotein1	0		0		
Infections	Toxoplasma gonodii	4	10/209 (4.7%)	0	–	0.04
	Cytomegalovirus	2		0		
	Herpes simplex virus	4		0		
	Rubella	0		0		
VD3 deficiency	≤ 10 ng/dl	2	9/209 (4.3%)	1	1/71 (1.4%)	0.25
	≤ 20 ng/dl	7		0		
Psychological disorders	Social trauma	6	9/209 (4.3%)	0	–	0.09
	Fear related to Pregnancy	3		0		
Obesity	≥25 kg/m²	2	2/209 (0.9%)	0	–	0.42
	≥30 kg/m²	0		0		
Thrombophilic factors	Protein S deficiency	5	5/209 (2.4%)	0	–	0.34
	Protein C deficiency	0		0		
	Factor V Leiden	0		0		
	Prothrombin G20210A mutation	0		0		
	MTFHR mutation	0		0		
Idiopathic		78	78/209 (37.5%)	34	34/71 (48%)	

Values are presented as mean ± SD and number %.

Table 6
Basic demographic and anthropometric characteristics of patients with two and more than two miscarriage.

Two miscarriages (n = 196)				More than two miscarriages (n = 84)			
Age	Height	Weight	Parity	Age	Height	Weight	Parity
30 ± 5	145.3 ± 14.4	73.4 ± 12	0.53 ± 0.81	30 ± 5.4	146 ± 15.2	70.4 ± 10.4	0.27 ± 0.66

Values are presented as mean ± SD.

Table 7
Comparison between the etiologic factors of women with two miscarriage and women with three or more miscarriages.

Etiology/ causes of RM	Sub-causes of RM	Two Miscarriages (n = 196)	N%	Three or more Miscarriages (n = 84)	N%	p value
Uterine abnormalities	Bicornuate uterus	2	10/196 (5%)	0	6/84 (7%)	0.3
	Fibroids/ myometrial fibroids	3		3		
	Cervical polyps	3		2		
	Cervical weakness	1		1		
	Utero-placental insufficiency	1		0		
Endocrinological disorders	Hypothyroidism (TSH ≥ 4.0μIU/mL)	68	89/196 (45.4%)	16	20/84 (23.8%)	0.3
	Hyperprolactinemia (Prolactin ≥ 17.9 ng/mL)	2		0		
	Diabetes mellitus	11		0		
	Polycystic ovarian syndrome	5		0		
	Single ovarian cysts	3		4		
Genetic abnormalities	Maternal	0	0	2	2/84 (2.3%)	0.3
	Paternal	0		0		
	Embryonic	0		0		
Autoimmune defects	Antiphospholipid antibodies (APA)	1	3/196 (1.5%)	0	2/84 (2.3%)	0.5
	Anticardiolipin antibodies (ACA)	0		0		
	Anti thyroid antibodies (ATA)	0		1		
	Antinuclear antibodies (ANA)	1		1		
	Lupus anticoagulant (LAC)	1		0		
	β2 glycoprotein1	0		0		
Infections	Toxoplasma gonodii	2	7/196 (3.5%)	2	3/84 (3.5%)	0.3
	Cytomegalovirus	1		1		
	Herpes simplex virus	4		0		
	Rubella	0		0		
VD3 deficiency	≤ 10 ng/dl	2	7/196 (3.5%)	1	3/84 (3.5%)	0.3
	≤ 20 ng/dl	5		2		
Psychological disorders	Social trauma	4	6/196 (3%)	2	3/84 (3.5%)	0.3
	Fear related to Pregnancy	2		1		
Obesity	≥25 kg/m^2	0	0	2	2/84 (2.3%)	0.4
	≥30 kg/m^2	0		0		
Thrombophilic factors	Protein S deficiency	0	0	5	5/84 (5.9%)	0.3
	Protein C deficiency	0		0		
	Factor V Leiden	0		0		
	Prothrombin G20210A mutation	0		0		
	MTFHR mutation	0		0		
Idiopathic		68	68/196 (34.7%)	34	42/84 (50%)	

Values are presented as mean ± SD and number %.

Table 8
Pregnancy outcome index of RM women (Primary vs. secondary and two vs. three or more).

	Primary vs. secondary RM		Two vs. ≥ three RM		
	Primary	Secondary	Two RM	≥ Three RM	Total
Pregnancy	209	71	196	84	280/280
Live births	154 (73.6)	58 (81.6)	145 (73.9)	67 (79.7)	212/280 (75.7)
Gestational period	37.31 ± 5.47	38.65 ± 1.51	37.91 ± 3.52	37.27 ± 4.32	37.78 ± 3.61

Values are presented as number (%) or mean ± SD.

7. Discussion

RM is a multi-factorial disorder with a huge proportion of patients with unidentified etiology that creates complexity in its management and leads to psychological trauma and frustration in affected couples as well as in physicians. As a result, researches have been carrying out to find out the unknown etiology of RM in order to develop advanced treatments as well as precautionary approaches. In our prospective cohort study, the incidence of primary and secondary RM was found to be 73.70% and 26% respectively. A previous study also reported almost the same incidence of different types of RM in that order (Singh et al., 2017). Our study was in accord with the earlier studies that also reported a higher incidence of primary RM (Jivraj et al., 2001, Li et al., 2002, Jaslow

et al., 2010). Conversely, Shapira et al. (2012) reported higher incidence of secondary RM. We explored that known factors affect 60% of cases. Endocrinological disorders remained as one of the most widespread abnormalities among the RM patient in our study and influenced 38.9% cases. Our finding is almost consistent with some research studies that reported endocrine disorders in 34.3% (Lee et al., 2016) and 46.6% (Vomstein et al., 2016) RM patients. However, there are researches contrary to our study that reported the endocrine defects in 4.98% (Le et al., 2018), 6% (Babkeret al., 2013) and 10% (NICE, 2012) RM cases. A different study reported endocrine pathology in 13.5% RM cases (Jaslow et al., 2010). Further studies reported that 17–20% RM patients were distressed by endocrinological abnormalities (Ford and Schust, 2009, Jeve and Davies, 2014, Singh et al. 2017).

The replacement of thyroid hormone therapy with levothyroxine improved the outcome of pregnancy in child bearing women affected with subclinical hypothyroidism (Reid et al., 2013, Ke, 2014). Polycystic ovarian syndrome (PCOS) as the most frequent endocrinopathy among women of reproductive age increases the risk of miscarriage. PCOS management with metformin or regulation of body weight appears to decrease the risk of miscarriages. Uterine alterations brought about RM among 5.7% of cases in our study. However, there are research reports according to which uterine alterations account for 10–15% cases of RM (Ford and Schust, 2009, Jeve and Davies, 2014). Another study reported the prevalence of structural uterine abnormalities in 6.6% cases which is almost in consistence with our investigation (Dobson and Jayaprakash, 2018). Uterine alterations have been reportedly detected in up to 19% of women experiencing RM (Li et al., 2002, El Hachem et al., 2017). The damaging consequences of uterine malformations on pregnancy are well recognized. Research studies have recommended uterine imaging only in those patients that have undergone two spontaneous consecutive miscarriages since no variations have been observed in the occurrence of uterine abnormality among human females who spontaneously aborted twice and those who suffered with three or more pregnancy losses. In our study, antiphospholipid antibodies (APLA) including lupus anticoagulant, anticardiolipin antibodies, antiβ2 glycoprotein 1 antibodies were reported in 1% cases. Antiphospholipid syndrome (APS) represents a defective autoimmune state characterized by APLA production, vascular thrombosis or morbid pregnancy (Miyakis et al., 2006). Conversely, a study reported the prevalence of APLA in 7.4% cases (Dobson and Jayaprakash, 2018). Another study reported that 16% of human females affected by RM were diagnosed APLA positive (Noble et al., 2005). One more study reported that 11.29% patients were affected by APLA (Le et al., 2018). Most of the APLA positive women had merely undergone early miscarriages and this suggests that all women irrespective of the gestational age of fetal loss ought to be screened for the presence of these auto antibodies. The fetal loss rate in female patients with APLA is approximately 80%. In the present study, APS incidence was less compared to other various studies. In the management of patients with APS, aspirin and heparin has been treatment of choice that leads to successful live births in about 75% of treated women (Kutteh and Hinote, 2014). Among hereditary thrombophilias, prothrombin G20210A (3%) and Factor V Leiden (8%) mutations are most frequent in Caucasian population (Poter and Scott, 2005, Jaslow et al., 2010). Antithrombin III deficiency has been found in 1.5% RM cases, while protein S deficiency and protein c deficiency in 3.5% and 1.1% cases respectively (Jaslow et al., 2010). In the present study none of the RM women was seen affected by prothrombin G20210A, Factor V Leiden and MTFHR mutations, however, protein S deficiency was found in 1.8% cases. In a previous study, Factor V Leiden G1691A and prothrombin G20210A mutations also were found not associated with RM in Kashmiri women population (Shafia et al., 2017). Only 20% couples

in our study had undergone karyotyping. Their karyotyping reports were normal except two couples where in each case the maternal chromosome 9 had increased heterochromatin region in the long arm [46 + XX, 9(q h +)]. According to our study, 0.7% couples underwent RM due to maternal chromosomal defects. No paternal chromosomal abnormalities were seen. Conversely, the rate of chromosomal aberrations was reported to be 7.75% among Kashmiri RM couples. The paternal and maternal chromosomal alterations were seen as 2.11% and 5.63% (Zargar et al., 2015). In our study, we found that 25% patients had higher age (≥35 years). The same percentage (25.35%) of advanced age has been reported among RM patients of Kashmiri women population (Zargar et al., 2015). The higher age of female partner has been reported to serve as an independent risk factor for spontaneous pregnancy loss (Risch et al., 1988, Abdalla et al., 1993, Andersen et al., 2000, RCOG Green Top Guideline, 2011, Patki and Chauhan, 2016). Vitamin D3 deficiency was reported in 3.6% RM patients in our study. This finding has been supported by the study of Ghaedi et al., 2016 who reported higher prevalence of vitamin D3 deficiency (33.3%) in women with RM. Moreover, in the present study reproductive tract infections were found associated with RM and affected 3.6% patients. In our study we reported stress as one of the factor responsible for RM. Our data is support by Qu et al. (2020) who reported higher prevalence of depression and anxiety in RM women particularly during early stage of pregnancy. Another study also reported higher level of depression and anxiety in RM women compared to women with no history of miscarriage (Tavoli et al. 2018). RM remained idiopathic in a large section of women giving rise to a challenging situation that augments emotional and physical morbidity in affected couples as well as clinicians as a result of the therapeutic dilemma since the information about reasons for RM and its accurate management is deficient. Nevertheless, the probability of successful pregnancy among couples with idiopathic RM in future might be 50–70% usually depending on the maternal age along with the number of earlier pregnancy failures (Lund et al., 2012, Kling et al., 2016). Such patients should be supported psychologically and reassured of the possibility of successful pregnancy in the future. Clifford et al. (1997) reported the significantly lower rates of miscarriage owing to any cause in women attending a specialized clinic during early pregnancy as compared to non-attendees. The rate of live birth was almost similar in each group. Lund et al. (2012) achieved the live birth rate in 66.7% RM affected women in five years after their management with progesterone, immunoglobulin, heparin, steroid or aspirin. Similarly, in Korean RM women the overall rate of live birth reported was 86.8% irrespective of therapeutic regimens such as intravenous immunoglobulin (IVg), low molecular weight heparin (LMWH), or low dose aspirin (LDA) (Lee et al., 2016). In our study, the overall live birth rate achieved was 75.7% after the management of RM patients with single/combined therapeutic regimens such as Levothyroxine, progesterone, folic acid, hCG, LMWH, LDA, VD3, genetic counselling, psychotherapies. Combined therapy was preferred since many cases had multiple defects. The rate of live birth compared between groups (i.e., primary (73.6%) vs. secondary (81.6%) and two miscarriages (73.9%) vs. three or more (79.7) was almost similar. However, secondary RM cases seem to have better pregnancy outcome index that needs to be evaluated further with larger studies.

8. Conclusion

In conclusion, the major factor of RM was endocrionopathy. However, the association of VD3 deficiency, psychological disorders, obesity, protein-S deficiency and increased heterochromatin region in long arm of maternal chromosome 9 with RM was

reported for the first in this population. In our study 40% patients represent a heterogeneous group experiencing idiopathic RM. The overall live birth rate achieved was 75.7% with highest pregnancy outcome in secondary RM patients after the management. The reproductive outcome in women with idiopathic RM may be very much improved via effective and productive psychiatric therapy, antenatal counseling, psychosomatic support, tender care love and reassurance of live births in subsequent pregnancies. Furthermore, exhaustive well structured researches are necessitated in etiology, reproductive immunology and medicine for the management of this disorder.

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Author contribution

SA collected patient details, samples, performed experiments and wrote the paper and developed tables. SM provided laboratory facilities and helped in experimentation. MNA, HAE, FAA helped in writing the manuscript and prepared figures. ST provided blood samples and helped in performing laboratory tests and SA also performed statistical analyses of the data. MNA, HAE, FAA helped in project administration and funding acquisition. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

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