Introduction:

Pregnancy loss is a distressing condition for both the patient and Obstetrician. It can occur at any gestational period but most commonly during early pregnancy. The etiology for early pregnancy loss and late pregnancy loss are most often different. Early pregnancy loss is defined as a non-viable intrauterine pregnancy with either an empty gestational sac or a gestational sac containing an embryo or fetus without cardiac activity within the first 12+6/7 weeks of gestation. In the first trimester the terms miscarriage, spontaneous abortion and early pregnancy loss are used interchangeably as there is no consensus on terminology in the literature [1].

Early pregnancy loss occurs in 10% of all clinically recognized pregnancies and approximately 80% of all cases of pregnancy losses occur within the first trimester [2]. Pregnancy loss when occurs repeatedly is termed recurrent pregnancy loss (RPL). According to European society of Human reproduction & embryology (ESHRE), recurrent pregnancy loss is a distinct disorder defined by two or more failed clinical pregnancies [3]. Guidelines recommend evaluation only for RPL as a wide variety of etiological factors have been described in the literature and evaluation of RPL revealed causes only in 50% [4]. But there are no recommendations for initiation of investigations after first or single pregnancy loss.

Whenever a woman suffers pregnancy loss an explanation is sought for the same from the treating Obstetrician. Sometimes women approach the clinicians after having suffered pregnancy loss and request for investigations, but the clinical practice recommendations are in place to investigate after two or more pregnancy losses and not for single pregnancy loss.

A significant proportion of women (20%) who experience a miscarriage become symptomatic for depression and anxiety [5]. This warrants diagnostic work-up and interventions. There are no studies with regard to initiation of investigations after first early pregnancy loss. In this context, this study aims to find out the etiological factors in women with first early pregnancy loss and to compare it with women who had two or more than two early pregnancy losses (RPL). This study will establish the need, if any, to investigate a woman after one pregnancy loss for possible etiological factors. This will also find out the common causes of early pregnancy loss in this population and ensure adequate timely intervention for treatable causes without waiting for the subsequent pregnancy loss.

MATERIALS AND METHODS

1. Study design and settings

This cross sectional analytical study was done in the Department of Obstetrics and Gynaecology, Jawaharlal Institute of Postgraduate medical education and research (JIPMER), Puducherry, South India, between January 2018 and August 2019. Processing of various samples was done in the department of biochemistry (thyroid function test), clinical immunology (antiphospholipid antibodies), pathology (protein C and protein S). Two groups of women with 105 subjects in each group were enrolled. (Group A- First early pregnancy loss; Group B- Recurrent pregnancy loss).

2. **Participants**

Inclusion criteria-

Group A- Pregnant Women admitted with first early pregnancy loss (Gestational age ≤14 weeks) or Non- pregnant Women attending OPD with history of one early Pregnancy loss and requesting investigations for pregnancy loss.

Group B- Women with two or more than two early pregnancy losses (RPL)

Exclusion criteria-

Age <18 years and > 35 years, prior live birth, known cases of Hypertension, Diabetes mellitus, Hypothyroidism and auto immune disorders

3. Sample size calculation

The sample size was calculated using OpenEpi software version 3.0 using 95% confidence level and power of 80%. As there were no prior studies, we assumed that the difference in proportion of identifiable causes in two groups that is women with two or more than two pregnancy losses (RPL) and women with first early pregnancy loss to be 20%. The proportion of identifiable causes which is 50% among women in RPL [4] the proportion of identifiable causes in group A is 30% the, sample size is 95 in each group and with 10% dropouts the final sample size is as follows. Group A-105 ; Group B-105. Sampling technique- Purposive Sampling technique**.**

4. Study procedure:

Women fulfilling the inclusion criteria were explained about the protocol of the study and a written informed consent was taken from each participant enrolled in the study. Group A-Women with first early pregnancy loss. Group B-Women with two or more than two pregnancy losses (RPL) . Demographic data including age, occupation, education, socio-economic status was collected by interviewing the patient. Clinical profile including gravidity, parity, past obstetric history, family history, treatment history was documented on a proforma after interviewing the patient and from the medical records. A general physical examination was carried out and height, weight, measured.BMI was calculated. A complete systemic examination including thyroid, breast, Respiratory, Cardivascular , abdominal and Gynaecological examination was performed. Parameters noted in this study were age, BMI, socioeconomic status, number of pregnancy losses, clinical assessment to find out the cause of pregnancy loss, thyroid function test, 75g oral glucose tolerance test, urine culture sensitivity, cervical swab culture sensitivity, ultrasonogram to assess uterine anomalies, fetus assessment and PCOS. If no cause was found, thrombophilia profile for acquired thrombophilias and congenital thrombophilias (lupus anticoagulant, beta 2 glycoprotein antibody IgM and IgG, anti cardiolipin antibody IgM and IgG, protein C and protein S) was done. Investigation for protein C and protein S deficiency were done 6 weeks after pregnancy loss to avoid false negatives during pregnancy.

Primary outcome measures were Proportion of women with Identifiable causes for first early pregnancy loss and recurrent loss.

Secondary outcome measures were proportion of women with various etiological factors.

5. Statistical Analysis:

Data was collected and entered into statistical software SPSS version 15 Continuous variables like height, weight ,age, BMI, hormonal levels were expressed as mean (standard deviation) or median (Interquartile range) as per distribution of data and compared across two groups using unpaired T-test (normal/parametric distribution) or Mann whitney test (nonparametric distribution). Catagorical variables (outcome) like proportion of women with endocrine causes and other non-endocrine causes were described as frequency and proportions and compared between groups by chi square test.p value <0.05 was considered as significant.

RESULTS:

One hundred and five patients were recruited in Group A (pregnant women admitted with first early pregnancy loss or non pregnant women attending OPD with history of one pregnancy loss) and one hundred and five patients were

recruited in Group B with recurrent pregnancy loss. Four women in Group A (first pregnancy loss) and two women in Group B (RPL) were in non pregnant state, rest of the women were recruited immediately after pregnancy loss as inpatients.

The demographic profile of subjects is shown in table 1. The mean of age women with first early pregnancy loss (group A) was 25 ± 4.2 years and mean BMI was 22 kg/m^2. Seventy three percent of women with first pregnancy loss had normal weight, 21% were pre-obese and only one woman was obese (class I). Majority of patients belonged to class III and IV kuppuswamy socioeconomic status classification (34% and 67% respectively). The mean gestational age at pregnancy loss in group A was 10 weeks. There was no statistically significant difference of age, BMI, socioeconomic status and gestational age at pregnancy loss between women in both the groups.

Table 2 shows the comparison of causes of first pregnancy loss with that of RPL. The proportion of known causes in group A women with single pregnancy loss was 58% as compared to 43% in group B(women with RPL) and the *difference was statistically significant.* Endocrine causes were the commonest in both the groups and the proportion of endocrine causes *in first pregnancy loss (36%) was significantly more* than RPL group (21%) with p=0.023. Combined etiology was the second commonest (group A 15.23% vs group B 19% ; p=0.46). The percentage of anatomical, infectious and combined causes were similar between both the groups.

Thrombophilia evaluation was done for unknown causes (103) in both the groups. (Group A-44; Group B 59). Eighteen percent of women in each group were positive for thrombophilia with p value of 0.47. Thus, the proportion of thrombophilia positive women in both the groups were similar (Table 3) . Of the acquired thrombophilias more than 50% were APLA positive in both the groups.

When Thrombophilia evaluation was considered to be a known cause for pregnancy loss, in women with first pregnancy loss the proportion of known cause increased from 58% to 65% and 43% to 54% in RPL group. The proportion of identifiable causes in both the groups were similar after addition of thrombophilia evaluation (group A 65% vs group B 54% ; p=0.09) (Table 4a).

APLA was done for all the women with unknown causes (44 in group A and 59 in group B).Beta 2 glycoprotein was positive only in one woman in group B and Anti cardiolipin antibodies were positive in 3 women in group A and 4 women in group B .Lupus anticoagulant was positive in 3 women in group A and 6 women in group B. Congenital thrombophilia screening being costly and because of limited funds it was performed for 23 women with first pregnancy

loss and 27 women with RPL. Protein S deficiency was present in 4 women in group A and 5 in group B. (Table 4b). There was no significant difference between both groups.

Discussion:

The present study was a descriptive analytical study to know the etiology of first early pregnancy loss and to compare the proportion of identifiable causes between first early pregnancy loss and recurrent pregnancy loss. The study included 105 women in group A (first early pregnancy loss) and another 105 in group B (RPL). We found that the proportion of identifiable causes in first early pregnancy loss was similar to that of RPL (p=0.09). In 65% (n=69) women in group A and 54% (n=57) women in group B, various etiological factors were identified.

There are no studies in the literature that evaluated causes first early pregnancy loss. In the present study, about 40% of pregnancy loss both in first pregnancy loss and RPL group was found to be among the age group of 21-25 years . Previous study by Nybo Anderson et al showed that as the age increased, the percentage of RPL increased [6]. We did not find a similar trend in the present study. The incidence of RPL in their study in the age group of 40-44 years was 51% as compared to 11% in 21-25 years. We did not recruit women >35 years in our study because pregnancy loss occurs more commonly in this group and the number of pregnant women would be less for analysis. The most common age group of antenatal women in our population is 21-25 years, that might be the reason we found the maximum incidence of RPL in this age group.

Bhandari et al in their study on obese women with RPL found that majority of women (48.6%) had normal weight, 31 % were pre-obese and 19% were obese [7]. Matjila et al in their study on medical conditions in RPL found in their study that majority of the women were obese (42%) [8].

MB Cavalcante et al performed a meta- analysis on obesity and recurrent miscarriage and reported forty seven percent women with RPL in normal weight category, while 29% pre-obese and 22% were class I obese [9] . In our study also, similar to Bhandari et al [7] and meta-analysis by Cavalcante et al [9] , majority of women had normal weight (71%) and 21% women were pre-obese, which was comparable to previous studies, but only 3.8% women were obese, which was less as compared to previous studies . The difference in the findings may be due to different population charecteristics. Bhandari et al performed their study in UK and Matjila et al on south African women. The incidence of obesity as such is in India is less as compared to west.

Based on previous studies, endocrine causes were the commonest among known causes of RPL. DM was found in 26%

women [10], hypothyroidism in 9-12% [10,11] and PCOS in 7.8% women with RPL [12]. In the present study also, we found that endocrine causes (21.9%) were commonest among RPL women which was comparable to previous study [13]. DM, hypothyroidism and PCOS comprised 16.2%, 4.8% and 0.95% respectively in women with RPL in our study. The prevalence of hypothyroidism and diabetes was found to be higher in previous studies than the present study. The incidence of PCOS in RPL women was found to 0.95% in our study. PCOS in RPL varied widely between 4.8-80% as described in literature, so more studies are required to come to a consensus [14].

Salim R et al found uterine anomalies in 5% of women with RPL, whereas in our study it was only 0.95% [15]. Infections as an etiological factor was found to be less (0.95%), which was comparable to previous studies in the literature [16]. In the present study, 19% women had combined etiology and only one study in the literature by Lee GS et al has reported combined etiology (48%) contributing for RPL, but the authors did not clarify causes included in the combined etiology [11].

Similar to the previous studies, in 56% women with RPL, the cause of RPL was unknown [4] .

There are no studies to find out the etiology of first early pregnancy loss. The various etiological factors found in present study for first early pregnancy loss are shown in figure 1. Endocrine causes were significantly higher in first pregnancy loss than RPL. The proportion of other causes were similar to recurrent pregnancy loss. We found that proportion of identifiable causes in first early pregnancy loss were more than that of RPL which was an unanticipated finding as there are no studies or recommendations for evaluation of first pregnancy loss in the literature so far.

Previous study by Vora S et al showed that in women with unknown causes of RPL, 75% were thrombophilia positive. Forty six percent were positive for acquired thrombophilia and 37% were positive for congenital thrombophilia. They screened for lupus anticoagulant, anticardiolipin antibodies, ß2 glycoprotein 1 antibody, annexin V, protein C, protein S, antithrombin III, factor V leiden, PT gene G20210A, MTHFR C677T, EPCR 23 bp insertion and PAI 4G/3G polymorphisms [17]. Previous study by Patil R et al in women with unexplained RPL showed that 40% of RPL women were positive for thrombophilias [18]. In the present study we found that 18% of RPL women were positive for thrombophilias . We could investigate only 103 women of unknown RPL and first pregnancy loss , where as Vora et al tested 381 women only with RPL. To come to conclusion regarding the necessity of testing for thrombophilias number needed to test would be 281 with 95% CI when 24% positivity of Thrombophilas as reported by Patil R et al in 2015

[18].

Acquired thrombophilia constituted 10% and congenital thrombophiia constituted 6.7%, while one women had both congenital and acquired thrombophilia . The difference in the results might be because we screened only for APLA, protein C and protein S as compared to previous studies which screened for more causes of congenital thrombophilias thus explaining the incidence of thrombophilia being less in the present study. The proportion of women with first pregnancy loss positive for thrombophilia were comparable to women with RPL. There are no previous studies in literature for thrombophilia evaluation after one miscarriage. As per ESHRE guidelines [3], screening for thrombophilia in RPL *can be considered*, while RCOG [19] and ASRM [20] *recommend* screening for thrombophilia in RPL women.

Conclusions

Significant proportion of women (65%) with first early pregnancy loss had various etiological factors and endocrine factors were the most common cause. Among the identifiable causes for first early pregnancy loss anatomical factors were found in 4.76%, endocrine in 36%, thrombophilia in 18% and combined etiology in 15%.

Statistically significantly more women with first pregnancy loss were found to have known etiological factors when compared to women with recurrent pregnancy loss and endocrine causes were the most common. The thrombophilia positivity was found to be similar in both the groups.

Evaluation should be undertaken for women with first early pregnancy loss so that further pregnancy loss can be prevented to achieve optimum pregnancy outcome. Thrombophilia screening may be undertaken for women when the endocrine causes and anatomical causes are normal.

Limitations of the study

Thrombophilia evaluation was done only in women with unknown causes in both the groups. Congenital thrombophilia screening could not be done for all women with unknown causes because of high cost and limited funds.

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