Stress in Women with Reproductive Failure

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Aims: The study aims to measure the level of stress in women with recurrent miscarriage and infertile women undergoing in-vitro fertilization treatment using psychological, endocrinological and immunological markers of stress.

Methods: Group A (Recurrent Miscarriage Group, RM Group) of 20 women who had had at least three or more consecutive spontaneous abortions after negative biomedical diagnostic screening test; Group B (Infertility Group) of 40 infertile women undergoing in-vitro fertilization treatment after down-regulation with Gonadotropin-releasing Hormone (GnRH) agonist in a long protocol and Group C (Control Group) of 15 fertile women were asked to complete the stress questionnaires : the Perceived stress scale (PSS), the Fertility Problem Inventory (FPI), the Positive and Negative Scale (PANAS) and the SF-12 Health Survey. Blood samples were collected to measure natural killer (NK) cells and cortisol levels.

Results: The score of stress questionnaires were significantly high in women with reproductive failure compared to fertile control group (p=0.023 for PSS; p=0.011 for FPI; p=0.013 for negative scale of PANAS) and the score of MCS from SF-12 Health Survey was significantly lower compared to control group (p=0.001). However, no significant differences were seen between women with RM and infertile women undergoing IVF treatment. Women with reproductive failure have increased number of peripheral NK cells compared to controls (p=0.000). No significant difference was found in the serum cortisol level between the women with reproductive failure and fertile women.

Conclusions: Women with reproductive failure perceived higher levels of psychological stress. Level of stress in women with recurrent miscarriage is comparable to that of infertile women undergoing in-vitro fertilization treatment. Increased numbers of NK cells were found in the peripheral blood of women with reproductive failure.

Keywords: infertility; recurrent miscarriage; stress.

INTRODUCTION

Reproductive failure is a broad term that encompasses the inability to conceive or the inability to maintain a pregnancy. Therefore, reproductive failure includes both infertility and pregnancy loss, the latter being defined as either a single loss at any gestational age, or recurrent miscarriage.

Stress is defined as an interaction between people and the environment.[1] Three "Super-systems"-the endocrine, immune, and nervous systems engage in multiple interactions during the body`s response to stress. The concept of a stress trigger for reproductive failure in human is controversial and a matter of intense discussion and research effort. Several studies used questionnaire score to define stress perception and found positive association of higher stress score and reproductive failure[2-4] whereas some studies found no association.[5,6] Till date there is

no consensus on the most appropriate methods for measuring stress in women with reproductive failure. Furthermore, the efficacy of stress measurement based on participants self –report versus stress biomarkers is still questionable.

The aim of this study was to investigate the self-reported and biochemical levels of stress in women with unexplained recurrent miscarriage and infertile women undergoing in-vitro fertilization treatment. We hypothesized that women with recurrent miscarriage and infertility seeking treatment are experiencing high level of stress in comparison to fertile control group. Our study will answer whether there is any significant difference of stress level between women with recurrent miscarriage and infertility.

METHODS

This was a cross-sectional comparative study conducted at the Recurrent Miscarriage Clinic and Reproductive Medicine Centre in First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China. A total of 40 infertile women prior to in-vitro fertilization/ intra-cytoplasmic sperm injection (IVF/ICSI) cycle, 20 women with RM and 15 fertile control subjects who met the following criteria and

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consented to the study were successfully recruited from October 2009 to May 2010: age between 25-40yrs, regular menstrual cycles of 25-35 days, not taking any hormonal methods of contraception, absence of anti-nuclear factors or anti-phospholipid antibodies, without endometriosis and PCOS.

Group A: Consecutive recurrent spontaneous miscarriages: Last abortion at least 6 months before the study; no previous live birth; and with history of at least three or more consecutive miscarriages with normal biomedical screening tests.

Screening tests include: karyotype of both parents, ovarian function test (LH, FSH, E2, P, PRL,T), thyroid function test, endometrial biopsy and midluteal progesterone, blood group and antibody screen including anti-nuclear, mitochondrial, smooth muscle antibodies, anti-thyroid antibodies, anti phospholipid antibodies (IgM and IgG anticardiolipin antibodies, beta-2 glycoprotein), protein C activity, protein S activity, anti-thrombin III activity, activated protein C resistance, factor VIII, fibrin degradation product, plasminogen activator inhibitor, pelvic ultrasonogram, full blood count, infection-antibody screen (TORCH), and sperm investigation of partner (morphology and DNA fragmentation) thereby excluding genetic , endocrinologic, thrombophilic, uterine, autoimmune cause, infectious cause, or male factors.

Group B : Infertile women: With tubal and/or male factor infertility undergoing IVF/ICSI treatment cycle on day 3-5 of menstrual cycle, i.e. on the day of ovarian stimulation, after down-regulation with GnRH agonist (Triptorelin 1.0 mg s.c) on the mid-luteal phase (D20) of previous month in a long protocol; and no previous live birth.

Group C: Fertile control: With no history of consecutive miscarriage and have at least one live birth.

Assessment

Psychological assessment of stress:

It was taken with the 4 well-validated questionnaires. It took around 20-30 minutes to complete the questionnaires.

Stress-specific Questionnaire

• **The Fertility Problem Inventory (FPI)**[7] focuses upon the stress of infertility. It has 46 items and contains five scales measuring Social concern, Sexual concern, Relationship concern, Need for parenthood, and Rejection of childfree lifestyle. The instrument is scored using a 6-point Likert scale and a total measure of Global stress can also be calculated by summing the five scales. The overall score ranges from 46 to 276, where the higher the score, the higher the fertility-related stress.

• **The Perceived stress scale (PSS)**[8] is a measure of the degree to which situations in one's life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The 10-items version was used in this study. These questionnaires assess the respondent how often certain experiences of stress occurred in the last month, on a five-point scale (0=never, 1=almost never, 2=sometimes, 3=fairly often, and 4=very often). Scores range from 0 to 40. Higher scores on the PSS indicate higher levels of perceived stress.

• **Generic Quality of Life**[9] to measure general health status, the Short Form-12 (SF-12) has been chosen. It contains 12 items that measures physical and mental components of health (psychological distress and psychological well-being), and provides psychometrically-based physical component summary (PCS) and mental component summary (MCS) scores. The SF-12 is scored so that a high score indicates better physical functioning.

• **Mood**: The Positive and Negative Affect Schedule (PANAS)[10] consists of 10 positive affects (interested, excited, strong, enthusiastic, proud, alert, inspired, determined, attentive, and active) and 10 negative affects (distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid). Participants were asked to rate items on a scale from 1 to 5, based on the strength of emotion where 1 = "very slightly or not at all," and 5 = "extremely" during the past week.

Biochemical assessment of stress markers: After the women completed the questionnaires on psychological factors, an intravenous cannula was inserted, and blood was collected immediately after cannulation in the morning between 8:00-10:00am at random phases of menstrual cycle for RM and Control group and on day 3-5 of menstrual cycle for Infertile group.

Flow-Cytometric analysis of the Peripheral Blood Lymphocytes

Heparinized peripheral blood was obtained from the women under study and analyzed within 8 h of collection. Aliquots of 1ml of whole blood was added 5ml of erythrocytes lysing solution (OptiLyse® C, Immunotech, Beckman Coulter) and washed once with 0.9% normal saline solution. The cell preparation was incubated for 30 min at room temperature in the dark with the following mouse anti-human antibodies: PE Mouse Anti-Human CD56, FITC Mouse Anti-Human CD16 (BD) and CD3-ECD Conjugated Antibody (Beckman Coulter). Fluorescence-activated cell sorter (FACScan, Beckman Coulter, USA) analysis was used to assess the number of NK cells in the blood. Natural-killer cells were identified as CD3-CD56+CD16+, CD3-CD56+CD16-, CD3-CD56+.

Serum total Cortisol assay

Venous blood for serum cortisol was collected in serum separator tube (BD Vacutainer® SST™II), and measured by ARCHITECT Cortisol Chemiluminescent micro particle Immunoassay (*CMIA*) on the Architect i2000 System (Abbott, IL, USA).

Statistical Analysis

Analysis of variance (ANOVA) was performed using SPSS Windows version 13 (SPSS Inc.USA). The results were reported to be statistically significant if the P value was <0.05.

RESULTS

The mean±SD age between women with RM, Infertile women, and fertile control group did not differ significantly (32.65±3.58 years vs. 32.07±3.8 years vs. 32.91 ±3.96 respectively).The mean±SD number of spontaneous abortion in RM group was 4.05±1.5 , and the gestational age of miscarriage was between 5 and 12 weeks. The mean duration of infertility in infertile group was 4.9±2.7 and the mean±SD of parity for fertile control group was 1.13±0.3 (Table 1).

Table 1. Patients' characteristics

	RM (n=20)	Infertile (n=40)	Control (n=15)
Age (years)	32.65±3.58	32.07±3.8	32.91 ±3.96
Parity	0±0	0±0	1.13±0.3
No. of miscarriages	4.05±1.5	0±0	0±0
Years of Infertility	0±0	4.9±2.7	0±0

Values are Mean ±Std. Deviation.

The analysis results of psychological parameters are presented in Table 2. Significant differences were found between study and control groups on the level of psychological stress scores. The Perceived stress scale (PSS) score, FPI (Global stress) score, Negative affect score from PANAS, were all significantly higher in study groups than in control group (p=0.023, p=0.011, p=0.013 respectively). The Mental component summary (MCS) score from the Short Form-12 (SF-12) Health survey was significantly lower in the study group compared to control group indicating poor mental health or psychological distress in these population (p=0.001). However, no statistically significant differences were found between the women with RM and infertile women undergoing IVF/ICSI cycle in all the psychological scores.

Table 2. Questionnaire scores between study group and control group.

	RM (n=20)	Infertile (n=40)	Control (n=15)	F	p-value
PSS	18.8±6.0*	16.7±4.8*	13.8±4.4	3.98	0.023
FPI	165.0±18.8*	156.1±18.2*	145.1±20.6	4.77	0.011
PANAS(+)	26.1±8.6	25.2±5.2	27.4±8.8	0.52	0.596
PANAS(-)	23.5±8.8*	20.5±6.1*	16.6±4.1	4.60	0.013
PCS	47.9±7.8	48.0±7.1	50.8±7.2	0.85	0.430
MCS	40.5±10.7*	44.7±8.14*	52.1±4.2	8.38	0.001

Note: Values are Mean ±Std. Deviation.
*p<0.05, RM or Infertile subgroup vs. control group.
**p<0.05, RM subgroup vs. Infertile subgroup.

Our study showed the NK cells to be significantly high in women with reproductive failure compared to fertile control (p=0.000). However, there was no significant difference of serum cortisol level between study groups and control group. The mean ±SD of peripheral NK cell (CD3-CD56+) and serum total cortisol level between the groups are shown in Table 3 and Table 4 respectively.

Table 3. Levels of peripheral NK cells.

NK cells	RM (n=20	Infertile (n=40)	Control (n=15)	F	p-value
	22.03± 11.62* **	47.29± 8.42* **	10.41± 5.80	10.858	0.000

Note: Values are Mean ±Std. Deviation
*p<0.05, RM or Infertile subgroup vs. control group.
**p<0.05, Infertile subgroups vs RM subgroup

Table 4. Levels of serum cortisol.

	RM (n=20)	Infertile (n=40)	Control (n=15)	F	p-value
Serum Cortisol	10.50± 2.70	12.53± 4.0	11.25± 4.37	1.463	0.238

Note: Values are Mean ±Std. Deviation
*p<0.05, RM or Infertile subgroup vs. control group.
**p<0.05, Infertile subgroups vs RM subgroup

DISCUSSION

The current study showed there to be a higher perception of psychological stress among women with reproductive failure. Our data are in agreement with those of previous studies in which women undergoing IVF treatment often experienced high levels of psychological stress, anxiety and depression.[4,11,12] However, our study is in contrast with other studies where the authors reported that women entering an IVF-program are, in general psychologically well adjusted.[13, 14]

In relation to RM, our study results are comparable to previous studies showing positive link of stress and miscarriage.[2, 3, 15] However, not all studies have found such an association.[5] This discrepancy may be due to differences in population characteristics, differences in sample size and differences in the assessment of psychosocial factors. We used various standardized, validated psychological instruments to measure stress; PSS to measure the degree to which situations in one's life are appraised as stressful; FPI to measure infertility-related stress; PANAS to measure the mood state and SF-12 Health survey to measure physical as well as mental health (psychological distress and psychological well-being).

Surprisingly, none of the studies have compared the stress perception between women with recurrent miscarriage and infertility. We found the mean scores of questionnaires: PSS, FPI, negative affect of PANAS to be higher in recurrent miscarriage group than in infertile women undergoing IVF treatment, although the difference was not significant. This could be because the women participating in our

study were recruited from a special RM clinic, where a thorough biomedical diagnostic screening program is offered. Therefore, our patient group was a selected population with no known cause for recurrent miscarriage and having a strong desire for children.

Our study showed the NK cells to be significantly high in women with RM compared to fertile control women without a history of RM. This is similar to previous studies.[16,17] The study by King et al also shows the NK percentage, as the only significantly higher variable in the RM screening test negative subgroup.[18] They conclude that NK cells as a percentage of lymphocytes best discriminated RM and control population. However, Emmer et al failed to detect a significant difference in peripheral NK percentage between women with RM and controls, possibly due to the broader analysis of including women with two or more miscarriages (rather than more than three in this study).[19]

In regards to infertile women our result is similar to previous study showing increased NK cell number and activity in infertile women.[20, 21] However, the highest level of NK cell numbers in our infertile women under the standard long protocol of IVF treatment, after GnRHa down-regulation might not only be explained by stress but also could be the result of direct effect of GnRH agonist or a consequence resulting from the depression of estradiol by GnRH agonist. Estrogen has been found to decrease NK activity in mice that were treated with 17b-estradiol.[22] Furthermore, investigators have found evidence of a negative correlation between estrogen levels and NK function. Gabrilovic et al demonstrated a decline in NK activity with increasing estrogen levels that were obtained from pregnant women.[23] However, further studies are needed to draw this conclusion.

In the current study, we did not observe any significant difference of serum cortisol level between women with reproductive failure and control group, though the mean (SD) of cortisol level in the infertile group was relatively higher than RM and control group. A number of studies have shown than stress-sensitive hormones prolactin and cortisol are elevated in infertile women during IVF.[24,25] However, all these studies have compared the cortisol level during different phases of in vitro-fertilization treatment. Similary, Nepomnaschy et al measure urinary cortisol level and found that increased cortisol levels associated with a higher risk of early pregnancy

loss[26] whereas Nelson measured total plasma cortisol level and found no relation to spontaneous abortion.[5] Both of these studies assess the cortisol at the time of reproductive failure. Moreover, cortisol is secreted episodically and there is a circadian rhythm with a morning maximum, declining levels throughout the daytime, a period of low concentration around midnight, and an abrupt rise after the first few hours of sleep, which may account for some of the conflicting data reported.

Furthermore, immunological functions are known to be under the influence of various psychological factors.[27] Psychological stress might influence pregnancy outcome via a shift in the balance of Th1/Th2 cytokines at the feto-maternal interfaces.[28] Interestingly, psychological therapy has been reported to decrease in both psychological distress and NK-cell activity in infertile women, resulting in an increased pregnancy rate.[29] Psychotherapy has also been reported to improve pregnancy outcome in women with recurrent miscarriage possibly via the same mechanism.[30]

More population-based prospective studies are needed to reveal the psycho-neuro-endocrine-immune pathways linking stress and reproductive failure. We evaluate the stress in women with reproductive failure with new approach combining psychological (stress questionnaires), endocrinological cortisol, and immunological (NK cell) aspects simultaneously. The results of the current study provide a foundation and rationale from which future larger scale studies can examine relations among these variables and reproductive outcomes. Considering the fact that women with reproductive failure perceive high level of stress, they may be more likely to benefit from psychological intervention to improve the reproductive outcome. Despite these implications, the results of this study should be considered in light of small sample size.

CONCLUSIONS

Women with reproductive failure perceived higher level of psychological stress. Perceived stress in women with recurrent miscarriage is comparable to that of infertile women. Increased number of NK cells are present in women with recurrent miscarriage and infertile women undergoing GnRHa down regulated IVF/ICSI.

ACKNOWLEDGMENTS

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