Nepalese Medical Journal, (2018) Vol. 1, 17 - 20

Original Article

Acute Stress Response in Women with Recurrent Miscarriage and Infertility

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ABSTRACT

Introduction: Stress has been linked to reproductive failure which includes miscarriage and Infertility. Several studies showed high levels of psychological stress and stress biomarkers in women with reproductive failure. However, very few studies are done to measure the stress response. So the current study sought to assess whether women with reproductive failure have exaggerated acute stress response.

Materials and Methods: This was a cross-sectional comparative study. Blood samples were collected immediately after venipuncture, and again 20 min later from 15 fertile controls, 20 recurrent miscarriage patients, and 40 infertile patients to measure natural killer cells and cortisol levels.

Results: The percentage of peripheral blood NK cells (total CD3-CD56+) and serum total cortisol level did not change significantly across the two samples of immediately after venipuncture, and again 20 min later in the fertile controls ($p=0.358$,and $p=0.890$ respectively). However, there was a significant decline in the second sample in women with Infertility ($p<0.05$ for serum cortisol and $p<0.05$ for NK cell) and with recurrent miscarriage ($p<0.05$ for serum cortisol). There was a decline of NK cell in the 2nd sample in women with recurrent miscarriage though not to significant level ($P>0.05$).

Conclusions: Women with recurrent miscarriage and Infertility may be more vulnerable to acute stressor.

Keywords: Cortisol; Infertility; Miscarriage; NK-cell; Stress

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Submitted: 7th January 2018
Accepted: 19th April 2018
Published: 1st June 2018

Conflict of Interest: None
Sources of Support: None

Citation: Bhandari S. Acute Stress response in women with Recurrent Miscarriage and Infertility. Nep Med J 2018;1:17-20. DOI: 10.3126/nmj.v1i1.20393

INTRODUCTION

When a situation is perceived as stressful, the Hypothalamic-Pituitary- Adrenal axis becomes activated, causing a cascade of hormones to be produced that may negatively impact reproduction. The paraventricular nucleus of the hypothalamus releases corticotropin-releasing factor, which stimulates the pituitary gland to releases adrenocorticotropic hormone that results in the secretion of glucocorticoids from the adrenal cortex. Increase in serum concentration of glucocorticoids in humans and rhesus monkeys evidently suppresses the hypothalamic-pituitary–ovarian axis.[1]

Moreover, natural killer (NK) cells have been described to be up-regulated during stress.[2] Even mild psychological stress and moderate physical activity rapidly recruit large numbers of NK cells into the circulation, an effect which subsides shortly after the stress ceases.[3,4] The increased NK cell number and activity previously observed in recurrent miscarriage patients may results from mobilization of NK cells in response to stress of venipuncture which is more pronounced in patients with primary recurrent miscarriages than secondary recurrent miscarriages.[5] Psychotherapy has been reported to decrease in both psychological distress and NK-cell activity in infertile women, resulting in an increased pregnancy rate.[6] Stress elevation of NK activity and NK cell mobilization support a known pathophysiological link between NK cells and reproductive failure.

Considering the fact that the mobilization of NK cells represents one of the most reliable stress responses, it is surprising that

only a few attempts have been made to further characterize this phenomenon in women with reproductive failure. The majority of research has been done showing the effect of stress in causing miscarriage and Infertility but very few studies are there to characterize the response to stress, whether women with reproductive failure have same response to stress as normal fertile women. The aim of this study was to assess the acute stress response in women with reproductive failure. We hypothesized that venipuncture could be stressful, stress marker (NK cell, Cortisol) would be lower in the second sample drawn 20 minutes later, after patients adjust to the situation.

MATERIALS AND METHODS

A cross-sectional comparative study was conducted after approval from Recurrent Miscarriage Clinic and Reproductive Medicine Centre in First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China from October 2009 to May 2010. Permission was obtained from the institutional review committee. Seventy five women of age between 25-40 years were consented to the study. All the study population was grouped into i) Infertility group, ii) recurrent miscarriage (RM) group and iii) control group. Infertility group: 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 with no previous live birth.

Recurrent Miscarriage group: Recurrent miscarriage women with last abortion at least 6 months prior to the study, with no previous live birth, with history of at least 3 or more consecutive miscarriages with negative biomedical screening test which included: a) karyotype of both parents, b) ovarian function test (LH, FSH, E2, P, PRL,T), c) thyroid function test, d) endometrial biopsy, e) mid-luteal progesterone, f) blood group, g) anti-nuclear antibody, h) anti-mitochondrial antibody, i) anti-smooth muscle antibody, j) anti-thyroid antibodies, k) anti-phospholipid antibodies (IgM and IgG anticardiolipin antibodies, beta-2 glycoprotein), l) protein C activity, m) protein S activity, n) anti-thrombin III activity, o) activated protein C resistance, p) factor VIII, q) fibrin degradation product, r) plasminogen activator inhibitor, s) pelvic ultrasonogram, t) full blood count, u) infection-antibody screen (TORCH), and u) sperm investigation of partner (morphology and DNA fragmentation) thereby excluding genetic, endocrinologic, thrombophilic, uterine, autoimmune cause, infectious cause, or male factors.

Fertile control group: Fertile women as control with no history of consecutive miscarriage and have at least one live birth, without using any hormonal methods of contraception.

Experimental procedure

An intravenous cannula was inserted, and blood was collected immediately after cannulation, and again 20 minutes later between 8:00-10:00am. Venous blood for serum total cortisol was collected in serum separator tube (BD Vacutainer® SST™II), and measured by ARCHITECT Cortisol Chemiluminescent microparticle Immunoassay (CMIA) on the Architect i2000 System (Abbott, IL, USA).

Heparinized peripheral blood was obtained and analysed 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 $CD3^-CD56^+CD16^+$, $CD3^-CD56^+CD16^-$, $CD3^-CD56^+$.

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

A total of 75 females were included in the study, out of which 20 females were in recurrent miscarriage group, 40 were in infertile group and 15 were of control groups. Mean age of the study population was 32.54 years. Table 1 summarizes mean age, parity, number of miscarriages and duration of Infertility in the three groups.

Table 1: Patients' characteristics among study population

	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

The mean serum total cortisol level at first blood withdrawal was 11.42 µg/dl. (Table 2) The mean ±SD of serum total cortisol level and number of peripheral NK cell ($CD3^-CD56^+$) between the groups, in the first (immediately after cannulation) and second blood withdrawal (20 min later) are shown in Table 2 and Table 3 respectively. P value was not significant for serum total cortisol level among RM or Infertile subgroup vs. control group (P>0.05). P value was significant for NK-cells among RM or infertile subgroup Vs. control group (P<0.05).

The serum total cortisol level and the percentage of peripheral blood NK cells did not change significantly across the two samples in the fertile control group (p=0.358, and p=0.890 respectively). However, there was a significant decline in the second sample in infertile group: p=0.007 for serum cortisol and p=0.000 for NK cell. There was a significant decline of serum cortisol in RM group in the second sample (p=0.005) but the decline of NK cell was not significant (p=0.220); Figure 1 and Figure 2.

Table 2: Levels of serum cortisol in the first and second blood withdrawal.

Serum Coritsol	RM (n=20)	Infertile (n=40)	Control (n=15)	F value	P Value
First blood withdrawal	10.50±2.70	12.53±4.0	11.25±4.37	1.463	0.238
Second blood withdrawal	9.29±3.55	11.32±3.44	10.64±3.46	2.116	0.128

Table 3: Levels of peripheral NK cells in the first and second blood withdrawal.

NK cells	RM	Infertile (n=40)	Control (n=15)	F value	P Value
First blood withdrawal	22.03±11.62*	47.29±8.42*	10.41±5.80	10.858	0.00
Second blood withdrawal	20.05±10.54*	42.42±8.89*	10.31±5.17	87.187	0.00

Note: Values are Mean ±SD; *p<0.05 is significant, RM or Infertile subgroup vs. control group.

DISCUSSION

This study showed that percentage of peripheral blood NK cell and serum cortisol level did not change significantly across the two blood withdrawals i.e immediately after cannulation, and 20 minutes later in the fertile control group. However, the NK cell level and the cortisol level decline significantly in second sample when blood is again drawn from the same cannula 20 min later in women with reproductive failure. The declines were statistically significant in infertile women undergoing GnRH down-regulated IVF/ICSI cycle, whose NK cell level was the highest in the first blood withdrawal. But, NK levels were still significantly higher in infertile and RM group as compared to fertile control group in the second blood withdrawal. It could be that these women are exposed to the long-term stressful event as a result of recurrent miscarriage or infertility and its treatment, and such a chronic stress, may underpin the basis for these patients' persistently high NK cells, as seen in our study. This is in contrast to finding from Shakhar et al [5], who found that the level of NK cell in women with RM in the second blood withdrawal declined to values similar to those seen in the control group. The discrepancy may be due to the small sample size (14 primary recurrent miscarriages, 1 missing data). In addition, no any stress markers were used in the study to measure stress level across the two blood withdrawals so as to confirm the stress-induced NK mobilization, we used serum cortisol to measure stress level across two blood withdrawal.

Overall our results suggest that women with reproductive failure are hyper-responsive to acute stress. They perceive venepuncture as stressful, probably because they think blood test as critical for their diagnosis and treatment outcome. The physiological acute stress response in human is primarily characterized by an activation of both the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenocortical (HPA) axis resulting in increased plasma concentrations of catecholamines (epinephrine, norepinephrine) and glucocorticoids (cortisol). NK cells have been found to express highest β-adrenergic receptors[7] and they increase rapidly in response to acute psychologic stressors.[8-10] This response reflects a release of NK cells from various reservoirs such as the margins of the blood vessels into the blood. In our study although, we did not observe high level of cortisol in women with reproductive failure in the first blood withdrawal, but the significant subsequent decline of cortisol together with NK cells in the second blood withdrawal seen only in these population and not in the fertile control group, gives an indirect evidence of enhanced stress response to venepuncture in women with RM and infertile women undergoing IVF treatment.

Our finding is also in agreement with that of a previous study of Facchinetti et al.[11] who concluded that infertile women undergoing IVF-ET treatment have an increased reaction to stress. Infertile women were tested on the day of oocyte pick –up and were submitted to Stroop Color and Word test, a task measuring the ability to cope with a cognitive stressor, involving attentional and sympathoadrenal systems. Systolic (SBP) and diastolic blood pressure, as well as heart rate (HR) were measured at baseline, during the test, and 10 minutes after the end of testing to measure stress response. Although the sample size was small (16 pregnant, 33 not pregnant), every subject reported an increase of cardiovascular parameters and the women becoming pregnant showed a lower response of both SBP and HR than women who failed.

The mobilization of NK cell among women with reproductive failure, especially in the infertile women is probably more than a specific response to the blood test and may be representative

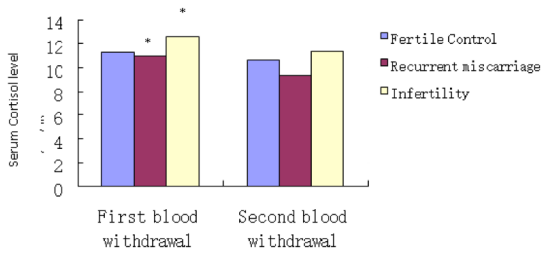

Figure 1: Serum total cortisol (mean±SD) in the first and second blood withdrawal in recurrent miscarriage, infertile and fertile control group. (*) denotes significant differences (p=<0.05): First blood withdrawal vs. Second blood withdrawal.

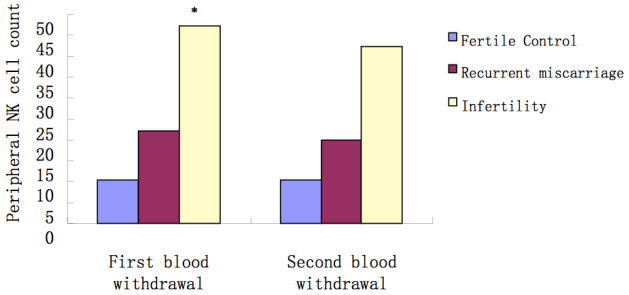

Figure 2: Percentage of NK cells (mean±SD) in the first and second blood withdrawal in recurrent miscarriage, infertile and fertile control group. (*) denotes significant differences (p=<0.05): First blood withdrawal vs. Second blood withdrawal.

of the response of these women to other stressors they encounter in everyday life. It could be that a greater sympathetic response to stressor leads to an enhanced increase in NK cell number and activity, accounting for negative impact on reproduction. Increased numbers of NK cells were found in the peripheral blood of women with reproductive failure[12-16]. However, data on NK cells underlying the influence of stress, as published by various groups, are still contradictory; most results were acquired by flow cytometry using peripheral blood lymphocytes. Decidual NK cells have an unusual phenotype, $CD3^{-}CD16^{-}CD56^{+++}$, distinguishing them from peripheral blood NK cells, which might explain the different observations on stress and NK cells in different experimental settings.

Despite several studies relating stress with reproductive failure, the question still remains as to whether psychological stress is a possible cause or it only represents a consequence of the reproductive failure. Nonetheless the response to stress is variable in different types of people. Interestingly, in murine models there is a genetic determinant in response to stress that causes infertility in A/J strain mice, and abortions in C3H strain mice.[17] These findings suggest that women with reproductive failure might have some specific characteristics that have escaped evaluation to date. Future studies should assess whether women with reproductive failure are more responsive to stressful stimuli, and whether these responses predict pregnancy outcome.

Considering the fact that women with reproductive failure are vulnerable to acute 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

Stress response is variable in different types of people. Women with reproductive failure have exaggerated acute stress response. Greater sympathetic response to stressor may increase the risk of miscarriage and Infertility.

ACKNOWLEDGMENT

I would like to thank my supervisor Professor Wang Qiong, all the nursing staff of Reproductive Medicine Centre, Gynaecology and Obstetrics Department, The First Affiliated Hospital, Sun Yat-sen University and to all the women who participated in this research.

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