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The effect of post-abortion care (PAC) on anxiety in women with spontaneous abortion based on MicroRNA-21 expression, cortisol level, and Fordyce happiness pattern

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ABSTRACT

Abortion is one of the most common complications in pregnancy, and the cause of its occurrence in many cases remains unknown. The high prevalence and consequences of anxiety in women with spontaneous abortion could highlight the importance and role of post-abortion care (PAC). Detection and identification of biomarkers related to abortion and anxiety can effectively diagnose and prevent complications. Among the known biomarkers, microRNAs and the cortisol level have high potential. Therefore, the present study evaluated the effect of post-abortion care (PAC) on anxiety in women with spontaneous abortion based on MicroRNA-21 expression, cortisol level, and Fordyce happiness pattern. In this randomized clinical trial, 72 women with spontaneous abortion were studied and randomly divided into two groups of intervention (n = 36) and control (n = 36). Data were collected through a demographic questionnaire and HADS. To assess PAC, the intervention group was consulted in 8 sessions of 60 minutes in the first 72 hours after abortion. Meetings were held twice a week for four weeks. Both groups were followed up immediately after and one month after the intervention. To evaluate biological factors, 4ml of blood sample was obtained from the subjects. Blood cortisol levels were measured by the Cortisol Competitive Human ELISA Kit (Thermo-Fisher, USA), and microRNA-21 evaluation was performed by Real-time PCR technique. Data were analyzed using SPSS16 software. Results showed that before the intervention, there was no significant difference in the mean score of anxiety between the control and intervention groups (P> 0.05); But at the time immediately and one month after the intervention, there was a significant difference in the mean score of anxiety (p <0.001). The results of biological factors evaluation showed that in the intervention group, serum cortisol levels and microRNA-21 expression decreased significantly (p <0.05). In general, PAC based on the happiness pattern can control the anxiety of women with spontaneous abortion. Therefore, it is recommended as an effective and non-invasive intervention in preventing women's psychological problems after spontaneous abortion.

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Introduction

The National Center for Health Statistics, the Centers for Disease Control and Prevention (CDC), and the World Health Organization (WHO) defined abortion as terminating a pregnancy before 20 weeks of giving birth to a fetus weighing less than 500 grams (1). Spontaneous abortion is the most common complication of pregnancy. Studies using human chorionic gonadotropin (HCG) sensitive methods show that the actual pregnancy loss rate after implantation is 31%. Among clinically diagnosed pregnancies, 15% are aborted before the twentieth week of pregnancy (from the last menstrual cycle) (2, 3).

Causes of spontaneous miscarriage include maternal age, history of abortion, smoking, moderate to high alcohol consumption, cocaine use, use of nonsteroidal anti-inflammatory drugs (except acetaminophen) during fertilization, fever, caffeine, very high (BMI> 25), and deficient (BMI <5.18) mother's weight, maternal diseases such as diabetes mellitus, celiac disease, polycystic ovary disease, Cushing's syndrome, thrombophilia, hypothyroidism, chromosomal abnormalities, uterine abnormalities, placental abnormalities, and severe trauma (4-6). The

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birth of a child helps to establish women's identity, and they attribute their social, psychological, and biological success to a large extent depending on their ability to have children. If they lack this power, they feel inadequate (7). Abortion is both a physically and mentally damaging experience (8). Women who have had abortions feel depression, anxiety, marital conflict, suicide attempts, drug abuse, grief, anger, guilt, feelings of emptiness and helplessness, low self-esteem, and sleep disorders (9, 10).

Recent evidence shows that more than half of women suffer from various psychological complications in the weeks and months following an abortion (11). A significant percentage of women offer high anxiety levels up to 6 months after the miscarriage and are at increased risk for post-traumatic stress disorder and obsessive-compulsive disorder (12). Anxiety is more common and more severe than depression during the 12 weeks after an abortion. Anxiety is pervasive, unpleasant, and ambiguous anxiety that is often accompanied by symptoms of the autonomic system such as headache, sweating, palpitations, chest tightness, and slight stomach upset (13). Anxiety harms tissue healing. If left unchecked or prolonged, it may lead to increased protein breakdown, reduced wound healing, increased risk of infection, altered immune response, disturbed electrolyte and fluid balance, and changes in sleep patterns (14, 15).

The risk of mental disorders in those who have had an abortion is 30% higher than in the general population (15). If the first pregnancy led to a miscarriage, anxiety, and depression became more common after the second child's birth, which means the outcome of the tragic experience has not been resolved (16). Disorders in the mother's psychological state may interfere with the process of attachment to the baby. This disorder may manifest itself as child abuse (17). The symptoms of anxiety and depression that follow a miscarriage continue into the subsequent pregnancy. Because many women become pregnant again 18 months after an abortion, the effect of spontaneous abortion on subsequent pregnancies is significant (18). Pregnant women with a history of miscarriage in less than a year are more likely to develop psychiatric symptoms and gestational distress, anxiety, depression, somatization, obsessive-compulsive disorder, interpersonal sensitivity, psychosis, suspicion, and hostility are more among them (17).

Shapiro *et al*. (19) found that women with a history of miscarriage showed higher anxiety levels in the first trimester of their subsequent pregnancies. Recent studies have shown that the primary psychological consequences of abortion are anxiety and post-traumatic stress disorder, and they support the view that abortion is a type of trauma. Since 2000, the United Nations has also included the variables of vitality, hope for the future, and satisfaction of individuals in society as key variables to determine the level of development of countries. In this way, if the people of a community do not feel cheerful, happy, and satisfied, that society cannot be considered developed, showing the importance of happiness and cheerfulness. According to the research on vitality and joy, for 50% of people, the feeling of pleasure is the most critical issue in life. Findings show that happiness is associated with positive outcomes such as physical and mental health and optimal performance. Increasing happiness affects the cognitive component of quality of life and improves the quality of life. Happy people feel more secure, make decisions more accessible, have a more cooperative spirit, and are more satisfied with their lives. Happiness can protect people against stress like a shield, and in addition, it generates energy, passion, and joy and ensures people's health.

Fordyce is one of the experts in the psychology of happiness and is a pioneer in the research and theorizing of joy in the world (20). He has reviewed several studies and developed a program called Happiness Education that includes 14 behavioral principles. In this study, the Fordyce happiness pattern was used to assess the importance of post-abortion care (PAC) (21). Nakano *et al*. (22) reported that PAC is beneficial for patients with post-abortion mental disorders. Gould *et al*. (23) found that training, counseling, and PAC effectively reduced post-abortion psychological complications.

According to the prevalence and importance of women's psychological problems after abortion and the importance of happiness in their lives (16), this study was conducted to investigate the effect of PAC based on the Fordyce happiness pattern on women's anxiety following spontaneous abortion. The result of PAC on anxiety was also assessed through important

biological factors (microRNA-21 expression and cortisol) that are directly related to anxiety (24, 25).

Materials and methods

Studied population

This study is a randomized clinical trial performed in two groups of intervention and control to investigate the effect of post-abortion care (PAC) using the Fordyce happiness pattern on anxiety in women with spontaneous abortion. The statistical population of this study includes the first pregnant women who were admitted to the gynecology and emergency department of the educational and medical center with definite symptoms of spontaneous abortion, who spent the first 24 hours after the abortion and were eligible for the study. According to previous studies (26), and a possible drop in samples, the sample size of 36 people in each group with a statistical power of 80% and a significant level of 0.05 was obtained. The formula for calculating the sample size is:

$$n = \frac{(Z1-\frac{\alpha}{2}+Z1-\beta)^2\ (\delta 1^2 + \delta 2^2)}{d^2}$$

According to the above formula, the number of samples in each group was 36 people. The required sample size was 72 people who were selected from eligible women referred to the hospital using available sampling. Then the samples were randomly assigned to experimental (n = 36) and control (n = 36) groups.

Inclusion criteria were literacy, desire to conceive, first pregnancy, mild upward anxiety (score eight and above), and no stressful events during the last six months. Exclusion criteria included absenteeism from more than one counseling session, adverse events during the study, re-pregnancy, use of sedatives and psychotropic drugs, and addiction to drugs, tobacco, psychotropic drugs, and alcohol.

Post-abortion care (PAC)

After obtaining written consent, a pre-test was performed in both control and intervention groups in the first 24 hours after abortion. The intervention was performed in 8 sessions of 60 minutes by using the Fordyce happiness pattern to reduce maternal anxiety and preventative care, such as providing birth control to prevent future unwanted pregnancies. The first session started within 72 hours after abortion (according to the research psychologist). Then it continued in two sessions per week for a total of four weeks. In both intervention and control groups, questionnaires were completed immediately after the intervention (one month after abortion) and one month after the intervention (two months after abortion). One person in the control group (due to breast cancer) and two in the intervention group (due to unwillingness to participate in counseling sessions) were excluded from the study. In this part of the research, the data collection tool was two questionnaires completed by face-to-face and telephone interviews. The first questionnaire included the Demographic Characteristics and Midwifery History Questionnaire and the second questionnaire included the Hospital Anxiety and Depression Scale (HADS).

Biological factors (MiR-21 expression and cortisol level) evaluations

The 4ml of blood sample was obtained from the subjects under fasting. Blood cortisol levels were measured by the Cortisol Competitive Human ELISA Kit (Thermo-Fisher, USA). For microRNA (MiR-21) evaluation, serum RNA was extracted using mirVana ™ PARIS kit (Ambion, France). The 1.5µl of the RNA solution was evaluated directly on a 1000A nanodrop spectrophotometer (NanoDrop, Wilmington, DE USA) and its absorbance was measured at 260 and 280 nm to evaluate the quality and quantity of extracted RNA. Electrophoresis on 1.2% agarose gel and ethidium bromide was used to determine the quality of isolated RNA. The presence of two bands, rRNA 28S and S rRNA 18, indicated good RNA quality.

Real-Time PCR was performed using special primers (miScript Primer Assay, Qiagen), and miScript SYBR Green PCR Kit (Qiagen). First, 2x QuantiTect Universal, SYBR Green PCR Master Mix 10x miScript Primer, 10x miScript Primer Assay, nuclease-free water, and synthesized cDNA of the template were brought to room temperature and each was mixed well separately. To each well of the Real-Time PCR plate kit, 22.5µl of Master Mix reverses transcription mix and 2.5µl of synthesized template cDNA for each sample were added. The initial PCR activation step was performed for 15 minutes at 95°C, which activates the enzyme HotStarTaq DNA polymerase. The periodic stage includes denaturation

for 15 seconds at 95°C, annealing for 30 seconds at 55°C, elongation for 30 seconds at 72°C. This step was repeated 39 times.

At the end of replication, with the help of the analyzer, the melting curve was performed on the reproduction product to confirm the specificity and identity of the original reproduced product. For this analysis, the plate temperature was slowly increased from 65°C to 95°C, during which the device continuously read the fluorescence signal of the amplified product. The machine then plots a curve or graph of fluorescence intensity versus temperature. By evaluating the specific and exclusive peak of the product under study, namely 21-miRNA, as well as the peak dimer of the primers, we confirmed the specificity of Real-Time PCR amplification (Figure 1).

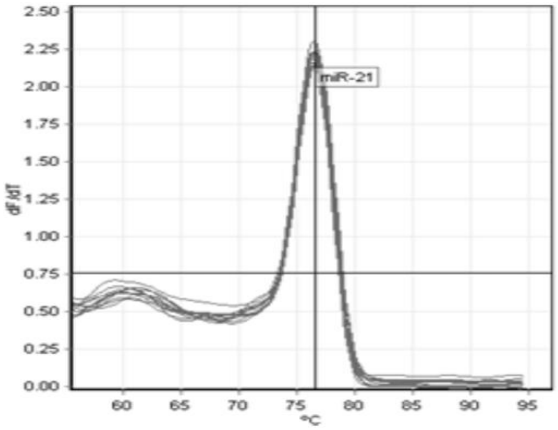

Figure 1. Melting curve diagram for miRNA-21

Statistical analysis

Statistical indicators such as mean, standard deviation and agreement tables were used to evaluate descriptive information. Chi-square, Fisher's exact tests, independent t-test and repeated data analysis were used to evaluate the inferential information. Data analysis was performed using SPSS software version 16 with a significance level of less than 0.05.

Results and discussion

Findings showed that the research units in the two groups of intervention and control were homogeneous regarding demographic characteristics, including age, level of education, employment status and economic status, and obstetric factors, including duration of marriage and estimation of gestational age (Table 1). The mean and standard deviation of age in the control group was 25.65 ± 4.32 years and in the intervention group was 24.83 ± 5.46 years.

The results showed that the mean and standard deviation of anxiety score in the control group before the intervention was 11.48 ± 2.94, immediately after the intervention was 9.25 ± 3.60, and one month after the intervention was 8.31 ± 2.56, while, in the intervention group, anxiety scores were 10.88 ± 2.67, 5.85 ± 2.52, and 5.00 ± 2.37, respectively. The results showed that over time, the level of anxiety in both groups decreased significantly. But this decrease was more significant in the intervention group than in the control group (Table2). The results of biological factors evaluation showed that in the intervention group, serum cortisol levels and microRNA-21 expression decreased significantly ($p < 0.05$) (Table 3). The results showed a significant difference in the mean post-test scores between the control and intervention groups; Post-abortion care (PAC) based on the Fordyce happiness model has reduced anxiety in women with spontaneous abortion in the intervention group (27). Many studies have confirmed the link between abortion and psychological problems such as anxiety and the positive effect of counseling on reducing it (27-29). Romero-Gutierrez *et al*. (30) recommend comprehensive counseling for women with miscarriages. So far, no research has been conducted on the impact of PAC based on the Fordyce happiness pattern on the psychological problems of women with spontaneous abortion. However, the effect of this method on anxiety and other psychological issues of other people in society and the impact of different types of PAC and support on abortion have been studied, which will be mentioned (30). A study by Constant *et al*. (31) was performed on the effect of text messages on women's support during home abortion treatment in South Africa on 469 women. They investigated the effect of SMS follow-up on reducing anxiety and emotional distress in women undergoing Preston abortion treatment at home. Two hundred thirty-five patients in the control group received routine care, and 234 patients in the intervention group received text messages during treatment in addition to routine care. The tools in this study were Hospital Anxiety and Depression Scale (HADS), Alder 12-item scale, and Accident Load Impact Scale (IES).

Table 1. Demographic and midwifery characteristics of the studied individuals

Variable		Control Group (n=36)	Intervention Group (n=36)	P-value
Age	<20 years old	7 (19.4%)	6 (16.7%)	0.68
	21-25 years old	12 (33.3%)	15 (41.7%)	
	26-30 years old	14 (38.9%)	10 (27.8%)	
	>30 years old	3 (8.3%)	5 (13.9%)	
Employment status	Employed	34 (94.4%)	31 (86.1)	0.42
	Housewife	2 (5.6%)	5 (13.9%)	
Economic situation	Good	11 (30.6%)	7 (19.4%)	0.53
	Medium	22 (61.1%)	27 (75%)	
	Weak	3 (8.3%)	2 (5.6%)	
Gestational age*	<12 months	20 (55.6%)	24 (66.7%)	0.46
	12-20 months	16 (44.4%)	12 (33.3%)	

* based on the first day of the last menstrual period

Table 2. Comparison of anxiety score of women with spontaneous abortion in control and intervention groups using repeated data analysis

Group	Before Int*	Immediately after Int*	1 Month after Int*	Intragroup F	Intergroup F
Control	11.48 ± 2.94	9.25 ± 3.60	8.31 ± 2.56	F = 22.410 P < 0.001	
Intervention	10.88 ± 2.67	5.58 ± 2.52	5.00 ± 2.37	F = 117.213 P < 0.001	F = 20.006 P < 0.001
Independent T-test	T = 1.095 df = 70 P = 0.277	T = 4.906 df = 68 P < 0.001	T = 5.567 df = 67 P < 0.001		

* Int=Intervention

Table 3. Comparison of cortisol level and MicroRNA-21 expression in women with spontaneous abortion in control and intervention groups at before, immediately after, and one month after the intervention

Variable	Control Group			Intervention Group		
	Before	Start	After	Before	Start	After
Cortisol Level (nmol/L)	25.31	20.73	18.47	26.16	20.99	10.35*
MicroRNA-21 expression	5.21±0.7	4.12±0.9	3.87±1.1	5.33±0.5	4.62±0.3	1.73±1.2*

*: P<0.05

The results showed that in the intervention group, anxiety decreased (P = 0.013), and its subjects experienced less emotional stress than the control group (P = 0.015). Participants in the intervention group also reported fewer bleeding (P <0.001), fewer pain (P = 0.042), and fewer side effects (P = 0.027). Finally, 99% of the people in the intervention group recommended such a follow-up SMS to their friends in similar circumstances. Nekcevic *et al*. (32) conducted a study entitled "The influence of medical and psychological interventions on women's distress after miscarriage" a prospective study of women who underwent routine screening at 10-14 weeks of gestation. A forgotten abortion was diagnosed. The intervention group consisted of 66 women divided into two groups of 33 (medical counseling group and psychological-therapeutic counseling group) and were evaluated for five weeks. The two groups were compared with a control group of 61 women who did not receive specific counseling. All participants were interviewed before and immediately after the intervention and four months later with a

questionnaire. The instruments used included the HADS and the Texas 17-item Grief Scale. The results showed that in the group of psychological intervention, in comparison with the group without psychological intervention and the control group, a significant decrease in the level of guilt, shame, and anxiety of the research units was gradually observed. In the group without psychological intervention, a reduction in stress and feelings of shame ($P < 0.001$) was observed over time.

Researchers believe that PAC and psychological counseling can help reduce post-abortion stress in women, in addition to research and treatment counseling (33). In both mentioned studies, as in the present study, the instrument for measuring anxiety was the HADS questionnaire. Counseling and support for women with an abortion have been effective in reducing psychological complications. These results were in line with the results of our research.

Evaluations of biological factors also confirmed the obtained results in this study. The results of these factors showed that in the intervention group, serum cortisol levels and microRNA-21 expression decreased significantly ($p <0.05$). Cortisol is the body's most famous glucocorticoid secreted from the cortical part of the adrenal gland. Cortisol is synthesized by the adrenocorticotropic hormone (ACTH), produced by the pituitary gland in response to stress and anxiety (34). Its primary mechanism causes high blood sugar or hyperglycemia. This increase is mediated by stimulation of hepatic gluconeogenesis, with the support of amino acids resulting from protein catabolism, especially at the level of skeletal muscle and fat, at adipose tissue (35). Therefore, lowering the level of this hormone in the blood reduces anxiety in the body. As the results of the present study show, PAC significantly reduced the level of this hormone in the intervention group. However, over time, levels of this hormone also decreased in both groups (34, 35).

Discovering and identifying biomarkers associated with abortion can be an effective aid in diagnosing and preventing complications (36). Among the known biomarkers, microRNAs have high potential (36, 37). Diagnosis based on molecular changes and changes in the expression of microRNAs that are altered in miscarriage can be beneficial in identifying or preventing people who are prone to miscarriage. MicroRNAs are a group of non-coding RNAs that regulate the expression of many genes in the body (36, 38). This setting can be done at the transcription level as well as after the transcription. The expression of this microRNA is highly regulated, and disruption of this order is associated with various diseases. Studies have shown that some of these microRNAs decreased expression during pregnancy and others increased expression, and these changes have a significant effect on the expression of their target genes (39). In addition to its role in abortion, MicroRNA-21 also plays a role in stress and anxiety. Increasing the expression of this microRNA indicates an increase in anxiety in individuals (36, 39). The results of the present study showed that PEC significantly reduced this microRNA in the intervention group.

Pregnancy is the most significant event in women's lives. It promotes mental health caused by the feeling of "motherhood" and raising a child (1). If it leads to abortion, it is both a physically and mentally terrible experience and can cause unpleasant psychological effects in women's lives. Also, a significant percentage of women show high levels of anxiety long after an abortion (30). Therefore, screening for anxiety should be recorded regularly as part of follow-up visits after abortion (15).

On the other hand, happiness can protect people from stress. The Fordyce Happiness Pattern leads to specific changes in people's cognitive and emotional states (40, 41). It helps them take a better view of life events and respond to life situations and situations with more optimism and adaptation (27). Therefore, PAC can reduce anxiety in patients with spontaneous abortion, according to the Fordyce Happiness Program. These findings, along with the results of other studies, show that post-abortion care is effective for women with spontaneous abortion. The findings of this study can be the basis of further research and help develop methods to improve the quality of care.

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