Increase Concentration of Transforming Growth Factor Beta (TGF-β) in Breast Milk of Mothers With Psychological Disorders

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Abstract- Several studies have shown an imbalance between proinflammatory and anti-inflammatory cytokines in depression and anxiety disorders. However, less attention has been paid to the role of cytokines in psychological disorder in mothers who breastfeed. This study looks at whether concentration levels of TGF-β2 are altered in anxious and depressive breastfeeding mothers. This study checked the concentration level of TGF-B2 in relation with psychological symptoms on 110 breastfeeding mothers; based on random sampling method with using of Beck Depression Inventory (BDI), General Health Questionnaire (GHQ) and Spielberger Stress Scale (STAI) in 2015 also TGF-β2 was measured in breast milk using ELISA. We used of Pearson Correlation Method, independent t-test and one-way analysis of variance (ANOVA) to analyze the data. Psychological symptoms (Anxiety and depression) showed positive correlation with TGF-Beta level in which relationships were significant (P=0.01). Psychological problems may be uniquely associated with the level of TGF-β in breast milk. More attention should be paid to the mental health of mothers during breastfeeding, and more research needs to be done in this subject to clarify the relationship between psychological variables with the level of TGF-β in breast milk.

Introduction

Cytokines in breast milk may have an important role in the development of baby's immune system. They also may act as mechanisms that lead to beneficial effects of breastfeeding in protecting children against allergic and infectious diseases (1). Transforming growth factor-b (TGF-β) is a central cytokine in the regulation of the immune system. In particular, evidence suggests that TGF-β may be a key immunoreactive agent to create the mucosal immune response including the production of immunoglobulin A (IgA) and induction of oral tolerance. Interestingly, human's milk contains large amounts of TGF-β (2-3) TGF-β2 is the main isoform in human milk among the Three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) found in mammals (4). The results of the recent systematic studies related to TGF-β concentration in breast milk are very diverse. TGF-β is potentially described with a history of mother's allergic diseases (5) or probiotic supplements (3-6-7).

The relationship between TGF-β levels in breast milk and other maternal factors including behavioral and psychosocial characteristics has not carefully studied yet. For example, psychosocial stress or depression symptoms are effective on secretion of neurotransmitters and neuroendocrine hormones, and they can change immune cells function and cytokines produced by TGF-beta and immune cells (8-9). In addition, other behavioral and psychosocial factors such as smoking, drinking, socioeconomic status, and social support can

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also change secretion of TGF-β (10) and the overall immunological performance (11-12). Empirical and animal evidence shows that the psychosocial problems of mother and behavioral factors impact on children's immunological susceptibility to allergic and infectious diseases.

As Kozyrsky *et al.*, reported in a prospective study conducted in Canada on a large population (n=3907), even according to the normal estimates of the risk of developing asthma, the children who are chronically exposed to maternal trauma are exposed to the risk of developing asthma 25.1 times more than others (13). Currently, potential mechanisms of children's psychosocial stress are focused on Poor Parent-Child Relationships. For example, constantly secretion of corticosteroid due to long-term exposure to maternal stress increases the risk for atopic diseases in infants (9-14). However, due to the high concentration of immune factors in breast milk and the importance of its potential role in enhancing the immune system of the baby, maternal psychosocial factors can change safety components of breast milk especially cytokines that include TGF-B and affect the immune system of the breast-fed infants? This study aims to find the relationship between TGF-B2 concentrations in breast milk and their psychological characteristics.

Materials and Methods

This is a cross-sectional study (descriptive-analytical study) carried out on 110 lactating women (about 14 women exit of study) referred to Akbarabad Health Center of Tehran for vaccination of infants aged 4-6 months at intervals of September to December 2015. The sample size was calculated using correlation Coefficient Estimation Formula between different quantitative psychological indicators and TGF-β, and with considering alpha=0.05, power (1-beta) was achieved equal to 80%, and the minimum accepted correlation coefficient was obtained as r=0.3, and the minimum sample size included 70 subjects. The samples were selected using simple random sampling method from the list of women referred to Health Center for vaccination of children. Inclusion criteria were infants' healthiness or lack of High-Risk situation. The full description of the study and its objectives were presented to mothers, and the mothers announced their satisfaction with signing the consent form by the Ethics Committee of Tehran University of Medical Sciences No 20260.

After announcing a willingness to participate in the

study, a researcher-made questionnaire was given to nursing mothers that included demographic data, history of allergic and asthma diseases, alcohol,drugs and tobacco consumption, sexual satisfaction, access to health resources and in the case of problems with treatment. Moreover, other standard questionnaires such as the Beck Depression Inventory (BDI), General Health Questionnaire (GHQ) and Spielberger questionnaire were completed by the selected samples.

Beck Depression Inventory (BDI) consists of 21 questions made to assess the attitudes and the symptoms of depression. The content of this questionnaire is in general depression symptoms but mostly focuses on cognitive content. Beck Depression Inventory is a Self-Assessment Test and is completed in five to ten minutes. The test materials are formed of 21 materials associated with different symptoms that subjects must answer them according to a four-point scale ranging from 0-3. These materials are in areas such as sadness, pessimism, sense of failure, guilt, sleeping disturbances, loss of appetite, self-loathing and so on. This means that 2 materials to emotions (affections), 11 materials to cognition, 2 materials to overt behaviors, 5 materials to somatic symptoms and 1 material has been allocated to interpersonal semiotics.

Accordingly, this scale determines varying degrees of depression from mild to very severe, and scores range from 0-63. Because of the importance of this instrument in the diagnosis of clinical intervention, many psychometric tests have been conducted on its psychometric properties. With reviewing the studies that had used this tool, Beck *et al.*, found that its reliability coefficient was ranging from 0.48-0.86 in terms of the gap between the number of tests and the type of the tested population. Beck *et al.*, in 1996 obtained test retest reliability coefficient within one week as 0.93. About the validity of the Beck Depression Inventory, several studies have also been conducted. The average correlation of Beck Depression Inventory with the Hamilton Rating Scale for Depression (HRSD), Zung Self-Rating Scale, MMPI Depression Scale, Multiple Emotional Symptoms of Depression and SCL-90 is more than 0.60. Various researches have also been conducted within our country that has tried to measure the psychometric properties of this instrument. Among these researches, Tashakkori and Mahriar's research can be pointed out that obtaining the reliability coefficient as 0.78 in Iran. In other studies, including Partovi's study in 1975, Vahabzadeh in 1973, and Chegini in 2002, Beck's inventory validity has been reported high and varying between 0.70-0.90 (15-16).

General Health Questionnaire (GHQ) includes 28 general questions from 4 subtests, each of which consists of 7 questions. The questions of each subtest are consecutive so that the questions 1 to 7 are related to the physical symptoms subtest, questions 8 to 14 are related to the anxiety and insomnia subtest, questions 15 to 21 are related to the social dysfunction and questions 22 to 28 are related to the depression subtest.

All items of GHQ have 4 options for which there are two types of scoring systems. In one of the traditional scoring methods, the test options are scored as 0-0-1-1, resulting in a score varying from 0 to 28. The second method is the Likert scoring method according to which, each of 4-degree questions in the test is as 0-1-2-3 4. As a result, an individual's total score will vary from 0 to 84. In both scoring methods, lower score is indicating of better mental health. In this study, we used the Likert scale scoring (17).

The cut-off score for the sensitivity and specificity of the test were found to be 7.2±93.7 and 7.84±7.2%, and the overall classification error rate is equal to 2.8±7.2% and the best cut-off score using the Likert's scoring method has achieved a score of 23 for the subjects under study. The sensitivity, specificity, and overall misclassification rate cut is 5.70±4.2%, 3.92±4.2%, and 3.12±4.2%. Investigating concurrent validity of this questionnaire with the checklist of disease symptoms consisting of 90 questions indicated a significant correlation between the scores of subjects in the sub-scales of the two tests. Pearson's correlation coefficient among the scales of the 28-item questionnaire with the total score implies that high and low anxiety level is predictive of the individuals' health or lack of health (18).

Spielberger's questionnaire has 40 questions. Questions 1 to 20 are related to state anxiety (manifest anxiety) with four options (never, sometimes, often, and very high). Questions 21 to 40 include trait anxiety with four choices (almost never, sometimes, most of the time, almost always). High correlations have been reported between the scale of anxiety trait and other tests that measure stress. The correlation of this scale with Taylor's Manifest Anxiety Scale (TMAS) is ranging from 0.79-0.83, and the correlation between anxiety traits with the list the attributes of affection is reported as 052-0.58. Anxiety State-Trait Inventory (ASTI) has a high internal consistency. The median of Alpha coefficients has been reported in various meetings in state scale of 0.92 and the trait scale of 0.90. For the selection of questions that assess a particular factor, the best set is the case that it's internal consistency

Coefficient has been calculated with alpha coefficient so that to be maximum. According to the reliability of questions that make up state and trait anxiety, state anxiety for questions 1-20 is 0.889 and trait anxiety is 0.864 for questions 21-40. Due to the high positive correlation coefficient, none of the 19 test questions were removed (19).

In addition to completing questionnaires, the mothers were asked to pour 2ml of their milk in the test tube, and give it to the laboratory to investigate the TGF-B. TGF-β2 concentration in breast milk was measured using TGF-β2 ELISAKIT kits, and milk samples were stored immediately in the freezer at temperature of -5° C. ELISA method: the collected milk was centrifuged at first, and then its lipid top layer was removed. The remaining water part was filtered using a 0.45-micron filter, and the filtered liquid was held at freezer at -70° C. When conducting the experiment, all samples were determined along with calibrators and the related controllers according to Kit manufacturer's instructions. Besides, the IgA secreted in breast milk was measured immunoturbidometric-ally. Statistical analysis was performed using the SPSS-20 software. Qualitative variables were reported as frequency (percentage), and quantitative variables were reported as mean±SD. To compare the levels of TGF-β between the two groups, the independent group's t test, and among several groups, ANOVA was used. $P<0.05$ was considered as statistically significant.

Results

In this study, 96 mothers (14 mothers exit of study because their sampling of breastfeeding was not enough for the exam) were participating. The features of nursing mothers are presented in Table (1) based on demographic characteristics questionnaire. According to Table (1), 9 infants were born unhealthy according to their mothers' reports. According to Mothers' announcements, 1 (1%) newborn had respiratory problems, 2 (1.2%) newborns had neonatal jaundice, 1 (1%) had neonatal infection, 1 (1%) newborn was premature, 1 (1%) newborn had thyroid problem and 3 (1.3%) newborns had no problem. Among the participants, there were no addict and alcoholic mothers. 1% of mothers had low satisfaction, 15% had the average satisfaction, and 84% had good, very good or excellent satisfaction to their marriage.

Table 1. Demographic characteristics of the nursing mothers under study

Variables	Mean±SD	Variables		Frequency (%)
Mother's Age (Year)	28.06 ± 7.99	Infant's Status at Birth		
Father's Age (Year)	32.71 ± 9.03	Healthy		87 (90.6%)
Child's Age (Month)	6.46 ± 11.1	Unhealthy		9 (9.4 %)
		Types of Allergic Diseases	Air pollution	1 (1%)
			Skin allergy	5 (5.2%)
			Food allergy	1 (1%)
			Olfactory sensitivity	2 (2.1%)
			Seasonal allergy	12 (12.5%)
			Allergic Diseases	28 (29.2%)
Frequency (%)				
Job of mother	Housewife	75 (78.1 %)	Smoking	Mother	1 (1%)
	Employed	21 (21.9 %)			
	Worker	8 (8.3 %)			
Job of Father	Staff	41(42.7 %)		Father	26 (27 %)
	Self-employed	43 (49 %)			
Type of Delivery	Vaginal delivery	21 (21.9 %)	Alcohol (Father)		5 (5.2%)
	Caesarean delivery	75 (78.1 %)	Drug use (Father)		1(1%)

In accordance with the Beck Depression Inventory (BDI), 23% of people were diagnosed with varying degrees of depression, but in accordance with the General Health Questionnaire (GHQ), only 4.9% of mothers were identified with depression disorder. Social dysfunction was the most and depression was the lowest frequent item in this inventory. According to Spielberger's state-trait anxiety inventory, the highest level of anxiety is the average anxiety of about 66% based on the classification of this test and mild and severs anxieties were still the most frequent in this study (Table 2). The amount of TGF-β was averagely about 278.73±311 (minimum and maximum were 102.4 and 2037.4 respectively). Investigating TGF-β correlation with the anxiety level and Beck and GHQ inventories scores showed that TGF-β is significantly correlated with state anxiety (P=0.006, r=0.309), trait anxiety (P=0.016, r=0.271), depression (P=0.0001, r=0.477), and general health (P=0.001, r=0.389).

Table 2. Frequency and percentage of psychological characteristics of nursing mothers under study

Disorder		Frequency (%)	Disorder		Frequency (%)
Depression	no symptoms of depression	74 (77%)	Trait anxiety scale	Mild Anxiety	17 (20.2%)
	Mild depression	16 (16.7%)		Moderate to low Anxiety	29 (34.5%)
	Moderate depression	3 (3.1%)		Upper Moderate Anxiety	27 (32.1%)
	Severe depression	3 (3.1%)		Relatively Severe Anxiety	10 (11.9%)
				Severe Anxiety	1 (1.1 %)
State Anxiety Scale	Mild anxiety	20 (22.7%)	General Health	Physical disorder	31 (32.3%)
	Moderate to low anxiety	29 (33%)		Social Dysfunction	53 (55.2%)
	Upper moderate Anxiety	29 (33%)		Depression	9 (9.4%)
	Relatively severe anxiety	7 (7.9%)		Anxiety and Insomnia	45 (46.9%)

Severe anxiety	3 (3.4%)

Using ANOVA and based on Spielberger's state and trait anxiety scale, the results suggest that TGF-β level increases significantly with increasing level of anxiety (*P*=0.001) Moreover, the results also showed a

significant relationship with Trait Anxiety so that with Increasing trait anxiety, TGF-β anxiety level was significantly higher (*P*=0.049) (Table 3).

Table 3. The relationship between depression, trait and state anxiety with the TGF-β in lactating women

	Mild anxiety	**Moderate to low anxiety**	**Upper moderate anxiety**	**Somewhat severe**	**Severe**	***P***
			levels of state anxiety			
TGF-β SD±Mean	102.93±245.57	242.95±379.35	399.11±452.82	675.84±1003.32	270.77±454.30	0.001
			Levels of trait anxiety			
	Mild anxiety	**Moderate to low**	**Upper moderate anxiety**	**Somewhat severe**	**Severe**	***P***
TGF-β SD±Mean	151.51±293.77	254±342.09	443.11±531.80	582.10±663.64	-	0.049
		Depression (based on beck questionnaire)				
	Yes			**No**		***P***
TGF-β SD±Mean	521.88±615.48			336 ±219.21		0.002

*Based on ANOVA test # based on t test for independent groups #

Based on the general health questionnaire, the results showed that TGF-β level was significantly higher in the group with anxiety disorder (*P*=0.010) Besides, TGF-β also showed a significant association with depression as TGF-β level was higher in depressed subjects (*P*=0.038)

But a significant relationship was not observed in the scale of social function (*P*=0.95) physical function (*P*=0.19) and the General Health Questionnaire (*P*=0.26) with TGF-β level (Table 4).

Table 4. The relationship between different aspects of General Health Questionnaire with the TGF-β level in lactating women

	Classifying based on disorders		
Disorders	**No**	**Yes**	***P***
	TGF-β SD±Mean	TGF-β SD±Mean	
Physical disorder	378.39 ± 310.78	487.53 ± 417.21	0.19
Anxiety and insomnia	316.10 ± 167.71	517.83 ± 451.97	0.01
Social dysfunction	418.30 ± 376.21	413.71 ± 336.83	0.95
Depression	388.25 ± 299.30	659.26 ± 639.69	0.038
Health disorders (based on total score of GHQ)	381.19 ± 329.23	474.69 ± 391.04	0.26

*Based on the Independent group's t-test

Discussion

In this study, there was a significant correlation between the level of concentration of TGF-β 2 with depression and anxiety, according to Beck Depression Inventory (BDI) and General Health Questionnaire (GHQ). In other words, with increased levels of TGF-β2, anxiety and depression scores increased in the

mentioned tests.

In investigating population-based epidemiology, Kondo *et al*., results (2011) showed that there is a strong correlation between the very low and poor self-rating scale of the health and postpartum depression with high concentrations of TGF-β2 in mothers whose milk was held until 3 months after delivery. Maternal history of allergic diseases, smoking habits, and drinking alcohol

was not associated with TGF-β2 concentrations. This study showed a positive and significant relationship between TGF-B2 concentrations with the psychological characteristics of people.

With increased levels of TGF-β2 in breast milk, maternal psychiatric conditions such as depression and the reduced level of perceived mental health were reported. From other important results of this study, the relationship between seasonal variations and hours of the day on the levels of TGF-β2 concentration in milk can be pointed out (20). There is a strong negative relationship between depression and the level of TGF-β2 concentration in breast milk. In other words, the physical and mental health of mothers reported by them through the General Health Questionnaire was associated with levels of TGF-β2 (21-22) Although, acute or chronic physical illnesses associated with the consumption levels of TGF-B milk have not been reported yet; however, the findings do not show the depressed mothers' breastfeeding can have a direct positive impact on children's immune system. The effect of TGF-β concentration of milk is still under discussion at the risk of atopic disease (3,4,6,23)

Depression can cause endocrine disorders and various immune systems leading to infections, allergies, and autoimmune diseases. Some of its molecular mechanisms have become evident (9). For example, the serotonin receptor plays an important role in the pathology of depression. Li *et al.*, reported the possibility of a significant interaction between serotonin receptors and the malfunction of immune system among the depressed patients (26). In a study, the multiple cytokine concentrations in plasma of the depressed and nondepressed subjects were studied. The results showed that TGF-β1 levels in depressed patients would rise (27). Similar findings have also been observed in patients with chronic fatigue syndrome with higher TGF-β1 levels (28) while Lee *et al.*, reported lower TGF-β serum levels in major depressive disorder patients (27).

The results obtained from different studies seem to be different. These studies cannot precisely explain the relationship between the concentration levels of TGF-β in milk with depression. Since TGF-β in milk is excreted through the mammary gland, the level of TGF-β in milk has nothing to do with plasma. Thorough, comprehensive and clear researches are required to investigate regulatory systems for identification of TGF-β in milk (29).

Other studies in this area suggest that dysregulation of the immune system is associated with suicidal behavior in Major Depressive Disorder (MDD). It is also believed that transforming growth factor of TGF-β1 in the regulation of inflammatory response and creating anti-inflammatory effects is an important agent. Lee and Kim, 2010 conducted a study to identify the role of TGF-β1 in the depression of people who had committed suicide. Polymorphism of TGF-β1 was investigated in condoms 10, 25 in 122 patients with suicidal MDD, 61 non-suicidal MDD patients, and 120 control subjects. Among them, in vitro production of TGF-β1 was measured in 48 suicidal MDD patients, 47 non-suicidal MMD patients and 91 patients in the control group. There were no genetic polymorphisms in condom 25 and three genotypes in condom 10. No significant difference was observed in the distribution of genotypes TGF -beta1 among the three groups. In vitro production of TGF-β1 in suicidal MMD patients (844.3 ± 7.329 PG/ml) and in non-suicidal MDD patients (0.853 ± 7.439 PG/ml) was considerably and significantly higher than the control group (0.683 ± 0.397 PG/ml). In vitro production of TGF-β1 among patients with allele or TGF-β1 genotype was not significant. Results showed that in vitro production of TGF- BETA1 plays an important role in MDD, but there is no relationship between TGF-β1 and suicidal behavior (30).

"At study, Pallavi (2015) compared the serum cytokine levels between adolescent depression patients and healthy controls and assesses the correlation between depression, anxiety scores and serum levels of eight cytokines. Depressed adolescents had significantly high levels of IL-2 ($P<0.001$) and IL-6 ($P=0.03$) as compared to controls. The female population skewed the result of one cytokine (IL-6) in patients. Anxiety scores showed positive correlation (only in female patients) with IL-1β, IL-10 and negative correlation with TGF-β1 and IL-17. The gender effect on the relationship between anxiety and cytokines was not straightforward. On comparing study groups on the medication/naïve status, IL-2 and TGF-β1 showed significant difference between the groups ($P<0.001$, $P=0.007$ higher in medicated). Depression in adolescents was associated with elevation of proinflammatory serum cytokines with a gender bias for females. Anxiety scores correlated negatively with TGF-β1 and IL-17" (31,32).

According to the research, it seems that some researches support the relationship between psychiatric disorders with levels of TGF-β concentration. Some research does not confirm this relationship, but in a study, we carried out, a significant relationship was found between depression and anxiety levels of TGF-β that our results are in line with some researches. Due to the limitations of the study, the following cases are

recommended:

1) It is suggested to investigate the levels of TGF-β concentration at intervals and different seasons and its association with psychiatric disorders.

2) It is suggested to make a prospective study to examine the physical and mental health of children in the long term 3) the larger sample size to be used in the study.

Currently, many public health officials and experts strongly recommend breastfeeding to all families because of its potential health benefits including the prevention of atopic and infectious diseases. However, the findings of our study and the results may have important implications for public health and clinicians that this issue is related to various factors in maternal levels of TGF-β. An important indicator of the control of cytokines in immune function in breast milk may be related to the mother's mental and physical health. According to a study we did, more research is needed to determine the complex courses of the relationship between mother's mental stressors, genetic traits, diet, and behavior of cytokines in breast milk and the role of these cytokines in the body of children is essential. According to the results of our project, the importance of mental health and relaxation during pregnancy must be considered, if mental stresses and other psychiatric disorders, these people should be put under psychological treatment.

Aknowledgement

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