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Effect of Moderate Exercise on Breast Milk Leukocytes in Exclusively Breast-feeding Mothers

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Abstract: Although it is well documented that moderate exercise enhances cell-mediated immunity, effect of moderate exercise on breast milk leukocytes has not been studied yet. So, the aim of this study was to investigate the effect of moderate exercise on breast milk leukocytes in exclusively breast-feeding mothers. Forty-seven exclusively breast-feeding mothers at 1st to 5th month postpartum were randomized into two groups. Group A received breast-feeding and nutritional counseling and engaged in moderate aerobic exercise for 4 weeks with a total of 12 sessions. Group B received only the same breast-feeding and nutritional counseling for 4 weeks. Both groups were evaluated pre and post-intervention. The outcome measures were maternal anthropometric parameters and breast milk total and differential leukocytes counts. Post-intervention, there was a non-significant difference in the maternal anthropometric parameters ($P > 0.05$) while there was a highly significant increase in breast milk total and differential leukocytes counts ($P = 0.001$) in favor of group A. There was a significant inverse relationship between maternal anthropometric parameters and lymphocytes count in group A. This study explored that moderate aerobic exercise during exclusive breast-feeding was associated with increased breast milk total and differential leukocytes counts.
Keywords: Moderate exercise, exclusive breast-feeding, breast milk, immune, leukocytes.

Introduction

The immature immune system of the newborn infant increases his/her susceptibility to infections[1]. In addition to transfer of immunoglobulin G to the fetus through the placenta, breast-feeding provides immunological support for the immunocompromised newborn infant against infections not only during the first months of life but also over the long term[2-4]. So, the American Academy of Pediatrics reaffirms its recommendation of exclusive breast-feeding for 6 months followed by continued breast-feeding with introducing complementary foods for 1 year or more[5].

The human milk immune system includes immunoglobulins, lactoferrin, lactalbumin, glycans, non-absorbable oligosaccharides, free fatty acids, monoglycerides, exosomes, cytokines, nucleic acids, antioxidants and maternal cells involving mature epithelial cells, progenitor cells, stem cells and leukocytes such as macrophages, neutrophils and lymphocytes[1,3,6,7].

Breast milk leukocytes arise from maternal blood leukocytes that home to the mammary gland and pass through the epithelial cells' junctures then they are secreted into the breast milk[8]. They perform immuno modulatory functions in the infant via phagocytosis, production of antimicrobial factors such as cytokines and immunoglobulins or antigen presentation[9-12]. These functions are thought to be exerted not only inside the gastrointestinal tract of the infant but also in remote tissues via the systemic circulation[9,13,14]. Breast milk leukocytes are influenced by several factors that include maternal and infant infections, breast-feeding status and stage of lactation[6].

Moderate exercise has favorable effects on the immune system through enhancing cell-mediated immunity[15]. Each session constitutes an increase in the immune system reducing the risk of infection over the long range[16].

All previous studies, that examined the effect of maternal exercise on the immunological factors in breast milk, had used a self-reported exercise. In addition, none of these studies analyzed the effect of maternal exercise on breast milk leukocytes. Therefore, this study aimed to investigate the effect of supervised and standardized moderate aerobic exercise on breast milk leukocytes in exclusively breast-feeding mothers. This study may expand the role of physiotherapy in the field of the women's health, breast-feeding counseling and exercise immunology.

Materials and Methods

1. Participants:

Forty-seven healthy, sedentary, nonsmoking, exclusively breast-feeding mothers at 1st to 5th month postpartum were selected from Mataria training hospital to participate in this study. They had had a single, healthy and full-term infant without complications. Their ages ranged from 20 to 35 years old and their body mass index (BMI) ranged from 20 to 35 Kg/m^2. The breast-feeding mothers were excluded from this study if they had poor lactation, cancer of the breast or the surrounding area, any previous breast or chest surgery, any cardiorespiratory disease, anemia, diabetes mellitus, immune disease, general, vulval or breast infection, retracted, cracked, inflamed or inverted nipples or any other condition that compromises lactation such as retained placental fragments, postpartum hemorrhage or receiving contraceptive pills. Also, breast-feeding mothers that had received any kind of medications or supplementations that could influence the immune function were excluded from this study.

The study was approved by Research Ethical Committee, Faculty of Physical Therapy, Cairo University (No: P.T.REC/012/00435). The study protocol was explained to all breast-feeding mothers, who had signed an informed consent form.

The breast-feeding mothers were randomly assigned to one of 2 groups using computer generated random numbers. Allocation was concealed in sequentially numbered opaque envelopes. Group A consisted of 23 breast-feeding mothers who received breast-feeding and nutritional counseling and engaged in moderate aerobic exercise for 4 weeks. Group B consisted of 24 breast-feeding mothers who only received the same breast-feeding and nutritional counseling for 4 weeks.

2. Measures:

Anthropometric measures:

Weight-Height scale was used to measure the weight and height of each breast-feeding mother before starting the study and after the end of the interventional program. Then, the maternal BMI was calculated according to the following formula:

$$BMI = weight\ (kg) / height\ square\ (m)^2$$

Breast milk collection and analysis:

The breast milk was expressed manually by the breast-feeding mother in a sterilized test tube with a cover. Immediately after collection, breast milk samples were placed in a cool box that had been stored at 4° C and were carried to the laboratory[17]. The milk samples were used immediately for analysis. Total and differential leukocytes counts were done using Beckman Coulter (Coulter MAXM, made in USA). It is a quantitative, automated, differential cell counter for in vitro diagnostic use. The Beckman Coulter method of sizing and counting particles uses measurable changes in electrical impedance produced by nonconductive particles suspended in an electrolyte. A suspension of blood cells passes through a small orifice simultaneously with an electric current. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. Each particle displaces its volume of electrolyte in the sensing zone. Beckman Coulter measures the displaced volume as a voltage pulse. The height of each pulse is proportional to the volume of the particle. The quantity of suspension drawn through the aperture is for an exact reproducible volume. Beckman Coulter counts and sizes individual particles at a rate of several thousand per second. This method is independent of particle shape, color and density.

3. Interventions:

Breast-feeding and nutritional counseling (for both groups):

All mothers received advices about proper breast-feeding technique (care of the breast during breast-feeding, infant feeding readiness cues, proper breast-feeding position, position of mother's hand, proper latch-on, signs of proper milk transfer and frequency and duration of feedings) and proper maternal nutrition and fluid intake during breast-feeding. During the 4 weeks follow up, all mothers were receiving the same average Egyptian daily diet.

Exercise program (for only group A):

Each breast-feeding mother in group A engaged in a supervised moderate aerobic exercise for 12 sessions (3 sessions/week for 4 weeks). Each exercise session consisted of warm up, active and cool down phases. The warm up phase started with walking on a treadmill (Biodex Gait Trainer 2, made in USA) at low speed for 5 minutes. The active phase lasted for 30 minutes at 60 to 70% of the maximum heart rate (HRmax). The treadmill speed was increased gradually and adjusted for each subject according to her prescribed intensity based on HRmax. HRmax was calculated according to the following equation: HRmax = 220−age [18]. The program ended with 5 minutes cool down phase on the treadmill during which the treadmill speed was gradually decreased until the heart rate returned to its resting level.

Statistical analysis:

Results are expressed as mean ± standard deviation (SD). According to test of normality, comparison between different variables in the two groups was performed using unpaired t test or Mann Whitney U test whenever it is appropriate. Pair-wise comparison (pre- versus post-assessment) within the same group for different variables was performed using paired t test or Wilcoxon Sign Rank test whenever it is appropriate. Correlation between difference in maternal anthropometric parameters and total leukocytes, macrophages, neutrophils and lymphocytes counts in both groups (A & B) was performed using Spearman's rho correlation coefficient. Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis. P value ≤ 0.05 was considered significant and < 0.01 was considered highly significant.

Results

Demographic characteristics:

The demographic characteristics of the exclusively breast-feeding mothers are shown in Table 1. None of these variables showed significant differences between both groups before the interventional program ($P > 0.05$).

Table 1. Demographic characteristics of the exclusively breast-feeding mothers.

	Group A (n = 23) mean ± SD	**Group B (n = 24) mean ± SD**	**P value**
Maternal age (yrs.)	29.74 ± 5.59	27.62 ± 4.45	0.158
Maternal height (m)	1.59 ± 0.05	1.60 ± 0.07	0.249
Months postpartum	2.93 ± 1.55	2.96 ± 1.52	0.958
Parity number	2.52 ± 1.24	2.42 ± 1.21	0.764
P > 0.05 = non-significant			

Anthropometric parameters:

After the intervention, the maternal weight and BMI showed a highly significant decrease (P= 0.002) within group A, while they showed non-significant differences (P > 0.05) within group B. Also, they showed non-significant differences between both groups after the intervention (P > 0.05). (Table 2)

Table 2. Inter- and intra-group comparison of maternal anthropometric parameters pre- and post-intervention.

		Group A (n = 23) mean ± SD	**Group B (n = 24) mean ± SD**	**P value***
Maternal weight(Kg)	Pre-intervention	76.54 ± 9.69	75.58 ± 11.56	0.760
	Post-intervention	74.98 ± 9.76	75.67 ± 11.59	0.827
	P value**	**0.002**	0.721	
Maternal BMI(Kg/m^2)	Pre-intervention	30.33 ± 3.83	29.45 ± 4.52	0.477
	Post-intervention	29.72 ± 3.95	29.41 ± 4.58	0.803
	P value**	**0.002**	0.773	

*Inter-group comparison; **intra-group comparison of the pre- and post-interventional program results.
P > 0.05 = non-significant; P < 0.05 = significant; P < 0.01= highly significant.

Breast milk total and differential leukocytes counts:

Within group A, the breast milk total and differential leukocytes counts showed a highly significant increase (P = 0.001). Within group B, the breast milk total and differential leukocytes counts showed a significant decrease (P < 0.05) except the neutrophils count which showed a non-significant difference (P > 0.05). Comparison of the post-interventional results of both groups revealed that there was a statistically highly significant increase in the breast milk total and differential leukocytes counts (P = 0.001) in favor of group A. (Table 3)

Table 3. Inter- and intra-group comparison of breast milk total and differential leukocytic counts pre- and post-intervention.

		Group A (n = 23) mean ± SD	**Group B (n = 24) mean ± SD**	**P value***
Total leukocytes ($cells/mm^3$)	Pre-intervention	3358.91 ± 2176.82	2409.88 ± 759.27	0.154
	Post-intervention	11295.26 ± 6491.57	1949.71 ± 629.13	**0.001**
	P value**	**0.001**	**0.040**	
Macrophages ($cells/mm^3$)	Pre-intervention	1696.43 ± 1162.22	1232.33 ± 403.26	0.209
	Post-intervention	5699.57 ± 3277.61	969.50 ± 310.71	**0.001**
	P value**	**0.001**	**0.028**	
Neutrophils ($cells/mm^3$)	Pre-intervention	1132.30 ± 777.39	785.58 ± 326.14	0.136
	Post-intervention	3946.30 ± 2379.72	649.62 ± 208.72	**0.001**
	P value**	**0.001**	0.083	
Lymphocytes ($cells/mm^3$)	Pre-intervention	323.35 ± 222.18	236.79 ± 72.04	0.259
	Post-intervention	1040.17 ± 638.14	189.33 ± 65.97	**0.001**
	P value**	**0.001**	**0.027**	

*Inter-group comparison; **intra-group comparison of the pre- and post-interventional program results.
P > 0.05 = non-significant; P < 0.05 = significant; P < 0.01= highly significant.

Correlation between different variables:

In group A, there were no significant correlations between maternal anthropometric parameters and total leukocytes, macrophages and neutrophils counts while there was a significant inverse relationship between maternal weight and lymphocytes count (r = - 0.415; P = 0.049). Also, there was a significant inverse relationship between maternal BMI and lymphocytes count (r = - 0.466; P = 0.025). (Table 4)

Table 4. Correlation between difference in maternal anthropometric parameters and total leukocytes, macrophages, neutrophils and lymphocytes counts in group A (n= 23).

	Maternal weight		Maternal BMI	
	r	***P value***	***r***	***P value***
Total leukocytes count	-0.356	0.096	-0.400	0.058
Macrophages count	-0.345	0.107	-0.393	0.063
Neutrophils count	-0.300	0.165	-0.350	0.102
Lymphocytes count	-0.415	**0.049***	-0.466	**0.025***
r = Spearman's rho correlation coefficient *P < 0.05 = significant.				

Table 5. Correlation between difference in maternal anthropometric parameters and total leukocytes, macrophages, neutrophils and lymphocytes counts in group B (n= 24).

	Maternal weight		Maternal BMI	
	r	***p value***	***r***	***p value***
Total leukocytes count	0.007	0.974	0.035	0.872
Macrophages count	-0.131	0.540	-0.101	0.638
Neutrophils count	-0.007	0.976	0.024	0.912
Lymphocytes count	-0.159	0.457	-0.127	0.555
r = Spearman's rho correlation coefficient				

In group B, there were no significant correlations between maternal anthropometric parameters and total and differential leukocytes counts (P > 0.05). (Table 5)

Discussion

The infant is born with an immature naive acquired immune system, a gut lacking microflora, a stomach whose function is less potent to combat pathogens, a locomotion mode that results in close contact with the most contaminated part of our environment and a tendency to explore the environment orally[19]. Breast milk has its own immune system and a broad range of soluble and cellular factors which support the infant's immune development and maturation[20].

Physical activity level affects the risk of infection by influencing immune function. Regular moderate exercise lowers the risk of infection while very prolonged bouts of exercise and periods of intensified training are associated with increased infection risk[21,22]. Although moderate exercise enhances the cell-mediated immune function, none of the previous studies had studied the effect of moderate exercise on breast milk leukocytes. Therefore, the present study was the first study that investigated the effect of supervised and standardized moderate exercise on breast milk leukocytes.

Regarding the anthropometric parameters, the results of this study agreed with a systematic review which studied the effect of postpartum exercise on mothers and their offspring. It reported that moderate exercise without specific dietary restriction does not promote greater weight or fat loss because exercise may advocate greater energy intake and/or lowered energy expenditure from non-exercise physical activity (so, preventing negative energy balance)[23]. Also, a meta-analysis of physical activity or diet or combination of both for weight loss in postpartum women found that the most effective methods in postnatal weight loss were diet alone or combination of both but not physical activity alone. It concluded that weight loss through a combination of diet and exercise was more favorite as this enhances maternal cardiorespiratory fitness and maintains fat-free mass while diet alone lowers fat-free mass[24].

Results of this study revealed that 4 weeks of moderate aerobic exercise produced a highly significant increase in total and differential leukocytes counts in breast milk of exclusively breast-feeding women. The mechanisms by which the moderate exercise caused a highly significant increase in breast milk total and differential leukocytes counts are still unknown. However, the results of this study can be supported with Groër and Shelton[25] who found that pro-inflammatory cytokines increased in mothers' milk in the early postpartum period with increasing exercise intensity and caloric expenditures. Since leukocytes recovered from expressed human milk have been shown to be capable of producing a number of cytokines[26], this indicates that the increased breast milk cytokines with increasing exercise intensity and caloric expenditures can be the result of the increased breast milk leukocytes that were found in the present study.

In addition, a former review article stated that each bout of moderate aerobic exercise causes an increase in blood concentrations of neutrophils, natural killer cells and antibodies[22]. Thus, an acute bout of exercise provides a positive increase to both the innate immune system (neutrophils and natural killer cells) and the acquired immune system (antibodies). This exercise-induced improvement in immune function is transient and returns to pre-exercise levels within 3 hours. However, it seems that each exercise bout boosts immune function against pathogens over a short time period which results in reduction of infection risk[27].

Moreover, a recent study reported that 6 weeks of moderate aerobic exercise increased the levels of particular antioxidant enzymes in blood of women with type-2 diabetes resulting in improvement of the body's antioxidant defense system and reduction of the oxidative stress[28]. Antioxidants are linked to immune cell functions and their deficiency can result in immunosuppression and influence T-cell-mediated immune response[29]. Consequently, it seems that the antioxidant effect of moderate aerobic exercise can improve T-cell-mediated immune response and increase T lymphocytes count.

In contrast, a previous study found non-significant differences between exercising and sedentary breast-feeding women at 3 months postpartum in their complete blood cell counts; differential leukocyte count; percentages and absolute counts of peripheral blood T cells, cytotoxic T cells, helper T cells, B cells and natural killer cells; neutrophil bacterial killing and oxidative burst activity and mitogenic responsiveness of lymphocytes. These findings may be caused by the physiological state of breast-feeding which may boost the immune response to a greater extent than exercise by decreasing the neuroendocrine response to stressors. However, significant limitations of that study are the cross-sectional design and use of self-reported exercise[30].

Also, Abdossaleh et al.[31] investigated the effect of exercise with different intensities on total and differential blood leukocytes counts in college judoists. In this study, subjects ran on a treadmill for 60 min at 45% of HRmax (low intensity) in first week and 60% of HRmax (moderate intensity) in second week and 75% of HRmax (high intensity) in third week. Blood was drawn before and immediately after exercise. They found that total leukocytes and neutrophils counts increased and lymphocytes and monocytes counts decreased after moderate intensity exercise. They explained the increased neutrophils counts after exercise by the vascular and muscle contraction. Also, secretion of catecholamines during exercise powered neutrophil rise from the margins of vessels and their entry into the circulation[32].

The post-interventional non-significant differences in breast milk neutrophils count in group B may be explained by the neutrophils' role in mammary gland protection. These cells have restricted functional ability once they are secreted into breast milk, so many researchers suggested that their main role is maternal protection[20,33].

The post-interventional significant decrease in total leukocytes count, macrophages count and lymphocytes count in group B may be clarified by Hassiotou et al.[6] who found that leukocyte levels in colostrum were considerable (13.2-70.4% leukocytes of total milk cells). Then, they rapidly lowered in transitional milk (0-1.7% leukocytes of total milk cells) and mature milk (0-1.5% leukocytes of total milk cells) when both the breast-feeding mother and her infant were healthy (which is the case in our subjects).

The significant inverse relationship between maternal anthropometric parameters and lymphocytes count in group A post intervention, can be explained by the highly significant maternal weight loss in this group. A recent study found that the milk protein concentration was affected by maternal BMI because there was more protein concentration in normal weight mothers compared with overweight mothers[34]. Since immunoglobulins (which are secreted by B lymphocytes) are proteins, this indirectly indicates that the greater weight loss can result in increased lymphocytes count and subsequent increase in immunoglobulins.

These findings provide new data about the effect of moderate exercise in increasing total and differential leukocytes counts in breast milk. Further studies are needed in order to enlighten the environmental, metabolic and endocrine mechanisms underlying these responses to moderate exercise. Although the current study provides objective data with statistically significant differences, there may be some limitations that include the short duration of follow up. So, further studies are needed to examine the long term effect of moderate exercise on breast milk leukocytes in exclusively breast-feeding mothers.

Conclusion:

These findings suggested that moderate aerobic exercise during exclusive breast-feeding may have positive effects on breast milk cell-mediated immunity through increasing its total and differential leukocytes counts.

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