THE EFFECT OF HORMONAL AND NON-HORMONAL CONTRACEPTIVE METHODS ON THE ANTIOXIDANT CONTENT OF HUMAN MILK

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Abstract- Different contraceptive methods are used by breastfeeding mothers. In general, these methods are classified into two major groups, hormonal and non-hormonal. A number of studies have shown that combined oral contraceptive pills may adversely affect the quality and the quantity of human milk. In other studies it has been shown that estrogen and progesterone seem to reduce the risk of cardiac and ischemic brain injury by enhancing anti-oxidant mechanisms. Since infants are at increased risk of oxidative stress and free-radical mediated diseases are partly related to deficient antioxidant state, we performed a study to investigate if contraceptive method has any effect on the maternal milk total antioxidant content. A cross-sectional study of total antioxidant capacity of mature human milk of two groups of healthy breastfeeding mothers who were on the hormonal (Lynestrol) or non-hormonal contraceptive regimen with 92 mothers in each group was performed. The total antioxidant capacity was determined by ferric reducing antioxidant power method. The body mass indexes (BMI) of hormonal and non-hormonal groups were 23.67±2.88 and 22.93±3.33 kg/m^2, respectively, with no significant difference. The hemoglobin concentrations of hormonal versus non-hormonal groups were 12.94±0.90 and 13.14± 0.76 g/dl which were not significantly different. The mean total antioxidant contents of the hormonal and non-hormonal groups were 575±139 and 583±135 µmol/l, respectively, with no significant difference. It seems that progesterone has no effect on the antioxidant contents of mature human milk; in other words, the Lynestrol has neither positive nor negative effect on the antioxidant capacity of the human milk.

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INTRODUCTION

Infants, especially premature ones, are at risk of "oxygen radical disease". This could be due to their exposure to supplemental oxygen and/or to a decrease in their antioxidant defense systems (1, 2).

Oxidant induced tissue injury may be a common underlying factor leading to several neonatal diseases including retinopathy of prematurity, intraventricular hemorrhage, necrotizing enterocolitis and chronic lung disease (3). Reactive oxygen species are intermediaries produced by normal oxygen metabolism. To protect themselves from the deleterious effects of reactive oxygen species, different organisms have developed a wide range of antioxidant systems to limit production of reactive oxygen species, inactivate them and repair cell damage. However, oxidative stress may occur when the balance between reactive oxygen species

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production and antioxidant defense is disrupted. The increasing number of diseases associated with oxidative stress suggests that oxidative balance may be precarious (4). Optimal protection is achieved only when an appropriate balance among free radicals and antioxidants is maintained (5). The oxidative destruction of polyunsaturated fatty acids, known as lipid peroxidation, is particularly damaging because it is a self-perpetuating chain reaction (6, 7).

Human milk has some antioxidant agents such as vitamin C and E, β-carotene, glutathione reductase and superoxide dismutase (8). These antioxidants are very important because they can prevent oxidizing activities that cause tissue damage. The composition of human milk varies over the course of lactation and in each individual. The nutritional status of the mother appears to influence the content of her milk (9, 10). For most drugs, their concentration in human milk is of the same order of magnitude as the plasma concentration (11). Since many drugs taken by a nursing mother can be passed on to her baby through her milk, it is important to know the effect of those that are usually taken by breast-feeding mothers. One of the most important drugs that are taken by nursing mothers is estrogen and progesterone as oral contraceptive pill (OCP) and progesterone-only compounds like Lynestrol. It has been reported that mothers who use progesterone only pills as contraceptive method have lower levels of lipid and protein and some minerals in their milk compared to those that have intrauterine device (IUD) (12,13).

Estrogen and progesterone, long considered as primary hormones in reproductive and maternal behavior, are now being studied as neuroprotective and neuroregenerative agents in stroke and traumatic brain injuries. Collectively, the hormones reduce the consequences of the injury cascade by enhancing anti-oxidant mechanisms. Estrogen seems more effective as a prophylactic treatment in females at risk for cardiac and ischemic brain injury, whereas progesterone appears to be more helpful in the postinjury treatment of both male and female subjects with acute traumatic brain damage (14).

In a study of antioxidant content of the milk of Nigerian women and the sera of their exclusively breast-fed infants, the researchers showed that the there was a significant correlation between the maternal antioxidant status and the antioxidant content of breast milk and between the infant antioxidant status and milk. The maternal and infant serum antioxidant levels were also significantly correlated. Their data suggest that the antioxidant status of exclusively breast-fed infants is dependent on the antioxidant status of their mothers (15). In another study the postnatal changes in plasma chain-breaking antioxidants in healthy preterm infants fed with formula and/or human milk was determined and plasma redox ratios of uric acid and ascorbic acid were compared in healthy preterm babies with those with respiratory distress syndrome (RDS) and chronic lung disease (CLD). They found that plasma allantoin and allantoin/uric acid ratios were elevated in CLD and both markers of oxidative stress enabled early prediction of development of CLD (16, 17).

Because of some adverse effect of hormonal contraceptive on the human milk and some protective effect of them as antioxidant, we decided to examine and compare the antioxidant capacity of the mature human milk in two groups of breast-feeding mothers who used hormonal or non-hormonal contraceptive methods. The BMI and blood hemoglobin concentration was also checked to exclude any probable malnutrition in the mothers.

MATERIALS AND METHODS

Three hundred and fifteen breast-feeding mothers were recruited from health care centers in south of Tehran (Akbarabad, Hakimabad, Meysam, Farman-farmayan, Shahid Ayat and Shahid Khaniabad centers).

Women with any pregnancy related or other long standing illnesses that could affect their nutritional status were excluded. Since the composition of milk is different in different months, we tried to use mature milk. By definition the mature milk is referred to the mother's milk after 30 days of perturbation (11). Eligible mothers (n=184) were sequentially recruited into two groups: hormonal (n=92) were those that used Lynestrol as contraceptive method and non-hormonal (n=92) were mothers that used contraceptive method other than hormonal. The sample volume was determined from a pilot study.

The hormonal and the non-hormonal groups were considered as the case and the witness groups, respectively. Mothers in the witness group were matched for their BMI and total hemoglobin concentration with respect to the case group. The study was conducted following the approval of our institutional review board, and informed consent was obtained from all subjects before inclusion in the study.

A blood sample was taken from each mother for determination of hemoglobin concentration. The hemoglobin concentration was measured by Drabkin method in which the hemoglobin reacts with ferrocyanate and the resulting cyanomethemoglobin is spectrophotometrically measured at 540 nm. At the same time a milk sample was taken for determination of total antioxidant status. The milk was centrifuged at 3000 g, for 10 minutes and after removal of the fat layer, the low fat milk was kept at -20° C until the day of analysis. The removal of fat is necessary for most calorimetric methods. Milk total antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay (18). The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method. In this assay, at low pH a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex reduced to the ferrous form, which is blue colored and monitored by measuring the change in absorption at 593 nm.

The change in absorbance is directly proportional to the reducing power of the electron-donating antioxidants present in the milk. The absorbance change is translated into a FRAP value (in µmol/lit) by relating the change of absorbance at 593 nm of test sample to that of a standard solution of known FRAP value. FRAP reagents included 300 mmol/liter acetate buffer, pH 3.6; 10 mmol/liter TPTZ (2, 4, 6-tripyridyl-s-triazine, Fluka Chemicals, Switzerland) in 40 mmol/liter HCl, 20 mmol/liter $FeCl_3$ and $6H_2O$. Working FRAP reagent was prepared as required by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3$, $6H_2O$ solution. Aqueous solutions of known Fe^{2+} concentration, in the range of 100–1000 µmol/liter ($FeSO_4$, $7H_2O$), were used as standards for calibration.

For the evaluation of FRAP, 300 µl freshly prepared FRAP reagent was warmed to 37°C and 10 µl of sample (unknown or standard) was then added and the absorbance was measured at 593nm. The concentration of unknown samples was determined by comparison to a Fe^{2+} standard curve. All solutions were used on the day of preparation.

Data was analyzed using the SPSS statistical package and the results are expressed as mean ± SD. Significant differences were assigned to $P < 0.05$.

RESULTS

The mean BMI of hormonal and non-hormonal groups were 23.67±2.88 and 22.93±3.33 kg/m^2 respectively, with no significant difference. The hemoglobin concentrations of hormonal versus non-hormonal group were 12.94±0.90 and 13.14±0.76 g/dl, which were not significantly different.

The mean total antioxidant contents of the hormonal and non-hormonal groups were 575±139 and 583±135 µmol/l, respectively, with no significant difference. Results are shown in table 1.

Correlation coefficients were calculated between blood hemoglobin, BMI and milk total antioxidant. Correlation coefficients between blood hemoglobin and the mother BMI, BMI and milk total antioxidant and between blood hemoglobin and milk total antioxidant were 0.18, 0.13 and 0.19, respectively. In other words there was a weak positive correlation between these variables.

Table 1. Comparison of BMI, hemoglobin and FRAP in breast feeding mothers who were on hormonal and non-hormonal contraceptive methods*

Variable	Hormonal group(n=92)	Non hormonal group (n=92)	*P* Value†
BMI (kg/m^2)	23.67 ± 2.88	22.93 ± 3.33	0.28
Hemoglobin (g/dl)	12.94 ± 0.90	13.14 ± 0.76	0.11
FRAP (µmol/l)	575 ± 139	583 ± 135	0.715

Abbreviations: BMI, body mass index; FRAP, ferric reducing antioxidant power.
*Data are presented as mean±SD.
† *t* Student test.

DISCUSSION

Although oxygen is crucial to a wide range of vital, life-sustaining biological activities, oxygen radicals can disrupt cell membranes, destroy cell enzyme function, alter DNA and cause cell death (19, 20). Tissue damage as indicated by plasma and urinary malondialdehyde (MDA) is increased in premature infants exposed to supplemental oxygen. While some scientists have examined the antioxidant defenses of premature infants, others have examined the relationship between tissue damage, antioxidant status, nutrient and drug intake in these infants (1). VanderJagt *et al.* have reported that the amount of total antioxidant of breast-milk of mothers who live in the different parts of the world is considerably unstable. They have measured total antioxidant capacity of breast-milk of Fulani women and reported an average amount of 1100 µmol/l. But in the same study, the average amount of total antioxidants of breast-milk in other races (European) has been reported 3100 µmol/l (15).

Since many drugs taken by a nursing mother can be passed on to her baby through her milk, we hypothesized that the drugs that alter the anti oxidative defense and are usually taken by breast-feeding mothers may alter the infant's antioxidant capacity. So we examined the effect of progesterone as Lynestrol on the antioxidant content of human milk and compared it with the milk from the mothers that did not take any hormonal contraceptive (as the control group). It should be mentioned that the effect of diet in the population under study could be considered as a confounding factor, however in this study the diet was not taken into account since the economical conditions seemed equal and the large number of subjects included in this study tended to minimize this effect. To evaluate the health of the mothers their hemoglobin and BMI was determined. We found that the total antioxidant capacity of breast-milk measured by FRAP method were 575 and 583 µmol/l in hormonal and non-hormonal groups respectively, with no significant difference (P=0.715). Although Stein *et al.* reported that estrogen and progesterone may have some positive effect on the antioxidant capacity (14), there are some reports against their idea (21) and we showed that progesterone has no effect on the antioxidant contents of mature human milk; in other words, the application of Lynestrol has neither positive nor negative effect on the antioxidant capacity of the human milk.

The total antioxidant capacity of breast-milk of these mothers in both groups was lower than the amount that was reported by Friel *et al.* (1). The difference may be attributed to different factors such as socioeconomic status, dietary habits, ethnicity, the stage of lactation, seasonal collection time and use of different drugs that may have different effect on the total antioxidant status of milk. Since the infant's antioxidant capacity is related to the antioxidant capacity of their mother's milk, and the antioxidant status of mother is related to her diet, it is important to evaluate the effect of different nutritional and drug regimen in lactating mothers. In other words, the examination of total antioxidants capacity of breast-milk may show the antioxidant status of mother and her infant.

The other important conclusion could be recommendation of higher antioxidant regimen for these mothers, because of lower levels of their milk antioxidant content with respect to some other mothers in the world. This can protect their infants against free radical related diseases.

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