Antioxidant and Radical Scavenging Activity of Human Colostrum, Transitional and Mature Milk

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Summary  Human milk from healthy women contains numerous nutrients such as antioxidants which are necessary for newborns. The aim of this study was to evaluate the changes of total antioxidant capacity (TAC) and free radical scavenging activity in human milk during the first six month period of lactation and also its relationship to maternal plasma. A total of 505 milk samples (colostrum, transitional and mature milks) collected from 115 healthy women with full term newborns. Blood plasma was obtained from 58 women at 3 months postpartum. The TAC of samples were measured by Ferric Reducing/Antioxidant Power assay and free radical scavenging activity were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. TAC was obviously higher in colostrums than transitional and mature milks. Similar results were observed for DPPH radical scavenging activity of the samples. There was a high significant correlation between the results of these two methods. The relationship between the antioxidant content of human milk and maternal plasma was also significant. These data suggest that using colostrum, with high antioxidant potential during the first days of life is vital; moreover, reduction in total TAC during the course of lactation may needs more attention about nutritional status.

Key Words: total antioxidant activity, radical scavenging activity, colostrum, transitional milk, mature milk

Introduction

Human milk has been considered as a package of essential nutrients (vitamins, minerals, essential amino acids and fatty acids) and is commonly known as the best kind of nutrition for neonates and infants for the first six months of life.

Studies documenting the protective effect of breast milk against various infectious diseases in infants are presented, including respiratory infections, diarrhea, otitis media, and infections in premature infants [1, 2]. In addition to numerous clinically significance of breastfeeding, It seems human milk has bioactive components that protect newborns from a hyperoxic challenge due to transition of life to an environment far richer in oxygen than intrauterine environment [3, 4].

Oxygen is potentially toxic, because of the production of Reactive Oxygen Species (ROS). Since these components
have the ability to interact with and alter essential cell molecules, they are extremely cytotoxic. Antioxidant defense mechanisms of the body may prevent the production of ROS or neutralize them [5]. When the production of ROS exceeds the capacity of the body’s antioxidant defenses to detoxify them, a condition known as oxidative stress occurs. It is well believed that oxidative stress is involved in the pathogenesis of numerous neonatal diseases such as bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis [6, 7].

Human milk has a number of enzymatic and non-enzymatic antioxidant constituents, like superoxide dismutase, glutathione peroxidase, catalase, vitamin E, vitamin C, β-carotene, which may protect newborns against ROS at the early stage of life [6, 8]. However, for the best benefits and functions, milk will always be recognized as a synergistic mixture of multiple interacting factors. In the other hand, the antioxidant status of breast milk seems to be affected by the maternal antioxidant status, which in turn, could influence the antioxidant status of the breast-fed infants [9]. Although data on the content of individual antioxidants in milk are available [10–12], there is a necessity in using methods for investigating the total antioxidant activity of milk.

To measure all the antioxidant activity present in biological fluids, various methods have been devised [13, 14]. These assays are useful in getting a global picture of related antioxidant activities in body fluids and how they change in different conditions. The aim of this study is to evaluate the total antioxidant and free radical scavenging activity levels in human milk during the first six month period of lactation and its correlation with maternal plasma total antioxidant capacity. This evaluation was done by using the ferric reducing/antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical method as simple and reliable experiments adopted for this investigation.

Methods

This study was approved by the Ethics Committees of the Birjand University of Medical Sciences. Informed consent was obtained from every mother. A total of 115 healthy women (26.9 ± 5.3 year old) with normal pregnancy and delivery were included in this prospective consecutive study. The primary requisite for inclusion was intent to breastfeed exclusively. Exclusion criteria included gestation <37 weeks, birth weight <2.5 kg, multiple pregnancy, major illness requiring intensive care admission, and major congenital anomaly.

We tried to take sample milk at 5 different times but due to missing some mothers at some points, our final samples were consist of 115 samples of colostrum at the first 2 ± 1 days of postpartum, 97 samples of transitional milk at 7 ± 3 days postpartum and 293 samples of mature milks in three times (102 at 30 ± 3 days, 100 at 90 ± 7 days and 91 at 180 ± 10 days postpartum), among them we had just 68 cases with 5 complete samples. The samples were taken by manual expression of each breast into 10 ml sterile containers between 9.00–11.30 at morning and then aliquoted into 2 ml screw-top cryovials. Samples were stored at −70°C until analysis.

Blood samples were drawn by venipuncture (5 ml) into heparinized tubes but due to some limitations we just took blood samples at 90 ± 7 days postpartum for one time. The samples were immediately centrifuged to obtain plasma, which was then aliquoted into 2 ml cryovials and stored at −70°C until analysis.

Determination of total antioxidant activity

The FRAP assay, developed by Benzie and Strain [15] as a direct method for measuring the total antioxidant power of biological fluids, was adopted in this study. At low pH, reduction of a ferric 2,4,6-tripyridyl-s-triazine [Fe (III)-TPTZ] complex to the ferrous 2,4,6-tripyridyl-s-triazine [Fe (II)-TPTZ] complex, which has an intense blue color, can be monitored by measuring the change in absorption at 593 nm. The working FRAP reagent was prepared by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 1 volume of 10 mmol/L TPTZ in 40 mmol/L HCl and 1 volume of 20 mmol/L FeCl₃. A proper amount of sample (20 μl of breast milk or 50 μl of plasma) was mixed with 1.5 ml of freshly prepared FRAP reagent and incubated at 25°C for 10 min then reading was taken at 593 nm. To omit milk turbidity in experiments, 20 μl of breast milk and adequate volume of acetate buffer, was used as sample blank. Aqueous solutions of FeSO₄·7H₂O (100–1000 μM) were used for the calibration and the results were expressed as FRAP value (μM Fe (II)) of the samples.

DPPH radical scavenging activity

The free radical scavenging activity of milk samples were measured by the DPPH method proposed by Brand-Williams, Cuvelier, and Beres with a slight modification [16]. Briefly, 100 μl of each sample was added to 2 ml of DPPH in ethanol solution (100 mM) in a test tube. After incubation at 37°C for 30 min, 1 ml of chloroform was added and centrifuged at 3000 g for 5 min. the absorbance of clear solution was determined at 517 nm using spectrophotometer. An ethanolic solution of DPPH (100 mM) was used as control and the percentage of DPPH radical scavenging activity was calculated according to the following equation:

Scavenging activity (%) = 
[(absorbance of the control – absorbance of the sample) /absorbance of the control]* 100.
Statistical analysis

Statistical analysis was performed using the SPSS 11.5 package. The data were expressed as means ± standard deviation (SD). A paired-samples t test was used for comparison of means at different times with the colostrum samples and also statistical comparisons between all groups were made by analysis of variance (ANOVA) with repeated measures. The correlation between parameters was determined by Pearson correlation analysis. p values less than 0.05 were considered significant.

Results

The total antioxidant capacity and radical scavenging activity of breast milk in different times of lactation was presented in Table 1. According to the paired-samples t test, there was a significant higher level of total antioxidant capacity measured by FRAP assay in colostrum in comparison to transitional and mature milks. The total antioxidant levels showed a trend to decrease from 1061.6 ± 500.6 μmol/L in colostrum to 724.7 ± 302.4 μmol/L after six months of study. In the DPPH test for radical scavenging activity, the colostrums were more potent (50 ± 20%) to reduce the stable radical DPPH in comparison with transitional and mature milks. Also statistical comparison were made by analysis of variance with repeated measures in cases with complete sampling (n = 68). These balanced data showed significant differences between the means of values at different times (p = 0.015).

There was a significant correlation between the results of FRAP and DPPH methods for total antioxidant capacity and radical scavenging activity of the samples of breast milk (r = 0.562, p<0.001) (Fig. 1).

The mean of maternal plasma antioxidant capacity was 842.0 ± 123.5 μmol/L ranged from 565.2 to 1193.4 μmol/L. This level was closely near to the mean of breast milk antioxidant capacity at 90 ± 7 days that was 862.7 ± 457.7 μmol/L and ranged from 121.5 to 3816 μmol/L and there was a significant correlation between the maternal plasma and breast milk antioxidant capacity (r = 0.267, p<0.05) (Fig. 2).

Table 1. Total antioxidant capacity, DPPH radical scavenging activity values of colostrum, transitional and mature milks

<table>
<thead>
<tr>
<th></th>
<th>Colostrum</th>
<th>Transitional milk</th>
<th>Mature milk</th>
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<tr>
<td></td>
<td>2 ± 1 days (n = 115)</td>
<td>7 ± 3 days (n = 97)</td>
<td>30 ± 3 days (n = 102)</td>
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<tr>
<td>Total antioxidant capacity (μmol/L)</td>
<td>1061.6 ± 500.6</td>
<td>915.3 ± 511.4*</td>
<td>816.3 ± 379.4*</td>
</tr>
<tr>
<td>DPPH radical scavenging activity (%) (μmol/L)</td>
<td>50.4 ± 19.7</td>
<td>40.8 ± 20.0*</td>
<td>41.9 ± 19.4*</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SD and * indicate significant difference in comparison with colostrums (p<0.05).
Discussion

In our research, the most significant result is thatcolostrums in comparison to transitional and mature milks hasmore total antioxidant activity decreasing during the courseof lactation. There are only few reports that measured TACand free radical scavenging activity of breast milk. Recently,thesame pattern for total antioxidant capacity was reportedby Quiles et al. [17]. They showed that the total antioxidantcapacity of milk registered significantly higher values forcolostrums compared with mature and transitional milk.These authors used 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS+) assay for total anti-oxidant capacity of breast milk. Ezaki et al. reported thatTAC tends to decrease with the passage of time [18]. Theyused biological antioxidant potential (BAP) test for mea-suring of TAC which is principally very similar to FRAPassay. Also, Fidanza et al. reported a high, but not significantantioxidant capacity incolostrums [19]. The reason for thisdifference is probably due to the type of the methods usedfor total antioxidant capacity of milk and the number ofsamples. They used ORAC (oxygen radical absorbentcapacity) assay with 30 samples whereas we used FRPASSay with 115 samples.

In the FRAP method the ability of the sample to reducethe ferric ion is used as a criterion on antioxidant capacity.Ascorbic acid, α-tocopherol, uric acid, Bilirubin andphenolic compounds were found to have ferric-reducingactivity, but this method was suggested to be unsuitable forproteins, glutathione and lipoic acid [15, 20]. DPPH is a-stable commercial available free radical using as a popularmarker for screening of free radical scavenging activity ofcompounds or biological samples. This method measureshydrogen atom or electron-donating activity and thesavaging activity related to the structure of the activesubstances [16]. Since the activities of antioxidant may varyin different biological systems, it is necessary to employseveral methods to measure total antioxidant capacity basedon different principles [14]. In our results, it is shown thatthere is a positive correlation between the results of theFRAP and DPPH assays. So these simple, speedy andinexpensive methods may be considered as practical indica-tors of total antioxidant activity of biological samples anduseful in all the studies concerning the evaluation of oxida-tive stress.

High antioxidant capacity incolostrums can be effectivewith preventing newborns from exposure to an environmentrich in oxygen after birth, 4 to 5 times as much as inter-uterine environment [4]. There is some evidence thatsusceptible newborns, especially preterm ones, are poten-tially vulnerable to oxidative stress due to the inefficiencyof their antioxidant defense system or increase in free radicalproduction [21]. In this situation damage to main molecules,lipids, proteins, carbohydrates and nucleic acids mayincrease [22]. In this way, oxygen therapy in newborns withrespiratory problems which can induce and intensify oxida-tive stress should be considered more cautiously. Also it hasbeen recently demonstrated that phototherapy for jaundicedneonates is related to increased oxidative stress and shouldbe used with care [23].

Hence newborn feeding with the breast milk especiallycolostrums can be useful to neutralize free radicals andimprove antioxidant system. It has been shown that 8-OHdeoxyguanosine levels as a sensitive marker for oxidativestress is lower in infants fed with breast milk compared withthose fed with formula [24]. In another report an increase wasalso found in the frequencies of sister chromatid exchangefor infants not breast-fed compared to those who were breast-fed [25]. In addition according to epidemiologic studies, a reverse correlation between breast feeding andsome diseases such as diabetes mellitus, cancer and cardio-vascular diseases was found [1].

In this study total antioxidant capacity of the breast milkshowed a significant decrease during the course of lactationwhich can be a natural result of decline in antioxidantstorage of the mothers. In addition, a large variation wasobserved between total antioxidant values. Mothers withlow values need more attention with regard to their nutrientintake, especially natural antioxidants during lactation.

Moreover, a significant correlation was observed betweenmaternal plasma antioxidant capacity and their breast maturemilk at 3rd month, which this situation may be observed incolostrum and transitional milk. This is in accordance withtheresult of Vanderjagt et al. and can be used as a predictorof breast milk antioxidant capacity [26]. Despite closenessbetween the mean values of maternal plasma and breast milkantioxidant capacity, the variation among breast milk valuesis remarkable. On the other hand, since the uric acidconcentration is lower in breast milk in comparison withplasma [27], equal and higher levels of breast milk anti-oxidant capacity indicate that milk is concentrated for someantioxidants.

Conclusion

These data suggest that usingcolostrum, with high anti-oxidant capacity and radical scavenging activity during thefirst days of life is vital; moreover, reduction in total anti-oxidant capacity during the course of lactation and its rela-tion to maternal antioxidant status needs more attentionabout nutritional status of mothers. More investigationsprefer to evaluate the in vivo efficacy of breast milks withdifferent levels of total antioxidant capacity.
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References
