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## A comparison of total antioxidant levels in breast-fed and formula-fed infants

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### Summary

**Aim:** We aimed to evaluate the effect of breast milk and formula on the total antioxidant capacity of the plasma.

**Material and Method:** The oxidative stress index was calculated by measuring plasma total antioxidant level and total oxidant level in 35 infants between the ages of 3 and 6 months.

**Results:** No significant differences were observed between the two groups in respect of age, gender, plasma total antioxidant status and total oxidant status. Plasma total antioxidant levels were higher in the breast-fed group than the formula-fed group ( $p=0.004$ ). Plasma total antioxidant status was lower in the breast-fed group compared to the formula-fed infants ( $p=0.019$ ). The oxidative stress index values of the breast-fed group were lower than formula-fed infants ( $p=0.006$ ).

**Conclusions:** Breast milk provides better antioxidant defence than does formula in infants before six months of age. (*Turk Arch Ped 2012; 47: 97-100*)

**Key words:** Breast feeding, total antioxidant status, infants

### Introduction

Breastmilk is a natural food with high bioavailability which can be digested easily and which contains all fluid, energy and nutritional elements required for growth and development of the newborn. Breastmilk and breastfeeding have many benefits in nutritional, developmental, psychological, social and economic aspects for both the baby and the mother (1).

Burning of carbohydrates and lipids for providing energy in biological systems cause formation of free oxygen radicals (2). The most important free radicals in living organisms are the radical derivatives of oxygen. These play an important role in fighting of the organism against foreign substances and infectious agents. If the balance between production of free radicals and antioxidant systems is disrupted, the structures and function of the proteins, lipids, nucleic acids and enzymes which are the basic structural elements of the body may become disrupted (2,3).

Protective enzymes and chemical compounds are synthesized to protect cells against the damaging effects of free oxygen radicals (2,4). If the dimensions of oxidative pressure are huge or the response is insufficient, oxidative damage occurs (2,5,6).

Newborns and especially preterm newborns have been shown to have insufficient anti-oxidative capacity against oxygen radicals (7). The amount and activity of antioxidant enzymes increase as late as in the last months of pregnancy and it has been reported that total antioxidant capacity increases rapidly after birth and reaches higher levels than observed in adults. It was found that healthy newborns are protected better against oxidative damage compared to adults due to bilirubin (8-9).

The highest contribution to the total antioxidant capacity comes from antioxidant molecules in the plasma. Feeding type may also affect antioxidant capacity. Bilirubin and karotenoids which are antioxidants are found to be higher in infants fed with breastmilk compared to infants fed with formula (10-12). The measurement of total antioxidant capacity gives more valuable information compared to individual measurements of antioxidants. There are limited number of studies on this subject. Therefore, we tried to determine the effect of feeding type on total antioxidant defense in infants younger than 6 months of age.

### Material and Method

Healthy infants aged between 3 and 6 months who were born at term and who had no history of perinatal asphyxia,

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infection or bleeding were included in our study. 16 of the infants constituted the group who were fed only with breastmilk since birth and 18 constituted the group who were fed only with formula because of different causes (absence of breastmilk, working mother, etc.). The subjects were not using any drug or vitamin except for vitamin D. Infants who had a history, signs and symptoms of acute or chronic disease and who were passive smokers were not included in the study. Our study was approved by the ethics committee on 10.14.2009 with the decision number 10/7 and informed consent was obtained from the families.

The total antioxidant capacity was measured in the serum using Erel method (13). In this method, 2,2'-azinobis - 3 - ethylbenzothiazoline - 6 - sulfonic acid radical (ABTS radical) is used. This method is based on the oxidation of ABTS molecule to ABTS+ molecule in the presence of hydrogen peroxide. ABTS radical loses its blue and green color according to the amount of antioxidant and antioxidant capacity. This change of color is evaluated by measuring the absorption at 660 nm. There is an inverse relation between the change of color and the amount of antioxidant in the sample. The speed of the reaction can be adjusted with Trolox which is a known method. The unit is Trolox equivalent/L.

The measurement of total oxidant level (TOL) was done using a full automated calorimetric method which was developed by Erel (13). The unit is  $\mu\text{mol H}_2\text{O}_2 \text{ Eqv} / \text{L}$ .

The oxidative stress index (OSI) is calculated by dividing the total oxidant level (TOL) to the total antioxidant level (TAL) and the unit is AU.

In this study, statistical analyses were done using NCSS 2007 package program. In addition to descriptive statistical methods (mean, standard deviation), Mann-Whitney-U test was used for comparison of paired groups, chi-square test was used for comparison of qualitative data and Pearson correlation test was used to determine the relation between variables in assessment of the data. A p value of <0.05 was considered to be significant.

## Results

34 infants were included in the study. The group fed with breastmilk was composed of 16 infants 9 of whom were male and 7 of whom were female. The group fed with formula was composed of 18 infants 9 of whom were male and 9 of whom were female.

There was no statistically significant difference between the two groups in terms of mean age and age (Table 1).

While mean TAL in the breastmilk group was found to be significantly higher compared to the formula group ( $p=0.004$ ), mean TOL was found to be significantly lower ( $p=0.019$ ). Mean OSI in the breastmilk group was found to be statistically significantly lower compared to the formula group ( $p=0.006$ ) (Table 2).

There was no statistically significant difference between the female and male infants in terms of mean age, TAL, TOL and OSI in the breastmilk group (Table 3). Similarly, there was no statistically significant difference between the female and male

**Table 1. Mean ages and gender distribution in the group fed with breastmilk and the group fed with formula**

		Breastmilk group (n:16)		Formula group (n:18)	p
Age (months)		4.03±0.94		4.39±0.7	p=0.085
Gender					
Male	9	56.3%	9	50%	$\chi^2:0.13$
Female	7	43.8%	9	50%	p=0.716

**Table 2 . Mean TAS (1), TOS (2) , OSI (3) values in the group fed with breastmilk and the group fed with formula**

	Breastmilk group	Mama grubu	p
Total antioxidant levels	2.68±0.58	2.09±0.4	0.004
Total oxidant levels	25.95±9.79	34.58±9.05	0.019
Oxidative stress index values	10.44±4.75	17.94±9.5	0.006

TAS<sup>(1)</sup>: Total antioxidant level, TOS<sup>(2)</sup>: Total oxidant level, OSI<sup>(3)</sup>: Oxidative stress index

**Table 3. The relation between age and mean TAS<sup>(1)</sup>, TOS<sup>(2)</sup>, OSI<sup>(3)</sup> values in male and female patients in the group fed with breastmilk**

Breastmilk Group	Male	Female	p
Age(months)	3.61±0.49	4.57±1.13	0.06
Total antioxidant levels	2.44±0.28	2.98±0.75	0.138
Total oxidant levels	27.85±9.66	23.51±10.14	0.368
Oxidative stress index	11.65±4.41	8.88±5.04	0.266

TAS<sup>(1)</sup>: Total antioxidant level, TOS<sup>(2)</sup>: Total oxidant level, OSI<sup>(3)</sup>: Oxidative stress index

**Table 4. The relation between age and TAS<sup>(1)</sup>, TOS<sup>(2)</sup>, OSI<sup>(3)</sup> values in the groups fed with breastmilk and formula**

		Breastmilk group	Formula group
Total antioxidant levels	r	0.275	0.11
	p	0.302	0.663
Total oxidant levels	r	0.172	-0.005
	p	0.525	0.986
Oxidative stress index	r	0.015	-0.102
	p	0.957	0.687

TAS<sup>(1)</sup>: Total antioxidant level, TOS<sup>(2)</sup>: Total oxidant level, OSI<sup>(3)</sup>: Oxidative stress index

infants in terms of mean age, TAL, TOL and OSI in the formula group ( $p=0.05$ ). Thus, mean TAL, TOL and OSI values were not affected by gender in both study groups.

When TAL, TOL and OSI values by age were compared between the group fed with breastmilk and the group fed with formula, no significant difference was found between the two groups ( $p=0.05$ ) (Table 4).

## Discussion

The metabolism fights against oxidative effects with its own defense mechanisms. Elimination and disarming of reactive oxygen species occur by enzymatic and non-enzymatic antioxidant mechanisms (14). Practically, TAL represents all of these compounds. In our study in which we evaluated the antioxidant and oxidative effects of breastmilk and formula in infants aged between 3 and 6 months, plasma TAL value was found to be higher in the group fed with breastmilk compared to the group fed with formula. TOL value was found to be higher in the formula group compared to the breastmilk group. No significant relation was found in TAL and TOL values in terms of age and gender in all groups in our study.

The effect of feeding type on the oxidative-antioxidative system and on development of diseases in newborns and infants is continuing to be an area of research. In the study performed by Ayçiçek et al. (15) in which they evaluated 44 healthy infants fed with breastmilk or formula, the total antioxidant capacity value in the breastmilk group was shown to be higher compared to the formula group. Again in the same study, total peroxide level which shows oxidative stress was found to be high in infants fed with formula. In addition, albumin, uric acid and bilirubin which are compounds of the non-enzymatic antioxidant mechanism were measured separately in both groups and found to be slightly higher in the breastmilk group compared to the formula group. Interestingly, it was observed that this slightly higher value markedly increased the total antioxidant capacity. Sommerburg et al. (11) found karotenoids which are strong antioxidants to be higher in newborns fed with breastmilk in the study in which they investigated plasma karotenoid levels in newborns fed with breastmilk and formula. Again in the same study, the karotenoid content of colostrum was found to be 5 fold higher compared to the mature breastmilk. Ezaki et al. (16) compared the breastmilk of the mothers of preterm newborns with formula in terms of antioxidant capacity and found that the total antioxidant capacity of breastmilk was higher. Even in cases in which breastmilk had to be frozen and kept for very small, preterm and sick newborns, total antioxidant capacity of breastmilk was found to be higher compared to formula. This may partly help to explain why preterm babies fed with breastmilk develop necrotizing enterocolitis and retinopathy of prematurity with a lower rate compared to babies fed with formula (16-19). Friel et al. (20) found no relation between feeding type and measurement results in the study in which they measured catalase and

dismutase enzyme activities in infants to evaluate oxidative pressure. Granot et al.(21) measured blood malondialdehyde which is a free radical triggering lipid peroxidation in a study in which they compared infants aged between 2 and 4 months fed with breastmilk and formula and found lower malondialdehyde levels in the group fed with formula. They related this finding to the fact that breastmilk contains higher long-chain fatty acids. In our study, the total oxidant level and oxidative stress index in the group fed with formula were found to be higher compared to the group fed with breastmilk. This high oxidative stress in the group fed with formula was thought to be related to the free iron contained in formulas. Free iron is a strong catalyzer increasing the formation of oxygen radicals with a 4000 fold higher rate. Relatively high free iron contained in formulas and absence of an iron binding antioxidant like lactoferrin contained in breastmilk may be responsible of the oxidant effect found in formulas (15,21-23).

Necrotizing enterocolitis, sepsis and respiratory diseases are observed with a lower rate in infants fed with breastmilk (18,22-24). Disruption of the oxidative and antioxidative balance may increase the development of these kind of diseases. When all these observations and findings are combined with our study, it is observed that more studies are needed to demonstrate the effect of feeding with breastmilk and formula on oxidative-antioxidative system and development of diseases.

Many studies performed so far have shown that feeding with breastmilk is protective against many diseases. One of these protective mechanisms is clearly antioxidant defense. The results of our study showed that breastmilk increased antioxidant defense. Since formula is being used increasingly more in our country, this benefit and similar benefits of breastmilk wait for being elucidated and investigated more extensively.

**Conflict of interest: None declared.**

## References

1. Samur G. Anne Sütü. Ankara: T.C. Sağlık Bakanlığı Temel Sağlık Hizmetleri Genel Müdürlüğü Beslenme ve Fiziksel Aktiviteler Daire Başkanlığı, 2008.
2. Scandalios JG. The rise of ROS. Trends Biochem Sci 2002;27:483-6.
3. Yiğit S, Yurdakök M, Kilin K, Oran O, Erdem G, Tekinalp G. Serum malondialdehyde concentration in babies with hyperbilirubinaemia. Arch Dis Child Fetal Neonatal Ed 1999;80:235-7.
4. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043-6.
5. Yesilkaya A, Altınayak R, Korgun DK. The antioxidant effect of free bilirubin on cumene-hydroperoxide treated human leukocytes. Gen Pharmacol 2000;35:17-20.
6. Dani C, Mertelli E, Bertini G, et al. Plasma bilirubin level and oxidative stress in preterm infants. Arch Dis Child Fetal Neonatal Ed 2003;88:119-23.
7. Kılınç K, Kılınç E. Oksijen toksitesinin aracı molekülleri olarak oksijen radikalleri. Hacettepe Tıp Dergisi 2002;33:110-8.
8. Wiedemann M, Kontush A, Finckh B, Hellwege HH, Kohlschütter A. Neonatal blood plasma is less susceptible to oxidation than adult plasma owing to its higher content of bilirubin and lower content of oxidizable fatty acids. Pediatr Res 2003;53:843-9.

9. Robles R, Palomino N, Robles A. Oxidative stress in the neonate. *Early Hum Dev* 2001;65 Suppl:75-81.
10. Korkmaz A, Yurdakök M. Hiperbilirubinemili yenidoğan bebeklerde serum bilirubin ve ürik asit düzeyleri arasındaki denge. *Çocuk Sağlığı ve Hastalıkları Dergisi* 2001;44:338-41.
11. Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M. Carotenoid supply in breast-fed and formula-fed neonates. *Eur J Pediatr* 2000;159:86-90.
12. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 1987;235:1043-6.
13. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004;37:277-85.
14. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 1994;344:721-4.
15. Ayçiçek A, Erel O, Koçyiğit A, Selek S, Demirkol MR. Breast milk provides better antioxidant power than does formula. *Nutrition* 2006;22:616-9.
16. Ezaki S, Ito T, Suzuki K, Tamura M. Association between total antioxidant capacity in breast milk and postnatal age in days in premature infants. *J Clin Biochem Nutr* 2008;42:133-7.
17. Hanna N, Ahmed K, Anwar M, Petrova A, Hiatt M, Hegyi T. Effect of storage on breast milk antioxidant activity. *Arch Dis Child Fetal Neonatal Ed* 2004;89:518-20.
18. Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. *Lancet* 1990;336:1519-23.
19. Cunningham AS. Breast feeding, antioxidants and the retinopathy of prematurity. *Am J Obstet Gynecol* 1987;156:1040-1.
20. Friel JK, Friesen RW, Harding SV, Roberts LJ. Evidence of oxidative stress in full term healthy infants. *Pediatr Res* 2004;56:878-82.
21. Granot E, Golan D, Rivkin L, Kohen R. Oxidative stress in healthy breast fed versus formula fed infants. *Nutr Res* 1999;19:869-79.
22. Aslan R, Şekeroğlu MR, Bayiroğlu F. Serbest radikal türlerinin lipid peroksidasyonuna etkileri ve antioksidan savunma. *Y.Y.U. Sağlık Bil. Derg* 1995;2:137-42.
23. Crissinger KD. Understanding necrotizing enterocolitis-promising direction. *Pathophysiology* 1999;5:247-56.
24. Oddy WH, Sly PD, de Klerk NH, et al. Breast feeding and respiratory morbidity in infancy: a birth cohort study. *Arch Dis Child* 2003;88:224-48.