A Comparative Study of Oxidant and Antioxidant Levels Between Human Milk With Other Types of Ruminant Animals

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Abstract

The present study was conducted in Mosul city to show the oxidants and antioxidants levels in different types of mature milk for (Human, buffalo, cow, sheep, goat) and compared them with each other, beside of showing the effect of age on the composition of human breast milk by measuring biochemical parameters which include: vitamin E, vitamin C, superoxide dismutase (SOD), glutathione S-transferase(GST), albumin, ceruloplasmin (Cp), glutathione(GSH), total bilirubin, uric acid, malondialdehyde (MDA), peroxynitrate(OONO-) and the metals (Copper (Cu), zinc (Zn) and iron (Fe)). The study included (144) sample represented in five groups according to types of milk: human milk (59 nonsmokers), buffalo milk(19), cow milk(23), sheep milk(22) and goat milk(21) .

The results showed significant increase in vitamin E for sheep, human, cow, goat and buffalo milk respectively, but showed significant increase for vitamin C in sheep milk and decreased in cow milk when compared with other groups. Also SOD showed high levels in human and goat milks, but low levels in sheep milk, while GST showed high significant levels in buffalo and cow milk, and lower in sheep milk. The GSH observed significant increased in human milk, but decreased in buffalo, beside of increased Cp in goat milk and decreased in human milk.

Moreover, it has been observed that there was significant increase in uric acid, for cow milk, but decreased in buffalo milk. On the other hand, the total bilirubin observed significant increased in sheep milk, but decreased in cow milk. The MDA observed significant increase in buffalo milk, but decreased in sheep milk and $ONOO^-$ levels observed significant increased in cow milk, but decreased in buffalo when compared with other types milk. After the determination of the elements, the results showed a significant increased in Cu of human milk and decreased in sheep milk, while Zn levels increased significantly in buffalo milk but decreased in human milk and iron increased significantly in cow milk but decreased in human milk. Depending on age increasing of lactating women, mature milks were divided into three subgroups (16-25), (26-35) and (36-42) year. It was found that vitamin E, SOD, GSH, T.Bilir., uric acid and iron were decreased significantly for mature milk with the age increasing for lactating women, but increased in: GST, Alb, MDA and Cu.

In conclusion, an increased antioxidants and decreased oxidants levels in: sheep milk, goat milk, cow milk, buffalo milk and human milk respectively. On the other hand, the study showed decreased antioxidant and increased oxidants levels in human breast milk with increasing aging of women and that might be affected on infants health.

Keywords: Human milk, Buffalo milk, Sheep milk, Cow milk, Goat milk, Antioxidants, Oxidants, age.

الخلاصة

أجريت الدراسة في مدينة الموصل لمعرفة تأثير العمر على مستويات الأكسدة ومضادات الأكسدة في أنواع مختلفة من الحليب الناضج (الإنسان والجاموس والبقر والغنم والماعز) والمقارنة بينهم خلال تقدير مستوى بعض المتغيرات الكيموحيوية التي اشتملت على: فيتامين E وفيتامين C وإنزيم سوبراوكسايد ديسميوتيز (SOD) وإنزيم كلوتاثايون S-ترانس فيريز (GST) والألبومين والسليروبلازمين(Cp) وكلوتاثايون (GSH) والبليروبين الكلي وحامض اليوريك والمالوندايألديهايد (MDA) وبيروكسي نيتريت (-ONOO) والمعادن(النحاسCu والخارصين Zn والحديدFe). وتضمنت الدراسة 144 نموذجا قسمت الى خمسة مجاميع حسب أنواع الحليب: 59 نموذج من حليب الامهات غير المدخنات، و 19 نموذج من حليب الجاموس و23 نموذج من حليب البقر و22 نموذج من حليب الغنم و21 نموذج من حليب الماعز.

دلت النتائج على وجود ارتفاع معنوي لفيتامين E في كل من حليب الغنم، الإنسان، البقر، الماعز والجاموس على التوالي، ولكن هناك ارتفاع معنوي لفيتامين C في حليب الغنم وانخفاض في حليب البقر عند مقارنة مع بعضهم البعض. لوحظ أيضا ارتفاع معنوي لمستوى SOD في حليب الإنسان والماعز ولكن انخفاض في حليب الغنم، اما GST لوحظ ارتفاعها معنويا لمستواها في حليب الجاموس والبقر ولكن انخفاضها في حليب الغنم. وأشارت النتائج أيضا الى ارتفاع معنوي لـ GSH في حليب الإنسان وانخفاضها في حليب الجاموس، بالإضافة الى ارتفاع معنوي في حليب الماعز للـ Cp وانخفاضها في حليب الإنسان.

علاوة على ذلك، لوحظ ارتفاع معنوي في حليب البقر وانخفاض في حليب الجاموس لحامض اليوريك. ومن جانب أخر لوحظ ارتفاع معنوي لبليروبين الكلي في حليب الغنم وانخفاضها في حليب البقر. كما أشارت النتائج الى ارتفاع معنوي لـ MDA في حليب الجاموس ولكن انخفاضها في حليب الغنم وارتفاع -ONOO في حليب البقر وانخفاضها في حليب الجاموس عند مقارنتهم مع أنواع الحليب الأخرى.

وعند قياس العناصر المعدنية لوحظ ارتفاع معنوي للنحاس في حليب الإنسان وانخفاضها في حليب الغنم ولكن لوحظ ارتفاع معنوي للخارصين في حليب الجاموس وانخفاضها في حليب الإنسان وارتفاع معنوي للحديد في حليب البقر وانخفاضه في حليب الإنسان.

بالاعتماد على زيادة العمر للمرأة المرضعة، قسم الحليب الناضج الى ثلاث أقسام (16−25)سنة، (26−35) سنة (36−42) سنة. إذ لوحظ انخفاضا" معنويا للمتغيرات: فيتامين E، SOD، GSH، البليروبين الكلي، حامض اليوريك وحديد، وارتفاع معنوي في:إنزيم GST ، Alb ، MDA و Cu ًفي الحليب الناضج مع زيادة عمر المرضعة.

نستنتج من ذلك، زيادة مستويات مضادات الأكسدة وانخفاض في الأكسدة لحليب كل من: الغنم، الماعز، الجاموس، البقر، والإنسان على التتابع، ومن جانب أخر لوحظ انخفاض في مستويات مضادات الأكسدة وزيادة الأكسدة مع تقدم العمر في حليب الثدي للإنسان والذي قد يؤثر فيما بعد على صحة الأطفال حديثي الولادة.

الكلمات الدالة: حليب الإنسان، حليب البقر، حليب الغنم، حليب الجاموس، حليب الماعز، مضادات الأكسدة، الأكسدة، العمر.

Introduction

Human milk is the optimal nutritional source for infants and children in the first months of life. In addition to the nutrients required for infant development, it contains immune defense and growth promoting factors [1]. Human milk is a source of multiple components, enzymatic and non-enzymatic antioxidant constituents which prevent oxidative rancidity[2].

The antioxidant enzymes found in human milk include superoxide dismutase (SOD), catalase, glutathione peroxidase [3] and coenzyme Q_{10} [4]. Some non-enzymatic antioxidants can be synthesized in the body such as iron-binding protein (Lactoferrin), while others have to be supplied with food including ascorbic acid, tocopherols, tocotrienols, carotenoids and selenium [3]. Antioxidants can scavenge radicals, hydrogen peroxide and other peroxides.

Fetal life is sustained under low oxygen tension; however, as breathing begins immediately after birth, a rapid increase in tissue oxygenation takes place[5] that might increase oxidants compounds. The antioxidant defense system matures during gestation. Term newly born infants are perfectly adapted to face this physiologic situation [6]. Hence, it has been shown that postnatal oxidative stress up-regulates expression of specific genes whose end products are beneficial in postnatal adaptation[7]. Premature neonates, however, are endowed with an immature antioxidant defense system and therefore are highly susceptible to the deleterious effects of reactive oxygen species (ROS) generated in the fetal to neonatal transition[7]. In addition, requirement of supplemental oxygen to treat respiratory immaturity worsens oxidative stress[8]. Under hyperoxia or during reoxygenation, mitochondrial superoxide anion and hydrogen peroxide production is enhanced. These ROS, in the presence of ferrous iron, result in many oxidants compounds (Radicals or non radicals) capable of causing damage to nearby molecules, such as phospholipids, proteins, and nucleic acids[9].

Some major reviews exist concerning the biochemical composition of human, cow, buffalo, goat and sheep milk and their variation[10, 11] but data concerning specific molecules with oxidants produced (MDA, ONOO), or antioxidants levels (Vitamins or enzymes antioxidants and other) are scant. Moreover, the effect of increasing age of lactating women on milk composition are absent.

Materials and Methods

a. Collection of human milk:

The composition of milk is varied in different lactating months, we collected samples of mature milk (30–60 days postpartum) from (59) milk donors of Al-Mansore health center- Mosul city, between December 2011 and October 2012. The detailed procedure was used for collecting breast milk which is described elsewhere in this study. The lactating women, aged (16-42) year, were without pharmacological treatment. Milk samples were collected by the mothers morning feeding (between 8 and 10 a.m.) in sterile plastic.

b. Collection of animal milk samples:

Eighty five fresh milk samples of the studied animals were collected in sterile bottles by direct milking, from four different non-dairy species include (buffalo milk 19), (cow milk 23), (sheep milk 22) and (goat milk 21) from Mosul city.

The samples were stored in container and delivered to laboratory within six hours after collection, and immediately frozen at –20°C until analysis. The biochemical parameters determination used manual methods (Table 1).

Table 1: Methods used to determinate biochemical parameters.

Measured parameters	Used method
Vitamin E	Emmerie-Engel reaction[12]
Vitamin C	2,4-dinitrophenylhydrazine derivatization method[13]
SOD	modified photochemical Nitroblue tetrazolum (NBT) method[14]
GST	1-chloro-2,4-dinitrobenzene(CDNB)conjugation[15]
Alb	Bromocresol green method(dye binding method) [16]
Cp	p-Phenylenediamine oxidase method[17]
GSH	Modified procedure utilizing Ellman`s reagent [18]
Total bilirubin	Diazo method[19]
Uric acid	Phosphotungstic acid method[20]
MDA	Thiobarbituric acid method[21]
$ONOO^-$	Nitration of phenol method [22]
Cu	Atomic absorption photometric method[20]
Zn	
Fe	

Body Mass Index (BMI) calculated by using the formula as weight (kg)/height^2(m^2) [23].

Statistical analysis

A thorough statistical analysis was performed to the experimental data gathered, using the (SPSS) version 15.0 for Windows (SPSS Inc., Chicago, IL, USA) and the results are expressed as mean $\pm$ standard deviation (SD) by applying Anova and Duncan test. Significant differences were assigned at $p \leq 0.05$[24].

Results and Discussion

1. Comparison of oxidants and antioxidants levels between many types of mature milk

The results of oxidants and antioxidants levels of many types of mature milk groups were listed in Table (2).

The results showed that there was significantly increased($p \leq 0.05$) in vitamin E for sheep, human, cow, goat and buffalo milk respectively, but showed significantly increased for vitamin C in sheep milk but decreased in cow milk when compared with other groups. These results were in a good agreement with other reported results[25] for vitamin E in human milk.

Vitamin E present in most milk types, but increased in sheep when compared with other groups, it's a very important antioxidant in protect lipid membranes from oxidants compounds. Vitamin E reacts rapidly with radicals and forms a stable tocopherol radical which can be reduced by ascorbic acid to tocopherol. Vitamin C also scavenges singlet oxygen, superoxide, peroxyl and hydroxyl radicals[26]. These antioxidant components are active in preventing lipid peroxidation and maintaining milk quality, and may also be used as ingredients in foods and pharmaceuticals to provide products for enhancing consumer health.

For SOD levels showed elevation in human and goat, but decreased in sheep milk, and GST showed significant increased in buffalo and cow milk, but low levels in sheep milk. The GSH observed significant increased in human milk and decreased in buffalo, beside of increased Cp in goat milk and decreased in human when compared with other milk types.

The increased Cp its important as a copper carrier protein because it binds copper tightly and prevent produced oxidants compounds by Fenton reactions or Haber-Weiss reactions[27].

Table 2: The oxidants and antioxidants levels of different types of milk.

Parameters	(Human milk) n=59		(Buffalo milk) N=19		(Cow milk) n=23		(Goat milk) n=21		(Sheep milk) n=22	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Vit.E(mg/dl)	2.07 **b**	0.56	0.57**e**	0.095	2.0 **c**	0.52	1.8 **d**	0.14	2.44 **a**	0.54
Vit.C(mg/dl)	4.88 **a**	1.5	3.66 **b**	0.45	2.94 **b**	0.8	5.5 **a**	1.49	5.77 **a**	0.3
SOD*	0.87 **a**	0.11	0.12 **b**	0.006	0.048 **c**	0.0049	0.76 **a**	0.19	0.0063 **d**	0.00051
GST (U/l)**	0.0113**c**	0.001	0.07 **a**	0.017	0.041 **b**	0.0008	0.003 **d**	0.00026	0.0006 **e**	0.000037
Alb (gm/dl)	4.78 **a**	0.35	4.89 **a**	0.17	4.72 **a**	0.44	4.84 **a**	0.36	4.83 **a**	0.49
Cp(mg/l)	23.09 **e**	6.99	29.74 **d**	6.37	46.7 **c**	9.12	96.6 **a**	30.48	72.05 **b**	24.13
GSH(µmol/l)	28.91 **a**	5.76	7.86 **c**	1.56	17.3 **b**	5.56	19.0 **b**	2.98	17.44 **b**	4.41
Total Bilirubin (mg/dl)	7.19 **c**	1.78	14.84 **b**	3.73	1.3 **d**	0.23	7.74 **c**	1.24	22.60 **a**	7.29
Uric acid (mg/dl)	47.86 **d**	14.78	38.37 **d**	5.57	177.29 **a**	2.58	94.02 **c**	29.83	113.07 **b**	36.24
MDA(µmol/l)	0.34 **a**	0.07	0.32 **a**	0.071	0.18 **b**	0.03	0.005 **c**	0.00053	0.0051 **d**	0.00025
ONOO-(µmol/l)	72.75 **d**	9.92	12.1 **e**	2.66	204.97 **a**	10.59	103.17 **b**	15.8	87.5 **c**	24.72
Cu(mg/l)	0.49 **a**	0.081	0.42 **a**	0.012	0.34 **b**	0.0013	0.37 **b**	0.0012	0.212 **c**	0.00095
Zn(mg/l)	0.63 **e**	0.16	2.8 **a**	0.15	1.57 **b**	0.0021	0.99 **c**	0.0022	0.79 **d**	0.0008
Iron(mg/l)	1.62 **d**	0.30	2.2 **c**	0.42	3.47 **a**	0.0095	2.85 **b**	0.0021	2.64 **b**	0.0013

-Different letters horizontally a, b, c, d, e indicate that the means are different significantly at $p \leq 0.05$ according to Duncan test.

* SOD: differences between absorbance before and after excitation by fluoresce light.

** U: a mount of glutathione S-transferase (GST) catalyzing the formation of one micromole of product per min under of optimum conditions.

As a consequence of aerobic metabolism, small amounts of reactive oxygen species, including superoxide radicals, hydroxyl radicals, hydrogen peroxide and peroxide radicals and its related radicals, are constantly generated within certain cells of certain organisms. The accumulation of peroxidants in the human body has been reported to be associated with disorders such as cancer, atherosclerosis, hypertension, and arthritis[28]. To avoid cellular damage by these peroxidants, most biological systems have developed inherent antioxidant systems, for example, SOD, GST, GSH, total bilirubin and uric acid, in order to protect from damage caused by the oxidant compounds[29]. Such systems, however, are not totally effective and do not prevent damage universally[30, 31], hence, there is an increasing interest world-wide in finding natural food-based antioxidants that are able to protect the human body from attack by free radicals and thus retard the progress of many chronic diseases, as well as retarding the lipid oxidative rancidity in all types of milk[32].

Moreover, it has been observed that there was significantly increase($p \leq 0.05$) in uric acid for cow milk, but decreased in buffalo milk and human, on the other hand, the total bilirubin observed significant increase in sheep milk, but decreased in cow milk when compared with other types milk.

Uric acid is a modestly water-soluble antioxidant with the ability to neutralize a broad spectrum of ROS, particularly singlet oxygen and free radicals. Its can accept a single electron to form a stable radical, and thereby neutralize a variety of ROS species[31], therefore it elevated with age increasing to defense against oxidants compounds.

Bilirubin is a powerful lipophilic antioxidant that protects membranes from lipid peroxidation and protects membrane proteins from oxidation[33]. Much of the power of bilirubin as an antioxidant comes from the extreme rapidity with which biliverdin (Oxidized bilirubin) is converted to bilirubin by bilverdin reductase[26].

The MDA observed significant increased($p \leq 0.05$) in buffalo and human milk, but decreased in sheep milk when compared with other types of milk, this indicated the marker of oxidative stress increased in buffalo milk but decreased in sheep milk. There are many factors such as storage temperature, dissolved oxygen, unsaturation degree of fatty acids, content of antioxidants, and metal prooxidants, etc., have an impact on oxidation intensity of milk lipids and others[34], therefore increased MDA could be a marker for oxidative stress and might be refer to increase lipid peroxidation in buffalo milk compared to other milk especially sheep milk occur oxidative stress[35].

On the other hand a significant increase in peroxynitrate ($ONOO^-$) in cow milk, but decreased in buffalo and human milk was a good indicator to increased superoxide radical anion accompanied with nitric oxide radical in cow milk compared to other types milk.

The Cu observed significant increased in human milk and buffalo, but decreased in sheep when compared with other kinds of milk, but Zn showed significant increased in buffalo milk, while decreased in human milk when compared with other milk. This result was in a good agreement with other reported results(36) for Zn in goat and cow milk.

The concentrations of Cu, Zn and Fe found in human whole milk in this study are well in agreement with values obtained in our previous studies(37, 38, 39).

The mineral content of ruminant types of milk depends on many factors such as the type of feed, stage of lactation, breed of animals and milk protein(40). Milk contains most of the necessary elements for a healthy diet, and in some groups, such as children and the elderly, it may constitute the main or even the only food(41, 42), but when accumulation of copper in turn leads to serious chronic liver injury and neurological dysfunction(43). Copper chelating agents are widely used to restore hepatic copper homeostasis, but they must be administrated over a lifetime and have little effect in severe cases.

The Iron observed significant increased in cow milk, but decreased in human milk. Iron and zinc deficiency in human milk are public health concerns during infancy, especially in developing countries(44). Iron deficiency in infancy may lead to poor psychomotor development(45), and zinc deficiency may lead to stunted growth and compromised immune function(46).

Several factors have been found to influence the extent of oxidation in the animal milk (Cow, goat, buffalo and sheep) including the presence of different prooxidants such as transition metals (Cu^{2+} and Fe^{3+}) and the level of natural antioxidants such as the tocopherols, uric acid, and ascorbic acid(42). The degree of unsaturation in milk fat has a significant influence on oxidative stability of milk, as a higher proportion of unsaturated fatty acid results in inferior oxidative stability of milk(47, 48).

In presences of free iron has strong pro-oxidant abilities, especially in cow milk, compared to human milk. Probably, the pro-oxidant activity of vitamin C results from the reduction of ferric iron to the ferrous form (equation 1) (49):

$Fe^{3+} + \text{vit C} \longrightarrow Fe^{2+} + \text{vit C}^{\cdot} + H^{+}$ ….. (1)

The intermediate vitamin C radical (vit $C^{\cdot}$) may reduce another ferric ion (equation 2) or may proceed to vitamin C and dehydroascorbate (DHA) (equation 3):

$Fe^{3+} + \text{vit C}^{\cdot} \longrightarrow Fe^{2+} + \text{DHA}$….. (2)

$2\ \text{vit C}^{\cdot} + 2H^{+} \longrightarrow \text{vit C} + \text{DHA}$…… (3)

As hydrogen peroxide is also produced during oxidation of vitamin C (equation 4) the requirements of the Fenton reaction (equation 5) are present and the reactive hydroxyl radicals are produced:

$vit\ C + O_2 \longrightarrow DHA + H_2O_2$….(4)

$Fe^{2+} + H_2O_2 \longrightarrow OH^- + {}^{\cdot}OH + Fe^{3+}$…….(5)

Therefore decreased free iron might be decreased pro-oxidant compounds.

The GST increase when present is best known for its role in detoxification of environmental carcinogens. However, they also catalyze specific reactions in a number of biosynthetic and catabolic pathways and play an important role in defense against oxidative stress by reducing peroxides and dehydroascorbate(26).

2.Effect of age increasing of lactating women on mature milk composition (oxidants and antioxidants):

Depending on the increasing age of lactating women, mature milks were divided into three subgroups (16-25),(26-35) and (36-42) year.

It was found that vitamin E, SOD, GSH and Fe were significantly decreased ($p \leq 0.05$) with age increasing of lactating women, but increased significantly in: GST, Alb, T.Bilir., uric acid, MDA and Cu .These results were in a good agreement with other reported results(50, 51) for vitamin E and Cu.

The composition of human milk may be influenced by different variables, such as genetic characteristics, dietary habits, nutritional and socioeconomic state of the mother, duration of lactation and length of gestation(52).

There is increasing evidences that aging is caused by the deleterious and accumulative effects of reactive oxygen species (ROS) generated throughout the life span(53). Therefore this might be decreased antioxidants compounds (Vitamin E and GSH) which defense against ROS and that may be decreased antioxidants in infant. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Our defenses against oxygen toxicity fall into the categories of antioxidant defense enzymes, dietary and endogenous antioxidants (Free radical scavengers), cellular compartmentation, metal sequestration and repair of damaged cellular components. The antioxidant defense enzymes react with ROS and cellular products of free radical chain reactions to convert them to nontoxic products. Dietary antioxidants, such as vitamin E, vitamin C and flavonoids, and endogenous antioxidants, such as urate, can terminate free radical chain reactions(54, 55). Beside of ,the need to attenuate the birth-related oxidative stress (Rapid increase of alveolar pO_2 and other physiologic processes involved in delivery)(56) explains the higher antioxidant requirements in the first weeks of lactation.

Albumin one of the most important protein in human plasma, is able to bind to (Cu^{++}) tightly and with iron weakly. Copper bond to albumin is still effective in generating radicals species (Hydroxyl radicals) in the presence of hydrogen peroxide by Fenton reactions, therefore increase it to protect human from oxidants compounds formation[57].

Iron decreased significantly with all groups among each other and that might be affect on infant since iron is an integral part of many proteins and enzymes that maintain good health such as oxygen transport and the regulation of cell growth. Almost two-thirds of body iron is found in hemoglobin, the protein in red blood cells that carries oxygen to tissues[58].

Recently, ROS have been recognized as important signaling molecules that control diverse signaling pathways involved in a variety of cellular responses such as programmed cell death, pathogen defense, and hormone signaling[59, 60]. In addition, oxidative stress causes dramatic inhibition of the tricarboxylic acid cycle and large sectors of amino acid metabolism followed by backing up of glycolysis and diversion of carbon into the oxidative pentose phosphate pathway[61]. Therefore, organisms have developed efficient systems to keep ROS levels in check and repair damage from attack by ROS.

Table 3: The oxidants and antioxidants levels in human mature milk at different ages using Anova test.

Parameters	(16-25) year n=23		(26-35) year n=20		(36- 42) year n=16		(p) value
	Mean	SD	Mean	SD	mean	SD	
Age (year)	20.0	2.52	30.33	1.63	38.8	1.48	0.0001
BMI (kg/m^2)	23.36	3.53	26.35	6.68	26.48	4.82	0.032
Vit.E(mg/dl)	2.03	0.86	1.72	0.83	1.48	0.42	0.021
Vit.C(mg/dl)	4.87	1.5	4.94	1.41	4.82	1.03	0.991
SOD*	0.14	0.01	0.0037	0.00023	0.0063	0.0014	0.038
GST (U/l) **	0.0062	0.0001	0.0014	0.00015	0.0022	0.00066	0.042
Alb (gm/dl)	4.14	0.35	4.87	0.33	5.1	0.14	0.021
Cp(mg/l)	28.86	6.9	25.67	4.21	22.43	6.6	0.44
GSH(µmol/l)	34.5	8.7	24.70	5.25	20.16	4.7	0.049
Total Bilirubin (mg/dl)	3.67	0.3	6.39	2.91	10.91	2.6	0.025
Uric acid (mg/dl)	44.14	11.7	53.57	9.84	66.5	12.8	0.044
MDA (µmol/l)	0.19	0.02	0.23	0.0087	0.57	0.07	0.011
$ONOO^-$ (µmol/l)	77.28	9.9	69.5	5.54	79.5	5.78	0.529
Cu (mg/l)	0.37	0.07	0.46	0.0071	0.69	0.12	0.013
Zn (mg/l)	0.66	0.15	0.57	0.1	0.54	0.09	0.816
Fe (mg/l)	1.8	0.3	1.6	0.22	1.1	0.19	0.021

-The means are different significantly at $p \leq 0.05$.
* SOD: differences between absorbance before and after excitation by fluoresce light.
** U: a mount of glutathione S-transferase (GST) catalyzing the formation of one micromole of product per min under of optimum conditions.

Determining how to avoid oxidative stress is an important factor for improving prognosis. Broadly, there are two ways to prevent oxidative stress. One is to avoid factors that trigger the production of free radicals and ROS. The other is to strengthen antioxidant capacity, which protects the body from cell damage by scavenging free radicals and ROS when they increase[62]. All cells in the body are exposed chronically to oxidants from both endogenous and exogenous sources but come equipped with an antioxidant defense system. Sheep milk comprise an important aspect of the antioxidant defense system with which humans have evolved.

Conclusions

This study is among few in literatures to determine oxidants and antioxidants content of breast milk and animal milk (Buffalo, cow, sheep, goat) and increased antioxidants with decreased oxidants levels in: sheep milk, goat milk, cow milk, buffalo milk and human milk respectively. On the other hand, the study showed decreased antioxidant and increased oxidants levels in human breast milk levels with increasing age of women and that might be decreased infants health[63].

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