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Micronutrient content and antioxidant enzyme activities in human breast milk

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ABSTRACT

Breast milk contains micronutrients that function as cofactors of antioxidant enzymes. High concentrations of iron (Fe) and copper (Cu) can increase the production of reactive oxygen species (ROS). This study aimed to assess the relationship between the activity of antioxidant enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST)) and the concentration of the micronutrients Fe, Cu and zinc (Zn) in breast milk. Breast milk samples were collected from 108 mothers (7–10 days postpartum, transitional milk). The samples were grouped into three groups according to the number of pregnancies (one, two and three or more pregnancies), also grouped according to the body mass index (BMI) suggested by the World Health Organization (WHO) in underweight, normal weight, overweight and obese. Breast milk Fe, Cu and Zn concentrations were determined by atomic absorption spectrophotometry and the activity of the antioxidant enzymes was determined by spectrophotometry. An increase in GPx, SOD and GST activities in relation to the number of pregnancies was found ($p = 0.05$, $p = 0.04$ and $p < 0.01$, respectively). An inverse relationship between GST activity and BMI was found ($p = 0.05$). A positive correlation was observed between Cu and Zn concentrations ($r = 0.52$, $p < 0.05$). A negative correlation was found between Cu concentration and catalase activity ($r = -0.22$, $p < 0.05$); Fe content was negatively correlated with GPx and GST activities ($r = -0.32$, $r = -0.22$, respectively, $p < 0.05$). The activities of antioxidant enzymes (GPx, SOD and GST) may be affected by the number of pregnancies and contribute to prevent oxidation of nutritional molecules in breast milk.

1. Introduction

Breast milk provides nutrients, antioxidants and microelements for the child's proper growth and development [1–4]. Trace element concentrations change with the mother's environment and diet [5,6]. A relationship between microelement concentrations and antioxidant enzymes in breast milk has been reported [1,4]. The antioxidant system found in breast milk includes superoxide dismutase (SOD-E.C.1.15.1.1)) [7,8], catalase (E.C.1.11.1.6) [2,9], glutathione peroxidase (GPx-E.C.1.11.1.9) [2,9], glutathione reductase (GR-E.C.1.6.4.2) [2,10], and glutathione S-transferase (GST-E.C.2.5.1.18) [2]. Zinc (Zn) participates in ~100 catalytic enzyme sites, in cell membranes and nucleic acids [11]. Infants have low capacity to regulate Zn absorption; however, breastfed children do not suffer from Zn deficiency [12]. Insufficient Zn consumption may cause nervous system and growth defects during the first months of life [13]. Zinc breast milk concentration decreases from early breastfeeding and stabilizes at the third month [14]. Copper (Cu) is essential in formation of bones, myelin, collagen, neuropeptides,

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hemoglobin and contributes to the electron transport chain [13,15]. Few studies report Cu toxicity in infants, attributed to regulation of Cu homeostasis at an early age [12]. Iron (Fe) content in breast milk is bound to lactoferrin [12]. Breast milk is low in Fe (0.2–0.5 mg L^{-1}). Supplements are recommended from the fourth month of lactation to prevent Fe deficiency, which can affect the infant's cognitive development [16,17]. However, excess Fe may reduce absorption of Cu [18] and promote growth of pathogenic bacteria, modifying the infant's microflora [17,19]. High concentrations of Fe and Cu can increase reactive oxygen species (ROS), associated to biomolecules' oxidation [20].

This study aimed to assess the relationship between antioxidant enzymes activity and trace element concentration in breast milk, as well as the relationship between the maternal characteristics, micronutrients and the activity of antioxidant enzymes of healthy women.

2. Material and methods

2.1. Sampling

Breast milk samples (n = 108) were obtained from healthy women from Baja California Sur, Mexico, during the second week postpartum (transitional milk). The samples were grouped into three groups according to the number of pregnancies (one, two and three or more pregnancies), also grouped according to the body mass index (BMI) suggested by the World Health Organization (WHO) in underweight, normal weight, overweight and obese. The milk samples were taken before the first breast feeding during the morning. The mothers were provided with sterilized Falcon BD polyethylene tubes, pre-washed with nitric acid and rinsed with deionized water. At the time of sampling, informed consent was obtained and a structured questionnaire for information on various anthropometric variables (age, body mass, and height) and the number of pregnancies was applied (anthropometric measurements were obtained during doctor's appointment on day 7th after parturition). Body mass and height of the mothers were used to calculate BMI = body mass (kg) / squared height (m^2). Samples were placed in 35 mL plastic tubes, refrigerated and transported to the laboratory to be stored (−80 °C) until analysis. Informed consent form and the project were approved by Consejo Nacional de Bioética (CONBIOETICA-09-CEI-009-2016060).

2.2. Antioxidant enzyme activity

The activity of superoxide dismutase (SOD, E.C. 1.15.1.1) was determined based on the inhibition of the reduction of nitroblue tetrazolium (NBT) to formazan [21]. Formazan is a pink product which can be detected spectrophotometrically at 560 nm. Results are expressed in units (U) per milligram of soluble protein. The substrate for catalase (E.C. 1.11.1.6) is hydrogen peroxide (H_2O_2); to quantify its activity, the decrease in H_2O_2 content is monitored on a spectrophotometer at 240 nm [22]. Enzyme activity is expressed in units of catalase per milligram of soluble protein. Glutathione peroxidase (GPx, E.C. 1.11.1.9) activity was determined using H_2O_2 as the substrate of the enzyme and recording the decrease in NADPH concentration at 240 nm [21,23] Results were obtained using the extinction coefficient of NADPH (6.22 mL^{-1}) and expressed in units of GPx activity per milligram of soluble protein. The activity of glutathione S-transferase (GST, E.C. 2.5.1.18) was assessed using the formation of the thioether glutathione dinitrobenzene complex from the conjugation of GSH with 1-chlorine-2, 4-dinitrobenzene (CDNB) [24]. Enzyme activity is expressed in units of GST per milligram of soluble protein. The enzyme glutathione reductase (GR, E.C. 1.6.4.2) is an NADPH-dependent enzyme which catalyzes the reduction of GSSG to GSH, which will be used by GPx for further reduction of H_2O_2 and lipoperoxides [5]. Activity of GR was determined by measuring the decrease in absorbance observed during the oxidation of NADPH to $NADP^+$ by GSSG [21,25]. Results are expressed in units of GR per milligram of soluble protein.

Table 1
Recovery (%) for the measured elements (copper, Cu; iron, Fe; zinc, Zn) with NIMGBW10017: Milk Powder as reference material.

Element	Standard	Read	Recovery %
Cu	0.51 ± 0.13	0.48 ± 0.15	94.11
Fe	7.8 ± 1.3	8.1 ± 1.6	103
Zn	34 ± 2	31.9 ± 3.1	93.82

2.3. Micronutrient concentration

The frozen breast milk was thawed and homogenized individually in a warm water bath before processing. The concentrations of micronutrients were quantified by atomic absorption spectrophotometry [26]. To validate the measurement, a sample of certified material (NIMGBW10017: Milk Powder) was digested simultaneously with the samples. For each 10 samples, a sample of this certified material was included. In addition, every 10 samples a control (reagents without sample), a blank (deionized water), and a sample containing a known quantity of the item being measured were included; the % recovery of the known amount in the latter was recorded (Table 1). Approximately 10 mL (≈ 10 g) of whole milk were weighed and transferred to Teflon tubes, digested with 2 mL of H_2O_2 and 6 mL of HNO_3 (Mallinckrodt JT Baker, USA) in a microwave oven (Mars 5X, CEM; Matthews, USA). The digestion product was brought to 50 mL with deionized water and subsequently filtered. Micronutrient concentrations were quantified using an atomic absorption spectrophotometer (HG 3000, GBC, Australia) (XplorAA, GBC, Braeside Australia). All analyses were performed in duplicate, using standards SRM1954 and GBW10017. The detection limits (DL) were 0.014 mg L^{-1} for Cu, 0.15 mg L^{-1} for Fe, and 0.016 mg L^{-1} for Zn. The recovery was calculated to be ≥90%. To exclude contamination of Cu, Fe and Zn, the material was previously washed in nitric acid, rinsed with deionized water and stored in plastic bags within closed plastic boxes until used.

2.4. Statistical methods

The normal distribution of the data was tested using the *Kolmogorov-Smirnov* test. Samples were grouped according to the number of pregnancies as one (n =36), two (n =36) and three or more (n =36). Samples were also separated into three groups depending on the BMI value following the classification suggested by the World Health Organization (2018); women with 18.5–24.9 BMI were considered normal weight, women with 25–29.9 BMI were considered overweight, women with a BMI > 30 were considered obese (34); in this study, there were no women with BMI < 18.5, considered underweight, therefore, all statistical analyses include only 3 described groups. Nonparametric statistics were used to analyze the differences between the groups using the Wilcoxon Mann Whitney test (WMW) to compare medians from two independent populations, due to their continuous data and biased distributions [27]. Spearman's rank correlation (Spearman's r) test was used to assess the correlation between antioxidant enzyme (SOD, catalase, GR, GPx, and GST) activities and micronutrient (Fe, Cu and Zn) concentrations [28]. The significance level (α) of 5% (p = 0.05) was taken to denote statistical differences for all tests.

2.5. Ethical aspects

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed

Table 2
Maternal characteristics (age, weight, BMI) grouped according to the number of pregnancies.

Parity Group		Age	Weigh (Kg)	BMI
E1				
(n = 36)	Median	22	71.50	28.16
	P 25	19	65.75	25.41
	P 75	25	80.00	30.36
	Range	17	38.00	15.62
E2				
(n = 36)	Median	25	72.00	28.98
	P 25	22	64.00	24.61
	P 75	31	82.00	32.73
	Range	16	45.00	20.80
E3				
(n = 36)	Median	30	83.00	33.04
	P 25	26	68.50	26.77
	P 75	34	94.00	36.48
	Range	23	73.00	28.26
		p = 0.001	p = 0.1	**p = 0.03**

Significant differences between groups ($p < 0.05$) are presented in bold.

consent was obtained from all individual participants included in the study.

3. Results

Maternal anthropometric characteristics are display in Table 2, grouped according to the number of pregnancies. Table 3 shows antioxidant enzyme activities and concentrations of Zn, Cu and Fe clustered by number of pregnancies. Significant differences in the activity of GPx, SOD and GST in relation to the number of pregnancies were found. The highest GPx activity (0.08 U mg^{-1} protein) was observed in women with 3 or more pregnancies; significant differences were found between women with two pregnancies and those with three or more pregnancies, according to the Mann-Whitney test ($p = 0.05$). Higher SOD activity (267 U mg^{-1} protein) was detected in women with 3 or more pregnancies and significant differences were reported between women with two pregnancies and those with three or more pregnancies ($p = 0.04$). Similarly, the highest GST activity (0.003 U mg^{-1} protein) was reported in group 3. The GST activity showed significant differences between women with one pregnancy and those with 2 pregnancies, as well as between women with two pregnancies and those with three or more pregnancies ($p = 0.05$ and $p < 1$). Table 4 shows the antioxidant enzyme activities and concentrations of Zn, Cu and Fe by BMI value. Significant differences in the activity of GST with respect to BMI were found. The highest GST activity (0.003 U mg^{-1} protein) was reported in women with BMI < 25; significant differences were observed between women with BMI < 25 and those with BMI > 25 and < 30 ($p = 0.05$). No significant differences were found in the concentrations of Zn, Cu and Fe in relation to the number of pregnancies and BMI value.

Spearman's rank correlation coefficients between the activity of antioxidant enzymes and the concentrations of Zn, Cu and Fe are shown in Table 5 ($p < 0.05$). Among microelements, the highest correlation was observed between Cu and Zn showing a significant positive relationship ($r = 0.52$, $p < 0.05$). We also found a negative correlation between Cu and catalase activity ($r = -0.22$, $p < 0.05$). A significant relationship between Fe and the activity of GPx and GST was observed ($r = -0.32$, $r = -0.22$, respectively, $p < 0.05$).

4. Discussion

According to WHO, breast milk is the best source of nutrients during the first six months of life [29] and has been supported by evidence that breastfeeding reduces the risk of morbidity and mortality in the infant, in addition to improving growth as well as the development and modulation of the immune system [30]. Knowing the concentration of

Table 3
Activity of antioxidant enzymes and concentration of micronutrients in breast milk of women from Baja California Sur ($n = 108$), grouped by number of pregnancies.

Variables	Number of pregnancies		Dif %	Comparison between pairs of groups Parity Group (Mann-Whitney)					
	Median			E1–E2		E1–E3		E2–E3	
				U	p	U	p	U	p
GPx	E1	0.04	−42.99	526	0.17	600	0.59	473	**0.05**
(U mg^{-1} protein)	E2	0.07	1.00						
	E3	0.08	21.22						
SOD	E1	132.37	−44.04	531	0.19	598	0.57	466	**0.04**
(U mg^{-1} protein)	E2	236.54	1.00						
	E3	267.00	12.88						
GR	E1	0.01	−63.42	525	0.17	615	0.71	551	0.27
(U mg^{-1} protein)	E2	0.02	1.00						
	E3	0.02	−6.03						
GST	E1	0.001	−54.50	466	**0.04**	541	0.23	355	< **0.01**
(U mg^{-1} protein)	E2	0.003	1.00						
	E3	0.003	23.58						
Catalase	E1	0.23	−12.73	587	0.5	624	0.79	584	0.47
(U mg^{-1} protein)	E2	0.27	1.00						
	E3	0.27	−1.14						
Zn	E1	3.46	−12.63	535	0.2	573	0.4	611	0.68
(mg L^{-1})	E2	3.96	1.00						
	E3	3.2	−19.19						
Cu	E1	0.57	−3.39	606	0.64	595	0.55	557	0.31
(mg L^{-1})	E2	0.59	1.00						
	E3	0.69	16.95						
Fe	E1	0.47	4.44	601	0.6	603	0.61	580	0.44
(mg L^{-1})	E2	0.45	1.00						
	E3	0.44	−2.22						

Glutathione reductase (GR), catalase, glutathione S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), zinc (Zn), copper (Cu), iron (Fe). Percentage of differences between medians (Dif %). Women grouped according to the number of pregnancies, (E1 = one pregnancy ($n = 36$), E2 = two pregnancies ($n = 36$), E3 = three or more pregnancies ($n = 36$). Significant differences between groups ($p < 0.05$) are presented in bold.

Table 4
Activity of antioxidant enzymes and concentration of micronutrients in breast milk of women from Baja California Sur (n = 108), grouped by body mass index (BMI).

Variables	Body mass index		Dif %	Comparison between pairs of groups BMI Group (Mann-Whitney)					
	Median			I1–I2		I1–I3		I2–I3	
				U	p	U	p	U	p
GPx	I1	0.058	23.10	346	0.6	505	0.71	814	0.37
(U mg^{-1} protein)	I2	0.047	1.00						
	I3	0.074	56.24						
SOD	I1	151.994	−7.40	355	0.74	505	0.71	901	0.88
(U mg^{-1} protein)	I2	164.142	1.00						
	I3	254.358	54.96						
GR	I1	0.024	28.12	358	0.74	473	0.44	909	0.94
(U mg^{-1} protein)	I2	0.019	1.00						
	I3	0.011	−42.72						
GST	I1	0.003	200.00	257	**0.05**	419	0.15	797	0.3
(U mg^{-1} protein)	I2	0.001	1.00						
	I3	0.002	100.00						
Catalase	I1	0.260	−3.70	338	0.51	493	0.6	915	0.98
(U mg^{-1} protein)	I2	0.270	1.00						
	I3	0.240	−11.11						
Zn	I1	3.240	−12.67	375	0.96	481	0.5	822	0.41
(mg L^{-1})	I2	3.710	1.00						
	I3	3.860	4.04						
Cu	I1	0.650	12.07	266	0.06	414	0.13	894	0.84
(mg L^{-1})	I2	0.580	1.00						
	I3	0.560	−3.45						
Fe	I1	0.470	14.63	296	0.17	508	0.73	781	0.24
(mg L^{-1})	I2	0.410	1.00						
	I3	0.460	12.20						

Glutathione reductase (GR), catalase, glutathione S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), zinc (Zn), copper (Cu), iron (Fe). Percentage of differences between medians (Dif %). Women grouped according to their body mass index (I1 = BMI < 25 (n =21); I2 = BMI > 25 < 30 (n = 36); I3 = BMI > 30 (n =51). Significant differences between groups (p < 0.05) are presented in bold.

Table 5
Spearman's rank correlation coefficients (r) between the activity of antioxidant enzymes and the concentration of micronutrients in breast milk of women from Baja California Sur (n = 108).

	GPx	SOD	GR	GST	Catalase	Zn	Cu	Fe
Zn							0.52	
Cu					−0.22	0.52		
Fe	−0.32			−0.22				

Glutathione peroxidase (GPx); superoxide dismutase (SOD); glutathione reductase (GR); glutathione S-transferase (GST); catalase; glutathione (GSH); copper (Cu); iron (Fe); zinc (Zn). Only statistically significant results are presented in the table (p < 0.05).

macronutrients and micronutrients in breast milk and how these change during the different stages of lactation due to factors related to maternal nutrition is relevant to the infant´s overall growth, nutrition and immune protection [31]. Breast milk is a complex mixture of micronutrients (Zn, Fe, Cu) and other molecules (proteins, fatty acids, vitamins, antioxidants) that, overall, contribute to the child´s proper growth and development [1,32,33]. Production of ROS and oxidative damage in breast milk can increase with reproductive effort, metabolic rate, foreign substances (xenobiotics) and maternal smoking [34,35]. Because ROS are highly reactive and unspecific, inducing oxidative damage to proteins, lipids, nucleic acids and other substrates [5], nutrients in milk may suffer structural and/or functional damage if exposed to increased ROS production. Enzymatic and non-enzymatic antioxidants that naturally occur in breast milk provide support to the neonate´s antioxidant defenses [36], but may also protect the nutrients in milk from oxidative damage, as suggested by the association between oxidative damage indicators and antioxidants reported in breast milk [35,37,38].

4.1. Relationship between antioxidant enzyme activities and maternal characteristics

The activities of SOD, GPx and GST increased in relation with the number of pregnancies (Table 3). This can be attributed to the reproductive effort and increased metabolic rate during consecutive periods of pregnancy and lactation, which increase ROS production [39]. Therefore, the number of pregnancies, or parity, can influence the markers of oxidative stress and cause an increase in the activity of antioxidant enzymes to mitigate oxidative damage [39]. Previous studies report that BMI correlates positively with ROS ($O_2^{\cdot-}$ r = 0.20, H_2O_2 r= 0.19), in addition it has been reported a decrease in antioxidant activity with increasing age [34], contrary to that reported in this study where women with the highest number of pregnancies were older and had higher BMI, in addition, those women with BMI < 25 had greater GST activity. Increased concentrations in contaminants, such as persistent organic pollutants (POPs) in serum and plasma samples have been reported during and after weight loss [40–43]. It is possible that the greater GST activity in women with BMI < 25 in this study is associated to post-parturition weight loss and mobilization of lipophilic contaminants.

4.2. Relationship between micronutrient concentration and maternal characteristics

The presence of Zn during the first months of life is important because it is a cofactor of more than 300 enzymes (including SOD) which participate in cell growth, the immune system, neurological development, among other functions [1,11,13,44]. Zn concentration in breast milk reported by Örun (2012) was 625 µg L^{-1} (0.625 mg L^{-1}), while in this study an average of 3.66 mg L^{-1} was observed; the main difference between studies may relate to the time of lactation [45]. Örun et al. (2012) analyzed milk samples taken during the second month of

lactation (diet of the breastfeeding mothers was not reported) [45], while samples from the second week of lactation were used in this study. According to Dórea (2012), Zn content in breast milk decreases rapidly during the first days and stabilizes until the three months of lactation [14]. However, other studies where milk samples were collected at two months of lactation have similar values to those reported in this study ($> 2\,mg\,L^{-1}$ of Zn) [14,45,46]. In the current study, there were no significant differences in the concentration of Zn between groups in relation to parity (number of pregnancies) and BMI ($p > 0.05$). Some authors suggest that factors such as maternal age, parity, smoking during pregnancy and taking supplements do not affect Zn concentration in breast milk [26,45].

Cu concentration reported in this study is $0.59\,mg\,L^{-1}$; similar results have been published in previous studies [46,47]. In a study from Tenerife, Spain, hair Cu content was inversely related to body mass and age [48]. However, BMI and parity do not seem to be determining factors in the Cu content in breast milk, according to the results from this and previous studies [16,26,49,50].

In this study, concentrations of $0.46\,mg\,L^{-1}$ of Fe in breast milk were found, below the average reported by Mahdavi et al. (2010) of $0.85\,mg\,L^{-1}$ in lactating women from Iran [47]. The differences in the content of Fe among studies may be due to differences in diet. The main source of non-heme Fe was reported from dietary vegetables; in addition, higher content of Fe was found in women in rural areas [47]. Of the lactating women included in this study, 88% are from an urban population. Despite the recommendations to supplement pregnant and lactating women with Fe, a clear relationship between supplement intake and Fe concentration in breast milk has not been found [47]. No differences were found between the number of pregnancies or the BMI and the concentration of Fe in this study.

4.3. Relationship between antioxidant enzyme activities and micronutrient concentrations

A positive correlation was found between the concentration of Zn and Cu ($r = 0.52$) (Table 4). Cu absorption increases when bound to GSH or organic acids present in breast milk. The presence of ions such as Zn and Fe, and even vitamin C, can affect the absorption of Cu [15]. Opposite to what could be expected, Zn concentration in the present study was positively correlated to Cu content ($r = 0.52$) in breast milk. Higher concentrations of Zn and Cu in serum compared to breast milk have been reported [51]. Zn concentrations are particularly high in breast milk, which provides the child with this essential element for growth and protection. However, the Cu:Zn ratio can be an indicator of oxidative stress, as well as of the immunological and nutritional status [52]. In the present study, the Cu:Zn ratio was 0.174, within the range of previously published reports, such as de Figueiredo et al. (2010), who found average values of 0.1702 and 0.199 in women supplemented and not supplemented with Zn, respectively [26]. Elevated levels (> 2) of the Cu:Zn ratio in blood have been related with various diseases, such as tuberculosis, leukemia, gastric cancer and breast cancer [53]. The Cu:Zn ratio is generally found at higher values in older people, which is attributed to the storage of Cu, but not of Zn, in the body over time. Cu can be considered as a pro-oxidant; higher Cu:Zn ratio could indicate a larger potential for degenerative pathologies associated to oxidative damage and aging [54]. Copper can participate in the Fenton reaction, in lieu of Fe. Copper changes from Cu^{2+} to Cu^{+}, with the consequent reduction of H_2O_2 to $^{\cdot}OH$, a still more reactive ROS [55]. Some proteins such as ceruloplasmin, which is a Cu transport protein, are present in breast milk; Cu binds to these proteins, which reduce its availability to participate in Fenton reactions and, thus, prevents oxidative damage and the consequent antioxidant response [56]. In this study, we found a negative correlation between Cu and catalase activity ($r = -0.22$). Cu inhibits catalase activity, presumably by the interference of the metal with the enzyme's functional group, which includes iron in its composition [57].

A negative correlation between the concentrations of Fe and the activity of GPx and GST ($r = -0.32$ and $r = -0.22$, respectively) was found. Fe is involved in ROS production, via the Fenton reaction, in which $O_2^{\cdot -}$ reduces Fe, transforming it from ferric to ferrous ($+3 \rightarrow +2$), Fe^{+2} reduces H_2O_2 producing $^{\cdot}OH$ [55]. The activity of GPx and GST can be affected by the decrease in the concentration of H_2O_2. However, the correlation of Fe with the antioxidant enzyme activities, although significant, is low, probably due to the presence of ferritin, a protein that maintains Fe in a soluble and non-toxic form, thus contributing to decrease the levels of free Fe, which can trigger oxidation reactions [5,17]. Therefore, the concentrations of Zn, Cu and Fe can not only influence the activity of antioxidant enzymes (SOD and catalase), of which they are co-factors [5], but can also affect the activity of other antioxidant enzymes (GPx, GST), either by decreasing availability of their substrate (H_2O_2) or by interfering with their functional groups [17,57].

5. Conclusion

Significant differences in the activity of GPx, SOD and GST in relation to the number of pregnancies ($p = 0.05$, $p = 0.04$ and $p < 0.01$, respectively) were observed in human breast milk; these can be attributed to the increase in metabolic rate during successive periods of pregnancy and lactation. In this study, there were no significant differences in the concentration of Zn, Cu and Fe that could be attributed to the number of pregnancies or BMI. A positive correlation was found between the concentration of Zn and that of Cu ($r = 0.52$); the presence of Zn ions can affect Cu absorption. Negative correlations between Cu concentration and catalase activity ($r = -0.22$), and between Fe concentration and the activities of GPx and GST ($r = -0.32$ and $r = -0.22$, respectively) were observed; the activities of GPx, catalase and GST may be affected by decreased concentration of H_2O_2. However, the correlations are low, possibly due to the presence of ferritin in the case of Fe, or of ceruloplasmin in the case of Cu, which limits the concentrations of the free elements. This coupled with the activity of the antioxidant enzymes SOD, catalase, GPx, GST, and GR, contributes to prevent the oxidation of nutritional molecules in breast milk.

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Declarations of interest

None.

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