

Study of the Concentration of Trace Elements Fe, Zn, Cu, Se and Their Correlation in Maternal Serum, Cord Serum and Colostrums

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Abstract A study of iron, zinc, copper and selenium concentration levels was carried out in three compartments namely, maternal serum (MS), colostrums and cord blood serum (CS) of healthy Indian mothers ($n = 42$) who delivered healthy normal neonates without any congenital anomalies at Bhabha Atomic Research Centre hospital, Mumbai. Fe, Zn, Cu in maternal serum, cord blood and colostrums were estimated by flame atomic absorption spectrometry while Se was determined by graphite furnace absorption spectrometry. It was seen that there was a significant difference in the level of trace elements in the three compartments. The average levels of Fe in the three compartments were $1,132 \pm 519$, $2,312 \pm 789$ and $1,183 \pm 602$ $\mu\text{g/L}$ while Zn was 514 ± 149 , 819 ± 224 and $7,148 \pm 2,316$ $\mu\text{g/L}$ respectively. Mean Cu values were $1,614 \pm 295$, 301 ± 77 and 392 ± 174 $\mu\text{g/L}$ respectively while Se values were 70 ± 15 , 36 ± 10 and 23 ± 8 $\mu\text{g/L}$ respectively. The results indicated a positive correlation of Fe and Zn concentrations in MS versus CS which were ($r = 0.386$), ($r = 0.572$) respectively and Fe levels in MS and colostrums

($r = 0.235$). A few inter element correlations were found within compartments. Zn and Se showed a negative correlation in both MS ($r = -0.489$) and colostrums ($r = -0.258$) while a positive inter correlation of Fe and Zn was seen in MS ($r = 0.44$) and in CS ($r = 0.54$). This study gave us an overview of the serum and colostrum values of mother and neonates in Indian population, data of which are scarce.

Keywords Maternal serum · Cord serum · Colostrum · Iron · Zinc · Copper · Selenium · Atomic absorption spectrometry

Introduction

Human milk is the first food human encounter and is the sole source of all nutrients required for the biological functions and growth during early stages of life [1]. Micronutrients such as iron, zinc, copper and selenium are extremely vital for fetal cell growth and decreased levels of these have been shown to be associated with many complications related to pregnancy and neonatal outcome. These metals are mostly bound to proteins, forming metalloproteins which are part of enzymatic systems which have structural and storage functions, or use the protein to be transported to their target site in the organism.

Fe has received the most attention in paediatric nutrition. Most of the Fe is in protein-bound complexes, and 60–70 % of it is present as part of the haemoglobin molecule and in serum most of it is attached to transport protein transferrin. Pregnancy imposes additional heavy iron requirements on the female, especially in the third trimester at which time daily iron needs increase from pre-pregnancy requirements of $\approx 1\text{--}1.5$ to ≥ 6 mg/day [2–4].

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Zinc participates in the synthesis and degradation of carbohydrates, proteins, lipids and nucleic acids. As it is required for cell division and differentiation it is an essential trace element administered during pregnancy to improve fetal growth. During pregnancy the requirements for zinc increases and many studies have shown that pregnant women are at risk of zinc deficiency [5–8].

Copper is known to be a component of a number of copper metallo enzymes such as catalase, superoxide dismutase, and cytochrome oxidase and its deficiency can lead to variety of nutritional and vascular disorders [9, 10].

Selenium (Se) is one of the essential trace elements in humans and is an essential component of more than 10 selenoproteins [11] with multiple biochemical functions. Selenium is a major cofactor of some important enzymes such as glutathione peroxidase (GSH-Px) which acts as a primary antioxidant enzyme and iodothyronine 5-deiodinase on which the deiodination reactions are dependent [12]. It is important for its role in regulating growth and development of the fetus and newborn and concentration levels are critical as both low and high levels have harmful manifestations.

The neonatal period is one of the most critical with respect to nutrition, therefore a study was carried out to estimate and correlate the concentration of trace elements iron, zinc, copper and selenium in three compartments namely maternal serum, cord serum and colostrums (3rd day postpartum). The correlation of these trace elements within the individual compartments and each element in every other compartment namely maternal serum, cord serum and colostrums were evaluated.

Materials and Methods

A randomized cross sectional study was done in the Department of Pediatrics, Bhabha Atomic Research Centre Hospital. This is a tertiary care multi-specialty hospital which caters to the needs of the employees and the families of Department of Atomic energy. The study was conducted over a period of 1 year on mothers and the newborns who are delivered at BARC Hospital. Mothers with age less than 40 years, with gestational age of more than 36 completed weeks who delivered normally as singleton live born healthy neonate without any visible congenital abnormalities were enrolled in the study. Also those mothers with multiple gestations; antenatal illness like toxemia of pregnancy, diabetes mellitus, hypothyroidism and those who received nutritional supplementation other than iron, calcium and folic acid were excluded from the study. All mothers were healthy without any major antenatal illness.

Ethical Issues

Necessary permissions and approvals were obtained from the Hospital Administration and Ethical Committee prior to starting the study. A well informed and valid consent was obtained from those participating in the study.

Sample Collection

The glass tubes used for collection of the blood and colostrum for trace elements estimation were cleaned twice with Suprapur® (Merck) Nitric acid in 1:1 dilution and then rinsed with double distilled water twice before storing them in acid cleaned polypropylene bags. 10 mL sample of venous blood was collected with help of a Teflon catheter from the mother's during labor and approximately 10 mL of cord blood at time of delivery was collected directly before placental separation. The blood collected was allowed to clot for 30 min at room temperature and then centrifuged at $2,500 \times g$ for 10 min. The obtained serum was stored in the trace element free glass tubes cleaned as mentioned previously. The same was further stored at -20°C until estimation.

Mothers were asked to rinse the breast with deionised water and allow to air dry immediately before taking colostrum samples of 5 mL each on day 3 post partum in trace element free test tubes and stored at -20°C until analysis.

Cold chain was maintained during the transportation of the blood as well as the milk samples to the Ultra Trace Analytical Facility of Analytical Chemistry Division of Bhabha Atomic Research Centre where the samples were later analyzed. The entire collection procedure was checked meticulously for iron, copper, zinc and selenium estimation and found to be essentially free from contamination.

Sample Analysis

Trace elements Cu, Fe and Zn were determined by Flame Atomic Absorption Spectrometry employing a GBC 906AA AAS unit with deuterium-arc background correction. The elemental hollow cathode lamp of Cu λ 324.7, Fe λ 248.3 and Zn λ 213.8 nm (GBC Australia), air-acetylene flame and nanopure water (18.3Ω) as diluent were used in this estimation. All measurements were performed using integrated absorbance mode.

Serum

The refrigerated samples were allowed to come to room temperature and suitably diluted with nanopure water to bring the mentioned element in the optimum analytical range. The samples along with calibration standards were aspirated into the air acetylene flame.

Colostrum

One milliliter of sample was treated with Suprapur HNO₃ (Merck) and H₂O₂ to digest and oxidize the organic matter. This procedure was repeated to ensure that all fat had been digested. A few drops of supra pure HClO₄ acid were added and fumed off to ensure complete oxidation and elimination of organic matter. 1 % HNO₃ in nanopure water was added and sample made up to 5 mL.

The results of Cu, Fe and Zn were expressed as µg/L. The coefficient of variation was from 2 to 5 % and the average accuracy was >95 % when analyzing the reference material.

Trace element Se was determined by Graphite Furnace Atomic Absorption Spectrometer (GFAAS) employing GBC 906AA AAS with GF 3000 electro thermal atomizer and an auto sampler PAL-3000. Pyrolytic graphite coated platform furnace tubes and HCL of Se λ 196 nm (GBC, Australia) were used in this estimation. All measurements were performed using peak height absorbance mode.

Serum and Colostrum

One milliliter sample of serum/colostrums was taken in micro centrifuge tubes and 100 µL of Suprapur HNO₃ (Merck) was added to it. The micro centrifuge tubes were heated in water bath at 70 °C for 5 min and centrifuged at 9,000 rpm for 10 min. Supernatant was decanted immediately and selenium was analyzed in supernatant using GFAAS. Palladium nitrate was used as matrix modifier. The results were expressed as µg/L. The detection limit for Se was <5 µg/L and the average accuracy was >95 % when analyzing the reference material.

Quality Assurance

The validation of each batch of serum and colostrums sample was thoroughly checked by Seronorm™ Trace Elements Serum-reference material (Nycomed Pharma AS, Oslo) and Reference Material 1549® (Non-fat milk powder) from the National Institute of Standards and Technology (Gaithersburg, MD, USA). (The *t* test at 95 % CL gave a value less than critical value $t_{0.05}$ is 2.78 $n = 5$).

All the samples were processed in Laminar Air Flow benches which are equipped with HEPA filters working at an efficiency of 99.7 % for 0.5 microparticles to minimize sample contamination.

Statistical Analysis

The observations were tabulated in a spreadsheet using Microsoft Excel®. Statistical analysis was done using SPSS® version 16.0. All the data was checked for normality using Shapiro–Wilk test. Non-parametric correlations were applied for analysis where the data failed normality. The tests used were Mann–Whitney test, linear regression, Chi square test, unpaired *t* test, Kruskal–Wallis test and ANOVA. A “*p* value” of less than 0.05 was considered statistically significant.

Results

Tables 1, 2 and 3 summarize the mean, standard deviation (SD), observed range and IQR of four essential elements namely Fe, Cu, Zn and Se in MS, CS and colostrums ($n = 42$) respectively. The literature values for these

Table 1 Results of analysis of maternal serum

Descriptive statistics	Element (µg/L)			
	Fe	Cu	Zn	Se
Mean	1,132	1,614	514	70
Median	1,165	1,550	520	72
SD	519	295	149	15
Min value	370	1,040	100.0	40
Max value	2,220	2,140	800	106
IQR	895	495	197.5	20.75
<i>Literature values</i>				
Ref. [17]		1,484–2,952	413–1,038	19.6–93.7
Ref. [14], [26]*	740 ± 210	2,360 ± 360	680 ± 100	62 ± 10*
Ref. [13], [18]*	1,021 ± 81*	2,042.2 ± 418	584.0 ± 115.2	85.5 ± 12.8
Ref. [19]		2,030 ± 520	650 ± 180	
Ref. [5]		1,210.0 ± 610.0	520.0 ± 220	
Ref. [15]	727.0 ± 73	1,873 ± 199	705 ± 112	
Ref. [25]		1,687 ± 353		68.3 ± 8.5
Ref. [16]	680 ± 260		850 ± 160	
Ref. [20]		1,715 ± 268	625 ± 183	

* refers to Elem. Conc.

Table 2 Results of analysis of cord serum

Descriptive statistics	Element ($\mu\text{g/L}$)			
	Fe	Cu	Zn	Se
Mean	2,312	301	819	36
Median	2,145	310	800	34
SD	789	77	224	10
Min value	830	150	350	22
Max value	3,710	450	1,340	60
IQR	1,115	100	290	13.75
<i>Literature values</i>				
Ref. [5]		210 \pm 110	830 \pm 390	
Ref. [14]	1,160 \pm 170	490 \pm 240	1,140 \pm 23	
Ref. [15]	949 \pm 72	1,016 \pm 119	887 \pm 98	
Ref. [13], [25]*	1,907 \pm 130	449 \pm 87*		37.02 \pm 8.9*
Ref. [19], [26]*		400 \pm 130	930 \pm 220	34 \pm 7*
Ref. [20]			870 \pm 95	

* refers to Elem. Conc.

Table 3 Results of analysis of colostrums

Descriptive statistics	Element ($\mu\text{g/L}$)			
	Fe	Cu	Zn	Se
Mean	1,183	392	7,148	23
Median	1,080	383	7,667	23
SD	602	174	2,316	8
Min value	320	50	1,300	11
Max value	2,410	740	10,900	42
IQR	802.5	210.5	3,236.5	10
<i>Literature values</i>				
Ref. [17]		186–2,628	1,869–22,050	34.2–187.6
Ref. [32]		110–990	2,960–23,090	22.9–54.2
Ref. [13], [33]*	849 \pm 154	590 \pm 50*	3,760 \pm 510*	15.2 \pm 2.3*
Ref. [19], [26]*		390 \pm 220	10,120 \pm 5,080	29 \pm 10*
Ref. [31]		570 \pm 336	6,040 \pm 3,590	

* refers to Elem. Conc.

elements in the different compartments are presented in these tables. The values for colostrums are of milk collected on 3 day post partum. Box and whiskers plots were drawn for easier comparison of the concentration levels in the three compartments.

A statistically significant difference was noted between Fe levels in MS and CS levels ($p \leq 0.05$) however no significant difference was seen in MS and colostrums levels (Fig. 1a).

Zn, Cu and Se levels showed significant difference in all compartments, (Fig. 1b–d), MS and CS ($p < 0.001$, $p < 0.001$ and $p < 0.05$), MS and colostrums ($p < 0.05$, $p < 0.05$, $p < 0.05$) and CS and colostrums ($p < 0.05$, $p < 0.05$, $p < 0.05$) respectively. The concentration levels of Cu and Se in colostrums are significantly lower while Zn level is higher than the levels in MS.

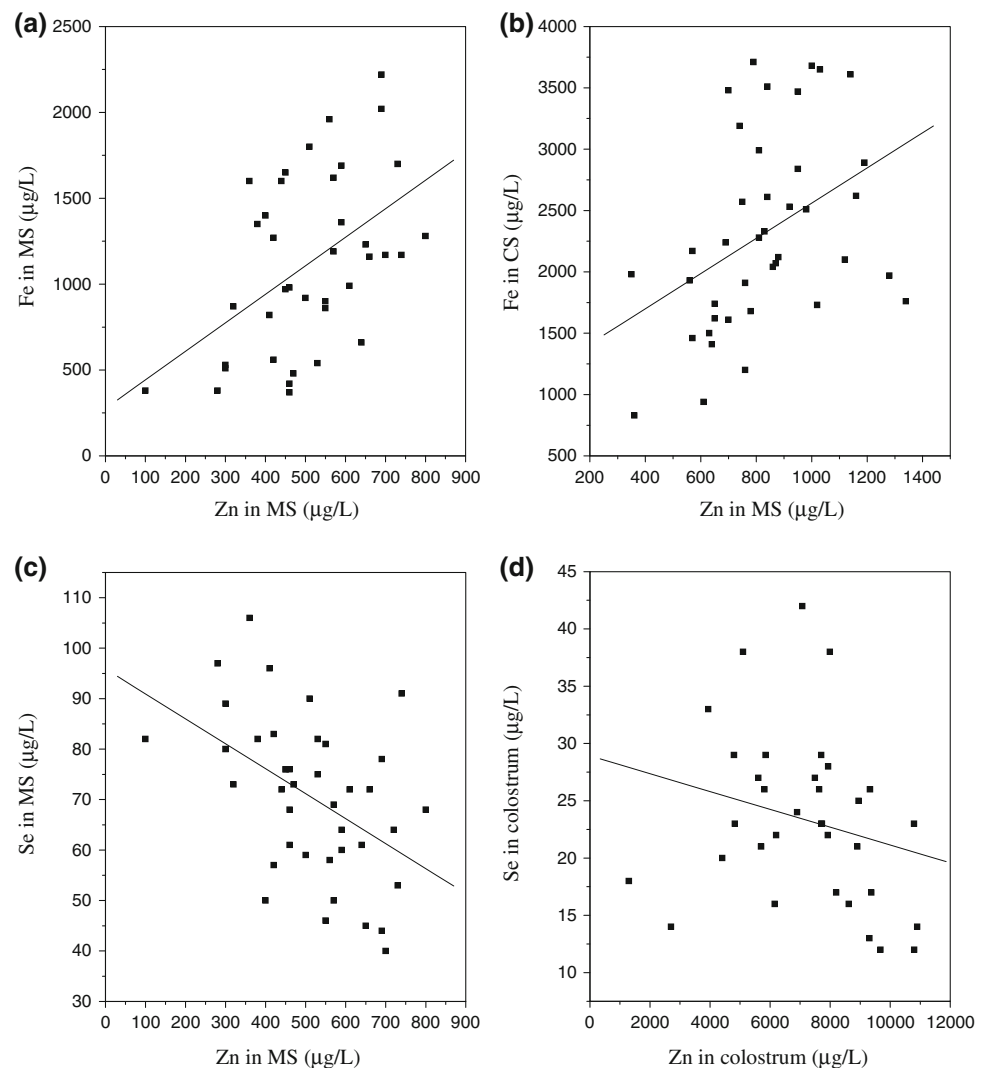
The CS have significantly higher concentration of Fe and Zn, than the MS and based on the median values the concentrations are 1.8 and 1.5 times higher, while the Cu and Se values are 0.21 and 0.48 times lower than the MS. There was no significant difference in Fe levels in colostrums and MS but Zn levels were 14 times higher and Cu and Se levels were lower by a factor of 0.25 and 0.32 respectively.

Discussion

Trace Elements in Maternal Serum

The mean serum iron level in the mother was $1,132 \pm 519 \mu\text{g L}^{-1}$ which were in comparison with the values noted

Fig. 1 Boxplots (median at center) of the **a** Fe, **b** Zn, **c** Cu and **d** Se concentration in maternal serum, cord serum and colostrums



in the non-anemic Indian mothers [13], but were higher when compared to those reported by other workers [14–16]. This was attributed to good socio-economic factors, proper supplementation with iron and good compliance of the mothers who follow up in this hospital during the antenatal period.

The mean maternal serum zinc levels reported in our study are 514 ± 149 µg/L and are higher than some reported in literature [5, 17, 18] but lower than the values denoted by others [14–16, 19, 20]. Review of literature [18] has indicated that pregnant women have lower serum Zn levels than non pregnant healthy women. This is a natural consequence due to an increased zinc uptake by the fetus and placenta and also due to the expanded plasma volume of the mother [21, 22].

Literature review [14, 17, 18] suggests that the serum copper levels of the mothers are significantly higher than the normal non pregnant reference range [23, 24]. Our mean Cu value is $1,614 \pm 295$ µg/L (Table 1), and is in agreement with the observations in the literature reports.

The higher levels of copper in MS are attributed to the increased mobilization of stored copper in tissues which is triggered by increased estrogen levels during pregnancy.

In a study carried out on a normal population similar to our study group the mean Se value was 100 ± 1.33 µg/L [27]. The mean MS selenium level noted in our study was 70 ± 15 µg/L which is similar to those reported by other workers. [18, 25, 26] where the mothers were in their 3rd trimester. This observation is attributed to the increased demand of Se for fetal growth along with increased blood volume at full term of pregnancy and in accordance with literature report which suggest that there is a diminution in the Se levels decrease as gestation pregnancy progresses and are lowest in the 3rd trimester [18].

Trace Elements in Cord Serum (Table 2)

The mean iron level in the CS was $2,312 \pm 789$ µg/L which is higher than the values reported by other workers

[14, 15]. In our study we found that there was a positive correlation between MS iron and CS iron ($p = 0.004$, $r = 0.386$). We also noted that the mean serum iron levels in CS were significantly higher ($p < 0.001$) to that of the corresponding MS iron levels. The high iron in cord serum compared with maternal serum, suggests that the process of active transfer of iron from the mother to the fetus is adequately maintained.

The mean neonatal CS zinc levels noted were $819 \pm 224 \mu\text{g/L}$, which were similar to those reported by other workers [5, 14, 15, 19, 20, 28]. The concentrations of zinc in CS are approximately 51 % higher than in the corresponding maternal sera. A positive correlation between the cord serum zinc and maternal serum zinc was seen in our study ($p = 0.001$, $r = 0.572$). Thus the level of zinc would be expected to be higher in CS than that in MS.

The mean CS copper levels obtained in our study were $301 \pm 77 \mu\text{g/L}$ which was comparable to values reported by [5, 19, 29] but lower than those reported by others [15, 25]. Copper levels in cord serum are much lower than in maternal serum. We found no statistical correlation between cord serum copper and maternal serum copper, however we found statistically significant difference between the maternal and cord serum copper levels ($p < 0.001$). Concentrations of copper in umbilical cord sera reached only 28 % of the normal concentration in mother despite the large copper stores of the liver.

The neonatal CS selenium levels noted in our study were $36 \pm 10 \mu\text{g/L}$ which were similar to those given by [25, 26, 28]. The concentrations of selenium in CS are approximately 50 % as compared to corresponding MS which is similar to the finding of [26] who noted cord serum selenium, was 55 % of that of the maternal serum selenium. The exact mechanisms responsible for the maternal–fetal selenium gradients are yet unknown. In our study we noted a significant difference between maternal and umbilical cord selenium levels ($p < 0.001$). Although it is mentioned in the literature that significant linear correlation between selenium concentrations in MS and CS exist, we noted both the entities to be closely associated though statistically not significant ($p = 0.055$).

Trace Elements in Colostrums (Table 3)

The colostrum iron levels in our study was $1,183.2 \pm 602 \mu\text{g/L}$ which is in agreement with value reported by [13] but considerably higher as compared to the those mentioned by Food and Nutrition Board [30]. The colostrum iron in our study had a weak positive correlation with the maternal serum iron ($p = 0.015$, $r = 0.235$). Also there was a significant difference ($p < 0.001$) between the colostrum iron and the serum iron of the mother. This linear relationship of maternal iron levels with colostrum iron content shows that

iron is transported into colostrum in direct proportion with the levels found in maternal circulation.

In this study the concentrations of Zn in colostrum was $7,148 \pm 2,316 \mu\text{g/L}$ which was approximately 10 times higher than in MS and 15 times higher as compared to the CS. There was a significant difference between MS zinc levels and colostrum zinc level ($p < 0.001$). There was no significant correlation observed in zinc content between the MS and the colostrum sample ($p = 0.068$). The reason for the higher concentration of trace elements in colostrum is not known however, it may be to meet requirements since newborn infants require larger amounts of these minerals due to the low volume of colostrum intake in their early life as compared with later days [17].

We found that the concentration of copper in colostrum was $392 \pm 174 \mu\text{g/L}$ which was similar to the concentrations in CS which is in close agreement to the literature report [31]. However there was no correlation found between the copper levels of maternal sera and colostrum ($p = 0.17$). This finding could be due to the developed mechanism in the mammary glands which could regulate the concentration of elements independently of the maternal status in order to supply the breast fed infant. A statistical significant difference was noted between the maternal serum, colostrum and cord serum copper levels ($p < 0.001$).

The mean colostrum selenium level obtained in our study was $24.3 \pm 9.9 \mu\text{g/L}$, which were comparable to those reported by [26, 32] but lower than those reported by [17]. The Se levels in colostrum showed a mean value 35 % of that of MS with a wide range of colostrum selenium value between 11 and $42 \mu\text{g/L}$. A statistical significant difference between MS selenium and colostrum selenium levels ($p < 0.001$) was seen.

The general absence of correlation and the difference in the concentration of individual trace elements suggests that homeostasis of trace elements is regulated through molecular processes which transport trace elements through the different compartments.

Inter-Correlation

Both zinc and iron contents of MS showed wide variability however, the magnitude of the variation was similar for both elements. We obtained a significant positive correlation ($p = 0.003$, $r = 0.44$) (Fig. 2a) between MS iron levels and MS zinc levels. Also positive statistical correlation was obtained between the CS iron levels and CS zinc levels ($p = 0.001$, $r = 0.54$) (Fig. 2b). No correlation of colostrum iron with other elements was observed in our study. A significant negative correlation between MS zinc and MS selenium levels ($p = 0.001$) ($r = -0.489$) (Fig. 2c). A weak negative correlation was noted between the zinc and the selenium levels in the

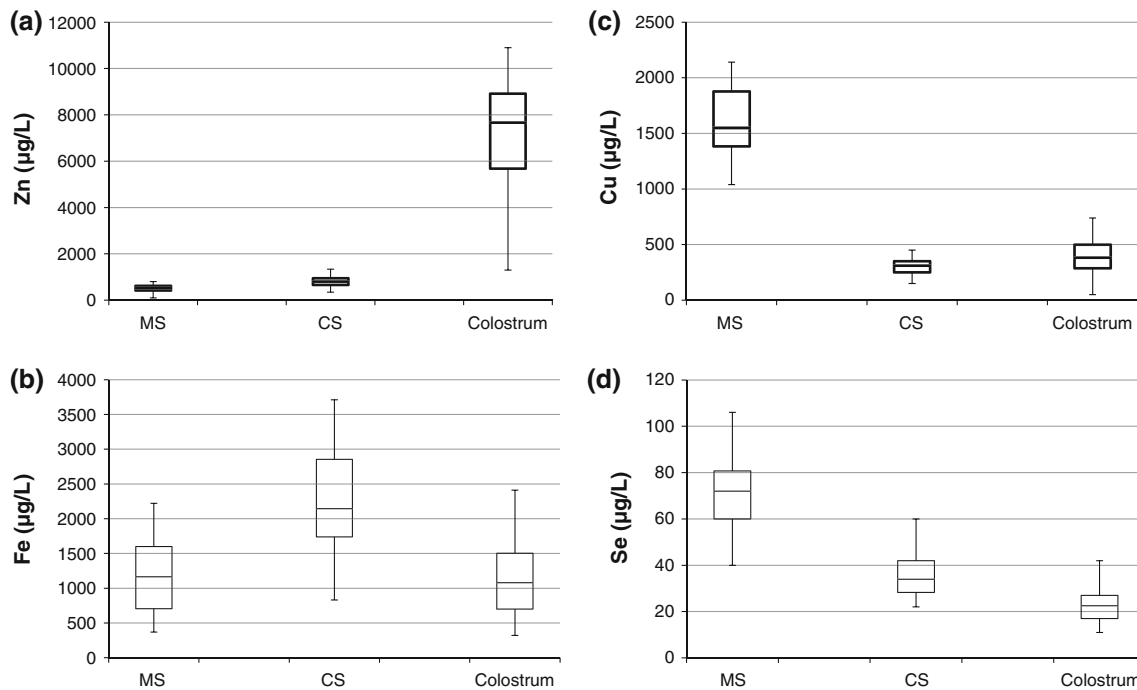


Fig. 2 **a** Correlation Fe versus Zn in MS ($r = 0.44$), **b** Fe versus Zn in CS ($r = 0.54$), **c** Se versus Zn in MS ($r = -0.487$), **d** Se versus Zn in colostrums ($r = -0.258$)

colostrum ($p = 0.035$, $r = -0.258$) (Fig. 2d). The reason for the above interactions however could not be found and further studies would be needed to evaluate the relationship between the two elements.

Conclusions

In this study we have evaluated 42 mothers and their neonates to study the inter-correlations of the trace elements (Fe, Zn, Cu, Se) in the three compartments viz. maternal serum, maternal colostrums and cord serum. The data in our study and trend of trace elements are consistent with earlier literature reports. The data in our study suggested that zinc and selenium levels were lowered in pregnant mothers hence supporting the premise of the need of their supplementation along with iron.

This study gave us an overview in the serum and colostrums values of mother and neonates in Indian population, data of which are scarce. It also highlighted the complex interactions of the trace elements between the maternal and the neonatal organism.

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