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Evaluation of factors affecting the physiological levels of copper and iron in sheep and cattle in some areas of Diyala Governorate, Iraq

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ABSTRACT

Background: Trace minerals are important components of many physiological functions, including growth, development, and the immune response.
Aim: This study aimed to evaluate copper and iron status in sheep and cattle in some areas of Diyala governorate, Iraq.
Methods: One hundred blood samples were collected, 50 from sheep and 50 from cattle in order to measure the levels of copper and iron in the serum.
Results: In sheep, the serum level of copper significantly increased among females compared to males, while among cattle, the serum level of copper significantly decreased in females compared with males. The level of iron showed a significant increase in females compared with males. Serum levels of copper were increased with the age of more than 1 year accordingly compared with sheep at the first year of life or less than a year. Among cattle, age plays no significant role in the level of copper. The levels of copper and iron were significantly increased among pregnant ewes compared with non-pregnant. The levels of copper were significantly increased in lambing compared with pregnant and non-pregnant ewes in the value of copper. The level of iron was significantly decreased in lambing sheep compared with pregnant and non-pregnant sheep. The levels of copper and iron showed no significant changes between pregnant and non-pregnant cows. Sheep from Abu Saida showed significant increase in serum levels of copper and iron compared with those from Baqubah and Saadia cities. Cows with good body scores have a significant increase in serum levels of copper and iron compared with those with medium and poor body scores. There was no linear correlation between iron and copper levels in serum and the studied parameters for cows and sheep.
Conclusion: The levels of copper and iron in the body of sheep and cattle are affected by age, gender, pregnancy, and soil composition.
Keywords: Copper, Iron, Trace minerals, Sheep, Cattle.

Introduction

The body needs trace minerals at extremely minute levels, but these elements are crucial for many different biochemical activities. Sheep need the following trace minerals: zinc (Zn), molybdenum (Mo), manganese (Mn), copper (Cu), iodide (I), iron (Fe), and cobalt (Co). It is commonly recognized that virtually all of the body's metabolic functions depend on trace minerals to function normally. They are necessary to preserve the health and production of animals since they are a component of many enzymes and regulate a vast array of biological processes. Adequate trace mineral levels along with optimal nutrition ensure that the body performs its essential functions, the most crucial of which are physiological, structural, catalytic, and regulatory. Previous studies stated that the most frequent reason to evaluate the trace mineral status of the animals is when their performance falls short of expectations (Suttle, 2010; Swecker and Van Saun, 2023).

The assessment of trace minerals is carried out in farm animals to ascertain if dietary deficiencies are common in animals or not, which have a direct effect on production and performance. Additionally, assessments of trace minerals are essential to determine the effectiveness of dietary supplements, which reflect the good management policies in large-scale farms (Byrne and Murphy, 2022).

As a co-factor in hundreds of enzymatic activities involved in the synthesis of collagen, the generation of red blood cells, the production of energy, the formation of hormones, and the defense against oxidative damage,

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copper is a necessary element for life (Jomova *et al.*, 2022). Many clinical signs, such as a pale coat, poor quality sheep fleece, anemia, spontaneous fractures, poor capillary integrity, myocardial degeneration, demyelination of the spinal cord, impaired reproductive function, decreased resistance to infectious disease, diarrhea, and generalized ill health, have been linked to copper deficiency, resulting in significant economic losses (Riaz and Muhammad, 2018).

The second most common mineral deficiency effect in grazing animals is hypocuprosis. Several studies on the mechanisms underlying the body's copper activity have focused mainly on the distribution of copper in different tissues, the changes that occur in the blood after various conditions, and the interactions between copper and different enzyme systems, vitamins, and minerals (Hefnawy and El-Khaiat, 2015).

Intracellular homeostasis and the appropriate balance of iron stores are therefore closely regulated. Iron is an essential nutrient and cofactor for the synthesis of hemoglobin and myoglobin as well as several cellular functions including oxygen transport, respiration, growth, gene regulation, and the proper functioning of iron-dependent enzymes (Cronin *et al*., 2019). Poor dietary intake of absorbable iron (or insufficient intake to meet increased demands during pregnancy) and/or iron loss from parasitic infections (e.g., hookworm) or blood loss are the causes of iron deficiency (Pena-Rosas *et al.*, 2015). The majority of dietary iron is nonheme iron, which is found in plant-based meals such as green leafy vegetables. However, the primary source of dietary iron for mammals is hemoglobin, which is found in foods such as fish and animal meats because it is more quickly absorbed and has a higher bioavailability (Cairo *et al.*, 2006).

The aim of the current study was to assess the effect of age, sex, and pregnancy on the levels of copper and iron in sheep and cattle in certain regions of the Diyala governorate in Iraq.

Materials and Methods

Study area and study population

This study was conducted on sheep and cows, reared in Baqubah, Saadia, and Abu Saida in Diyala province, Iraq.

Study design

In order to assess the levels of copper and iron in the serum, a cross-sectional study was designed (Al-Ezzy, 2016a), involving the collection of 100 blood samples, which were randomly collected from 50 sheep and 50 cattle from various sites in Diyala Governorate.

Sample collection

Five milliliters of jugular vein blood were taken according to Pugh *et al.* (2020) and collected in a dry, clean tube. The samples were centrifuged for 10 minutes at 3,000 rpm after being left to clot in a slanting posture at room temperature throughout the whole night (Sultan *et al.*, 2023; Hameed and Al-Ezzy, 2024). According to Hameed *et al.* (2024a,b), the clear sera were thoroughly aspirated using an automated pipette and then dispensed into clear, dry, labeled Eppendorf tubes, and kept at −20°C until testing.

Assessment of serum iron and copper

Copper biochemical analysis was performed using the spectrophotometer (AA-5) according to Dawson *et al.* (1968), Ogunsanmi *et al.* (2002), Akhtar *et al.* (2009), using a special copper kit. The working solution (working reagent) is prepared according to the recipe attached to the kit. Equal amounts of reagent A are mixed with reagent B. This reagent remains for 20 days at room temperature. When stored in the refrigerator before work, reagent A may precipitate in the form of grains. In this case, it must be placed in a water bath at a temperature of −2,530°C for 5 minutes. This will make it clear and suitable for mixing. After preparing the working reagent, we prepare three tubes to mix the sample in. After mixing, we wait 10 minutes; then, the samples are read by the device under a wavelength of 580 nm. The reaction color remains constant for 30 minutes. We apply the following equation:

Amount of copper (CU) = Wavelength of the model/ Wavelength of the standard x×3.15

Estimating the amount of iron in the serum

The spectrophotometer was used according to the method indicated by Doumas and Biggs (1972) using a special kit to examine the amount of iron in the serum.

Ethical approval

The Helsinki Declaration's guiding principles were followed in the conduct of this investigation. A thorough explanation of the study's objectives to each owner prior to its commencement. Complete consent forms were acquired from all owners. Before beginning work, the Department of Medicine at Diyala University in Iraq's College of Veterinary Medicine obtained approval from an ethical review commission (AL-Ezzy *et al.*, 2020; Al-Khalidi *et al.*, 2020a,b; Al-Zuhairi *et al.*, 2020a,b; Humadi *et al.*, 2020; Hameed *et al.*, 2020a,b; Hameed and Al-Ezzy, 2024; Hameed *et al.*, 2024a)

Statistical analysis

Data were expressed as (mean ± SE) (Al-Ezzy, 2016b,c). *T*-test was used for data analysis (Al-Ezzy *et al.*, 2016; Hameed *et al.*, 2024b). The Pearson correlation coefficient is used to measure the linear correlation between parameters (Fajer *et al.*, 2023a,b). The SPSS is used for the analysis of variables with significant levels ($p < 0.05$) (Hameed and Al-Ezzy, 2019; Hameed *et al.*, 2020b)

Results

The results of the current study showed the blood serum value of copper and iron (14 male 28% and 36 female 72%). A significant increase in serum copper in females compared with the serum value of copper of males, while the result of iron showed no significant changes between sex. As shown in Table 1, in cattle, the serum values of copper and iron (8 males 16% and 42 females 84%) significantly decreased in serum copper in females compared with the serum value of copper of males, while the result of iron showed a significant increase in serum iron in females compared with the serum value of iron of males (Table 1).

The results of blood serum value for copper and iron [12 (24%) ≤ 1 year and 38 (76%) ≥ 1 year] showed a significant increase in ages of more than 1 year compared with sheep in 1 and less year, but the result of iron showed a significant decrease in ages of more than 1 year compared with sheep in 1 and less year. However, the blood serum value of copper and iron [21 cows (1–2 year) 48.8% and 22 cows (3–4 year) 51.2%] showed no significant changes between ages in the examined cows (Table 2).

Among non-pregnant sheep (8/36; 22.22%), pregnant sheep (16/36; 44.44%), and lambing sheep (12/36; 33.33%), there was a significant increase in the serum levels of copper among pregnant compared with non-pregnant sheep. On the other hand, there was a significant increase in serum copper among lambing sheep compared with pregnant and non-pregnant sheep. There was a significant decrease in serum iron levels among lambing sheep compared with pregnant and non-pregnant sheep. There were no significant changes in serum iron levels between pregnant and non-pregnant sheep (Table 3).

The results of blood serum value for copper and iron (19 pregnant cows 44.19%, 24 non-pregnant cows 55.81%) showed no significant changes between pregnant and non-pregnant cows (Table 4).

The results of blood serum value for copper and iron (13) sheep (36.11%) from Baqubah, 9 sheep (25%) from Saadia, and 14 sheep (38.88%) from Abu Saida city showed a significant increase in serum copper and iron in sheep from Abu Saida compared with Baqubah

Table 1. Value of serum copper and iron according to sex of sheep and cattle.

Type of animal	Parameters	Copper (µmol/l)		Iron (µmol/l)	
Sheep	**Sex**	**Male**	**Female**	**Male**	**Female**
	Mean ± SE	15.69 ± 0.96^{a}	17.89 ± 4.18^{b}	28.45 ± 2.69^{a}	28.0 ± 1.16^{a}
	Number	14	36	14	36
	Percentage	28%	72%	28%	72%
	Total	50		50	
Cattle	Mean ± SE	32.34 ± 6.73^{b}	22.19 ± 2.37^{a}	35.86 ± 1.98^{a}	44.34 ± 2.28^{b}
	Number	8	42	8	42
	Percentage	16%	84%	16%	84%
	Total	50		50	

Values are M ± SE: a, b Similar letters indicate that there are no significant differences, and different letters indicate that there are significant differences. Significant changes in comparison between same elements at $p < 0.05$.

Table 2. Value of serum copper and iron according to age of sheep and cattle.

Type of animal	Parameters	Copper (µmol/l)		Iron (µmollL)	
Sheep	**Age**	**1 year and less**	**More than 1 year**	**1 year and less**	**More than 1 year**
	Mean ± SE	14.48 ± 3.3^{a}	18.10 ± 3.64^{b}	31.47 ± 2.01^{b}	27.72 ± 2.46^{a}
	Number	12	38	12	38
	Percentage	24%	76%	24%	76%
	Total	50		50	
Cattle	Age	1–2 years	3–4 years	1–2 years	3–4 years
	Mean ± SE	22.09 ± 3.71^{a}	22.30 ± 3.06^{a}	46.6 ± 3.62^{a}	42.18 ± 2.84^{a}
	Number	21	22	21	22
	Percentage	48.8%	51.2%	48.8%	51.2%
	Total	43		43	

Values are M ± SE: a, b Similar letters indicate that there are no significant differences, and different letters indicate that there are significant differences. Significant changes in comparison between same elements at $p < 0.05$.

and Saadia cities, but no significant changes between sheep from Baqubah and Saadia cities (Table 5).

The results of blood serum value for copper and iron (27), good body score (62.7%), (9) medium body score (20.9%), and (7) poor body score (16.4%) showed a significant increase in good body score compared with medium and poor body scores in value of copper, while in iron significant decrease in good and medium body scores compared with poor body score as shown in Table 6.

As shown in Table 7, the serum level of copper and iron was not correlated with age, sex, body score, and pregnancy status among studied cows. As shown in Table 8, the serum level of copper and iron was not correlated with age, sex, location, and pregnancy status among studied sheep. A negative correlation was reported between iron and copper levels in sheep.

Discussion

Because blood is less intrusive to collect than liver and blood measurements are highly connected with nutritional status of specific trace elements, blood measures are commonly utilized in evaluation (Herdt and Hoff, 2011). Mineral concentrations in whole blood frequently fluctuate gradually. There is a correlation between the amount of bioavailable copper in bovine diets and the concentration of copper in their livers (Ensley, 2020). Physiological requirements influence the concentration of copper in the liver in addition to bioavailable copper from food. Genetics has a role in copper metabolism, and breeds of sheep differ significantly in the amounts of copper in their plasma and liver (López-Alonso and Miranda, 2020).

When compared to males, females' serum levels of copper increase substantially. For feeding purposes,

Table 3. Value of serum copper and iron in non-pregnant, pregnant and lambing sheep.

Stage	Copper (µmol/l)			Iron (µmol/l)		
	Non pregnant sheep	Pregnant sheep	Lambing sheep	Non pregnant sheep	Pregnant sheep	Lambing sheep
Mean ± SE	14.92 ± 3.44[a]	18.82 ± 1.14[b]	20.78 ± 7.9[c]	29.44 ± 1.99[b]	28.80 ± 3.02[b]	24.84 ± 2.06[a]
Number of sheep	8	16	12	8	16	12
Percentage	22.22%	44.44%	33.33%	22.22%	44.44%	33.33%
Total of sheep	36			36		

Values are M ± SE: a, b, c Similar letters indicate that there are no significant differences, and different letters indicate that there are significant differences. Significant changes in comparison between same elements at $p < 0.05$.

Table 4. Value of serum copper and iron in pregnant and non-pregnant cows.

Pregnancy	Copper (µmol/l)		Iron (µmol/l)	
	pregnant cows	Non-pregnant cows	Pregnant cows	Non-pregnant cows
Mean ± SE	22.23 ± 3.36[a]	21.90 ± 3.44[a]	41.43 ± 1.95[a]	45.03 ± 3.54[a]
Number of cows	19	24	19	24
Percentage	44.19%	55.81%	44.19%	55.81%
Total of cows	43		43	

Values are M ± SE: a Similar letters indicate that there are no significant differences, and different letters indicate that there are significant differences, significant changes in comparison between same element, significance at $p < 0.05$.

Table 5. Value of serum copper and iron of female sheep in the Baqubah, Saadia and Abu Saida city.

The city	Copper (µmol/l)			Iron (µmol/l)		
	Baqubah	Saadia	Abu Saida	Baqubah	Saadia	Abu Saida
Mean±SE	16.06 ± 4.18[a]	17.01 ± 7.75[a]	21.01 ± 1.14[b]	27.86 ± 1.16[a]	26.50 ± 1.33[a]	30.51 ± 1.58[b]
Number of sheep	13	9	14	13	9	14
Percentage	36.11%	25%	38.88%	36.11%	25%	38.88%
Total of sheep	36			36		

Values are M ± SE: a, b Similar letters indicate that there are no significant differences, and different letters indicate that there are significant differences. Significant changes in comparison between same elements at $p < 0.05$.

Table 6. Value of serum copper and iron according to body score of cows.

Body score	Copper (μmol/l)			Iron (μmol/l)		
	Good = 3	Medium = 2	Poor = 1	Good = 3	Medium = 2	Poor = 1
Mean ± SE	23.7 ± 2.62[b]	17.73 ± 5.04[a]	22.14 ± 8.78[a]	42.28 ± 2.19[a]	38.8 ± 3.28[a]	59.34 ± 8.90[b]
Number of cows	27	9	7	27	9	7
Percentage	62.7%	20.9%	16.4%	62.7%	20.9%	16.4%
Total of cows		43			43	

Values are M ± SE: a, b Similar letters indicate that there are no significant differences, and different letters indicate that there are significant differences, significant changes in comparison between same element, significance at $p < 0.05$.

Table 7. Correlation between sex, age, pregnancy, body score and serum copper and iron levels among cows.

Parameters	Correlation coefficient	Iron level	Copper level
sex	*R*	0.178	−0.177
	p value	0.217	0.219
age	*R*	−0.143	0.065
	p value	0.321	0.655
pregnancy	*R*	−0.031	0.007
	p value	0.829	0.963
Body score	*R*	−0.022	−0.252
	p value	0.878	0.077
Iron	*R*		−0.340*
	p value		0.016

*Correlation is significant at the 0.01 level (2-tailed).

Table 8. Correlation between sex, age, pregnancy status, location and serum copper and iron levels among sheep.

Parameters	Correlation coefficient	Copper	Iron
Sex	*R*	−0.108	0.168
	p Value	0.456	0.245
Age	*R*	−0.138	−0.180
	p Value	0.338	0.211
Location	*R*	0.034	−0.074
	P Value	0.815	0.608
Pregnancy status	*R*	0.127	−0.225
	p Value	0.380	0.117
Copper	*R*		−0.146
	p Value		0.312

the animal's mineral status should be taken into consideration when preparing the final diets, as diets and feedstuffs lacking in trace mineral requirements can have detrimental effects on reproduction functions in both males and females of both species (Biru, 2021). According to Hussein's (2012) study, the levels of copper and iron in goats from the Iraqi region are unaffected by age or gender. The present study's findings on low levels of copper in men might perhaps be attributed to the dietary differences between males and females, particularly in cases when iron intake is elevated (Constable *et al.*, 2017). However, Khwedim (2013) noted that the copper content of the Iraqi soil is low. In cattle, the results indicated that the blood serum values of iron and copper significantly increased the serum iron value in females when compared to males, while the blood serum value of copper significantly decreased in females when compared to the serum value of males. The animal breeds have great role and directly affect the efficacy of copper metabolism (Sefdeen, 2017). The source of the high iron intake in ruminants surpassing several hundred micrograms was the increase in soil iron brought on by pollution, which also caused an increase in iron (Yildiz Küçük and Gökçek, 2024).

Cattle and sheep that were fed iron compounds or 10% dry matter from soils high in iron consistently saw an increase in iron levels and a decrease in copper in their livers (Sousa *et al.*, 2012). Conversely, in sheep and goats, the impact of iron on copper metabolism was noted. When iron was given at doses of 247 and 827 mg/kg dry matter, the greatest levels of iron and lowest levels of copper were seen in all tissues, including lung tissue, in grazing sheep (Grace and Lee, 1990; Mohammed *et al.*, 2016).

According to Suttle (2022), there are notable variations in the concentrations of copper in the plasma and liver of different sheep breeds. These variations may be due to genetic influences on copper metabolism (Adeniyi *et al.*, 2024). The results of blood serum copper values significantly increased in ages greater than 1 year when compared to sheep younger than 1 year. These results disagree with those of Suttle (2010).

Although all of the animals in this study are of post-weaning age, Mohammed *et al.* (2016) stated that "copper absorption was much higher in milk supplied to pre-ruminant lambs (75%–90%) than in post-weaned lambs and adult ewes (8%–9%)." However, breed-specific variations exist in sheep's copper absorption efficiency (Autukaite *et al.*, 2021). Using meals containing 12 to 20 mg Cu/kg dry matter, a study by Woolliams *et al.* (1982) found that Texel lambs store more than twice as much iron in their livers than pure black-faced lambs. The blood serum values for iron and copper in the cattle did not significantly alter between the ages of the cows that were investigated. When chelated amino acid complexes were used instead of inorganic feed sources, copper absorption was greater (Pal *et al.*, 2010). While less than 5% of copper may be absorbed via herbage, sheep, and cattle can receive 60%–80% of it from milk (Suttle, 2010). Due to species variations in Cu availability, the availability in cattle was calculated to be around 3.1%, but the projected value using equations based on sheep data was 4.0% (Suttle, 2010).

Ruminants seldom experience iron deficiency, with the exception of young ruminants (Joerling and Doll, 2019) and calves given milk. Up to the age of six, cows' liver and spleen have higher concentrations of iron (Nogara *et al.*, 2024).

When comparing pregnant and non-pregnant sheep, the serum copper value significantly increased in the former group and significantly decreased in the latter. A significant increase was found in lambing sheep's serum copper value when compared to pregnant and non-pregnant sheep. In addition to dietary considerations, there are several additional factors that influence plasma copper concentrations. According to Rodríguez *et al.* (2021), cows' serum has more copper during the estrus period on day 21 than nulliparous calves and less copper on the day of parturition in meat cows. According to Shoushtari *et al.* (2015), the plasma copper level peaked five weeks prior to birth. Moreover, infections raise serum copper levels (Besold *et al.*, 2016). The study's findings support the assertions made by Kapper *et al.* (2024) that there is a connection between an animal's mineral status and its reproductive cycle phases. For instance, during pregnancy, both the mother and the fetus experience fast development and cell differentiation as well as extremely sensitive dietary imbalances (Caton *et al.*, 2020).

For instance, during pregnancy, both the mother and the fetus experience fast development and cell differentiation as well as extremely sensitive dietary imbalances (Caton *et al*. 2020).The neurohormonal connection and the intricate way biomineral molecules work are to blame (Zatta and Frank 2007). Since sheep milk is high in iron, low iron in mother ewes plasma might be the result of increased iron excretion from milk (Molik *et al.*, 2008).

When comparing the blood serum values of sheep from Abu Saida with those from Baqubah and Saadia Cities, the results showed a considerable increase in both copper and iron. These findings are corroborated by other studies that looked at the concentration of minerals and how they relate to one another in the soil, feed, and serum of sheep and goats in Mexico. These studies found that there are mineral imbalances, with the excess of phosphorus and iron in the sheep and copper deficiencies linked to an excess of iron and copper in the soil and forages (Domínguez-Vara and Huerta-Bravo, 2008). It is possible that there is a difference in the diets followed by the breeders and what is available from the pastures, which led to these differences between different regions of the current study. In another context, Qudoos *et al.* (2023) indicated that there is another difficult factor represented in the recommendations made to animals in the fattening field system. These recommendations stated that grazing animals meet all their needs of minerals due to the high concentration of trace elements in pastures and what is available in soil and water. However, the amount of trace elements in fodder varies depending on the kind of soil, fertilization methods, species of forage, age of the plants, composition of the pasture's plants, climate, and season (Mirzaei, 2012). A study by Hussein (2012) reported that the average serum levels of copper and iron for healthy goats were 21.66 and 29.24 µmol/l, respectively. When compared to goats that seemed to be in good health, the anemic goats' average values showed a significant reduction. Thus, one of the factors that may have contributed to the variation in copper and iron contents across the study's many locations is the management and health conditions.

According to the studies by Sousa *et al.* (2012) and Mohammed *et al.* (2016), feeding sheep with different iron compounds or supplementing soil with iron at a rate of 10% of its dry matter results in high iron levels and a corresponding decrease in copper levels in the animal's body, particularly in the liver. Older cattle on farms have an adequate amount of iron; the primary issue is an excess of dietary iron (Suttle, 2010). These findings may help to explain the current study's findings.

Serum values for iron and copper (27/43 , 62.7%, with good body scores; 9/43, (20.9%), with medium body scores, and 7/43, 16.4% with poor body scores) showed a significant decrease in the good and medium body scores compared to the poor body scores, while the good and medium body scores significantly increased in the value of iron. These results are consistent with the findings of Nawito *et al.* (2015), who reported that an assessment of the nutritional status of animal herds revealed that the mean body condition score was below mean levels, but differed markedly between sheep and goats or lambs.

The findings of the current study are in accordance with Constable *et al.* (2017), who stated that "the clinical manifestations of copper deficiency in cattle occur yearly in approximately 0.9% of cattle herds in the United Kingdom." The primary cause of the largest loss is thought to be the animals' inability to develop copper deficiency, which impairs tissue oxidation and interferes with intermediate metabolism, leading to status or growth failure. Conversely, when more copper was given to the cattle, the physical state of the cows improved compared to the cows with little copper in the same field circumstances.

In the current study, there was no linear correlation between serum levels of copper and iron with age, sex, body score, and pregnancy status among studied cows. There was no linear correlation between serum level of copper and iron with age, sex, location, and pregnancy status among studied sheep. No previous study reported such correlation between iron, copper, and other parameters of the current study in sheep and cow. The absence of such linear correlation might attribute to the fact that to estimate the linear correlation among studied parameters, a large number of samples required, and study groups equally distributed regarding parameters under investigation, such as age, sex, location, and pregnancy status.

Conclusion

Although the serum levels of copper and iron in the body of sheep and cattle are affected by age, sex, pregnancy, and soil composition, there is no linear correlation between serum level of copper and iron with age, sex, body score, and pregnancy status among studied cows as well as age, sex, location, and pregnancy status among studied sheep.

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Conflict of interest

The authors disclosed that there is no conflict of interest

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The authors disclosed that they share the total cost of current research project.

Authors' contributions

Authors disclosed that they were equally contribute in planning, methodology, data collection and analysis, and writing of manuscript.

Data availability

All the required data were included with in the text.

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