Original Article

Relationship Between Some Heavy Metals and Antioxidant Markers on the Healthy Profile of Pregnant Women with Anemia in Kirkuk Governorate

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Abstract

Background: Heavy metals are defined as any metallic chemical element that has a high density and is hazardous at a lower concentration, including cadmium, arsenic, copper, lead, and mercury which mainly affect the reproductive system and are especially toxic to growing fetuses. **Objective:** The objective of this study is to assess the effect of heavy metals on pregnant women with anemia compared to healthy pregnant women. **Materials and Methods:** A total of 195 blood samples (105 pregnant women with anemia and 90 healthy subjects as control) with three trimesters were collected to estimate the levels of some heavy metals (arsenic, lead, cadmium, copper, and mercury) and some biochemical parameters includes total protein, albumin, globulin, albumin/globulin ratio, ischemia-modified albumin, free amino, carbonyl, total thiol, native thiol, di sulfide bond, LH and FSH in all studied groups in Kirkuk Governorate, Iraq and surrounding areas. **Results:** The results revealed that there were significant ($P \leq 0.05$) increases in the levels of Cu, As, total thiol, and IMA in all trimesters, Cd and Pb in the 1st and 3rd trimesters, carbonyl, carbonyl/tp, and total thiol/tp in the 2nd and 3rd trimesters, and disulfide bond and AGR in the 1st and 2nd trimesters, respectively. **Conclusion:** Statistical analysis of the results revealed that the levels of Cu increased significantly only in the 2nd trimester, Fe only in the 1st trimester, Cd and Pb only in the 1st and 3rd trimesters, while levels increased significantly in all pregnancy trimesters.

Keywords: Heavy metals, ICP, pregnancy with anemia, trace element

Introduction

Pregnancy is defined as a series of temporary, complex events that are delicately orchestrated and include fertilization, placentation, and partum.[1] Proper pregnancy depends on chronological transitions, and any deviation from this pattern might have negative effects on the health of the mother and fetus. Increased oxidative stress, a condition caused by a typical systemic inflammatory response and leading to higher levels of circulating reactive oxygen species (ROS), is prevalent during pregnancy.[2] The major source of ROS during pregnancy is the central organ that regulates this condition, that is, the placenta.[3] The higher metabolic rate, which also results in more oxidative stress in the placental tissue, allows for proper fetal growth and development. It also elevates the level of antioxidant enzymes to maintain the oxidative balance.[4] Protein modification causes oxidative stress, in which amino acids are targets for oxidative damage.[5] Protein thiol groups may scavenge oxidants, thus sparing antioxidants and/or cellular constituents from attack. The measurement of thiol groups in serum provides an indirect reflection of the anti-oxidative defenses.[6] Carbonyl groups can be formed when protein side chains are directly oxidized. They are formed rapidly in response to oxidative stress can be detected in plasma and have long-term stability making them ideal candidates for the early detection of diseases

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in which oxidative stress plays a role.[7] Heavy metals are defined as any metallic chemical element that has a high density and is hazardous at lower concentrations. Heavy metals include mercury (Hg), copper (Cu), lead (Pb), and arsenic (As) which mainly affect the reproductive system and are especially toxic to development and growing fetuses.[4] The placenta does not filter heavy metals from the mother to the fetus, where they are instead directly deposited in developing fetal tissue. Toxic metal exposure may cause infertility in women.[8] Arsenic is a grayish substance that is not poisonous, as it is insoluble in water which is not absorbed from the alimentary canal. However, it is continuously changing into white arsenic or arsenious oxide which is tasteless and highly poisonous. Arsenic may be dangerous during pregnancy. Dietary exposure to arsenic may result from agricultural regions that employ harmful metal-containing fertilizers.[6] Pregnant women exposed to arsenic have a higher risk of gestational diabetes and impaired sugar metabolism. The risk of fetal and infant mortality may rise with prolonged exposure to arsenic via drinking water. Pregnant women who are exposed to arsenic risk of mental retardation and developmental disability in newborn babies. Copper in metallic form is not poisonous but some of its salts are poisonous such as blue vitriol and sub acetate. Copper is a powerful inhibitor of enzymes. Sources of Cu are common in the diet, particularly in vegetarian diets, and can be found in the water due to Cu plumbing.[7] Many multiple vitamins contain relatively high doses of Cu. The hormone estrogen promotes the retention of Cu, and this is why women are particularly vulnerable to the problem of Cu toxicity. Copper toxicity may lead to a poor fertility rate.[6] Lead is a heavy metal that is found in the environment and can be absorbed in foods prepared with water transported through lead pipes or from processing plants that use equipment or painted walls that contain trace amounts of lead. The soluble compounds of Pb are poisonous. Lead may elevate into the body through inhalation, ingestion, or absorption through the skin and mucous membranes. Exposure of women to lead (Pb) during pregnancy has been associated with adverse outcomes such as miscarriage, early delivery, low birth weight, impaired fetal brain development, and hindered growth of the infant. Lead exposure presents health hazards for individuals of all ages, with young children and pregnant infants being particularly vulnerable to the toxic effects of lead. This exposure has been shown to have adverse impacts on the development of growing children, including their behavior and cognitive abilities.[8] There is a scientific suspicion that the element Cd (cadmium) may be a potential hazard during pregnancy. According to research, it has been shown that exposure to Cd may have detrimental effects on the placenta and result in decreased birth weight of infants. This metallic element finds use in several professional domains, including semiconductor fabrication, welding, soldering, ceramics, and painting. Women who are exposed to Cd in their work should adhere to all recommended safety measures and keep from bringing it home on their clothing. Pregnant women, in particular, may want to consider removing sources of Cd from their homes, such as fungicides containing cadmium chloride, certain fabric dyes, ceramic and glass glazes, and certain fertilizers. Cadmium at lower concentrations may produce an adverse effect during delivery. It causes abortion and the weight of the newborn child will be retarded.[8] Thus, the current study aimed to investigate the relationship between some heavy metals and antioxidant markers on the healthy profile of pregnant women with anemia compared to healthy pregnant women in Kirkuk Governorate.

Materials and Methods

Subjects and blood samples

This study was conducted between February and September 2022 in Kirkuk City, north of Iraq. The patients in the present study were pregnant women with anemia (n = 105) admitted to Azadi Hospital who either received regular treatment or not and those who were referred from the private sector or primary health centers, and normal, healthy pregnant women (n = 95). All participants were aged between 17 and 43 years and the pregnant women were divided into groups as in Table 1, including 12, 24, and 36 weeks of pregnancy.

The current study was conducted with the prior approval of the local ethics committee of the Kirkuk Health Department. All the patients mentioned above gave their consent in writing, and the objectives of the study were fully explained to them in detail before obtaining consent. None of the participants had a family history of diabetes mellitus, hypertension, or obesity. A vein was punctured to extract 5 mL of venous blood using a plastic syringe

Table 1: Samples groups in this study

Trimesters	Age of the case		Number of the cases			
	Patient age	Control age	In whole blood		In serum	
			Patients	Control	Patients	Control
1st (12 weeks)	17–41	18–40	15	15	20	15
2nd (24 weeks)	19–39	17–41	15	15	20	15
3rd (36 weeks)	17–43	18–43	15	15	20	15

and gauge 21 steel needles. From the same groups as previously, blood was drawn into gel tubes and heparin tubes. For the serum, the gel tubes' blood was allowed to coagulate at room temperature before being centrifuged at 704*g* for 10 min. Before analysis, the serum was collected and stored at −20°C. Samples of hemolysis were discarded.

Exclusion criteria

The collected samples are free from other complications except anemia to avoid any interference with the results, and patients do not suffer from other diseases outside the scope of the research topic.

Biochemical assay

The total protein (TP) and albumin (alb) levels of serum samples were determined by Lowry *et al*.[9] and Bromo Cresol Green (BCG)[10] methods, respectively. The serum globulin (glo) level was calculated using the equation: TP = albumin level – globulin level. The spectrophotometric determination of the free amino group level was determined by Zaia *et al*.[11] method. The color agent for free amino estimation is p-benzoquinone the absorbance was measured at 480 nm against the reagent blank. A standard curve was constructed with glycine in the concentration range from 10 to 90 mM.

Sulfhydryl groups were assayed according to the method of Ellman[12] as modified by.[13,14] Briefly, 10 µL of serum was added to 10 µL of 10 mM $NaBH_4$ in 1000 mL of water–methanol solution (with a volume ratio of 1:1), then 110 µL of buffer containing 0.1 mol/L tris, 10 mmol/L EDTA (0.5 mL formaldehyde add to the buffer solution, pH 8.2). Following, add 10 µL of 10 mmol/L DTNB in methanol. Blanks were run for each sample prepared as previously described in this article, with the exception that there was no DTNB in methanol following incubation for 15 min at room temperature, sample absorbance was read at 415 nm. The level of total thiol groups was determined using the TNB molar extinction coefficient of 14,100 M^{-1} cm^{-1}, and results are reported as micromole per litter (µmol/L).[13] Native thiol level was estimated: briefly 10 µL of serum was added to 10 µL of 10 mM NaCl in 1000 mL of water–methanol solution (with a volume ratio of 1:1), then 110 µL of buffer containing 0.1 mol/L tris,10 mmol/L EDTA(0.5 mL formaldehyde add to the buffer solution, pH 8.2) were mixed. Followed by adding 10 µL of 10 mmol/L DTNB in methanol. Blanks were run for each sample prepared as described previously in this article, with the exception that there was no DTNB in methanol following incubation for 15 min at room temperature, sample absorbance was read at 415 nm. Sample and reagent blanks were also subtracted. The level of the native thiol group was determined using the TNB molar extinction coefficient of 14,100 M^{-1} cm^{-1}, and results are reported as micromole per litter (µmol/L). Disulfhydryl group determined by the equation: Total thiol-native thiol/2. Protein carbonyls were estimated using the method of Levine *et al*.[15] carbonyl concentrations were determined utilizing a molar absorption coefficient of Ɛ370 = 22,000 M^{-1} cm^{-1} and using a UV–spectrophotometer and expressed as nanomoles of carbonyls per mg protein.[15] Ischemia modified albumin (IMA) involves adding cobalt chloride to a serum sample and then incubating to allow albumin cobalt binding. Dithiothreitol (DTT: a cobalt chelator) was used as a colorizing agent, and the IMA level was measured at 470 nm using a spectrophotometer.[16] Heavy metals were estimated by the ICP method based on the Tersa *et al*. and Tripathi *et al*. methods.[17,18] Reagents and Standard Solutions Ultrapure TCA was supplied by BDH. Working standards for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis were made using correct standard solutions from Perkin Elmer (USA) that had 1000 ppm of each element under test. Other reagents were all of the analytical kind. All work was done on a clean bench to limit the possibility of contamination from outside air and dust. The following procedures were followed: (1) An aliquot of 3 mL of each blood sample was treated with 3 mL TCA after being thawed at room temperature, and both volumes were mixed well in the vortex for 15 min then centrifugation for 10 min in 307*g*. The supernatant was kept in a polyethylene bottle and stored at 4°C until analysis. The blank of the reagents was carried out following the same procedure without a blood sample. At the Ministry of Science and Technology, blood samples were analyzed using ICP-OES, research and Environment Department, Baghdad, Iraq. ICP-OES (model: Horiba scientific) nebulizer type: glass concentric with a pressure of 300 kPa) was operated under the proper conditions, including selecting the best wavelength for each element (Cd 226.502 nm, Pb 405.783 nm, and 197.198 nm) with plasma argon flow rate of 0.02 L/min, auxiliary argon flow rate of 0.0 L/min, nebulizer argon flow rate of 0.4 L/min, integration time 30 s, read delay 0 s. The sample used in this part is whole blood digested by TCA. Iron concentration was estimated by biolabo kit, adding 1 mL of working reagent on 200 µL serum, then waiting 5 min at room temperature and reading at 600 nm. Estimation of copper concentration by LTA kit, adding 1 mL of working reagent on 66 µL serum, then waiting 10 min at room temperature and reading on 580 nm. Estimation of LH and FSH hormones by using the Mini Vidas technique using an IFLASH kit.

Statistical analysis

Graph Pad Prism V 9.0 (Graph Pad Software, San Diego, CA, USA) was used to do the statistical analysis, and the outcomes were presented as mean ± SD. The independent *t* test was used to compare the mean and standard deviation, and $P \leq 0.05$ was used to assess statistical significance.

Ethics approval

The study was conducted by ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol of the subject information and consent form were reviewed and approved by a local ethics committee according to document number 245 dated January 26, 2022.

Results

Tables 2, 3, and 4 showed the levels of all studied biochemical parameters of the 1st, 2nd, and 3rd

Table 2: Levels of all biochemical parameters of the 1st trimester in pregnant women with anemia compared with control

Parameters (mean ± SD)	1st trimester patients with anemia	Control	*P* value
TP (g/dL)	8.70 ± 1.054	8.44 ± 0.969	0.3101
Alb (g/dL)	3.940 ± 0.525	4.000 ± 0.674	0.7517
Glo (g/dL)	4.845 ± 1.112	4.506 ± 0.596	0.2740
AGR	0.8634 ± 0.2584	0.8850 ± 0.1633	0.7698
Free amino (mmol/L)	11.59 ± 3.982	10.92 ± 2.223	0.5569
Free amino/Tp	1.355 ± 0.5035	1.335 ± 0.3116	0.4472
Total thiol (µmol/L)	908.3 ± 193.9	685.3 ± 86.40	0.0001*
Total thiol/Tp	103.4 ± 17.63	83.00 ± 12.66	0.003*
Native thiol (µmol/L)	430.8 ± 167.1	352 ± 109.5	0.1110
Disulfide bond (µmol/gm)	227.7 ± 101.5	166.1 ± 38.40	0.0269*
Carbonyl (nmol\mL)	11.86 ± 4.577	12.91 ± 3.628	0.4734
Carbonyl/TP	1.595 ± 1.595	1.388 ± 1.388	0.2960
LH (mg/dL)	>0.2	>0.2	–
FSH (mg/dL)	>0.2	>0.2	–
Cu (µg/dL)	147.4 ± 44.6	164.7 ± 11.89	0.1389
Fe (µmol/L)	29.18 ± 8.129	19.37 ± 7.839	0.0006*
Cd (µg/L)	3.355 ± 3.510	1.91 ± 1.982	0.0469*
Pb (µg/L)	2.679 ± 2.328	1.211 ± 0.716	0.0280*
As (µg/L)	5.105 ± 1.452	0.618 ± 0.694	0.0001*
IMA	0.850 ± 0.151	0.570 ± 0.0932	0.0001*

Tp: total protein, LH: luteinizing hormone, FSH: follicle-stimulating hormone, ISM: ischemia-modified albumin, SD: standard deviation, * significant differences ($P \leq 0.05$) between patients and control

Table 3: Levels of all biochemical parameters of the 2nd trimester of pregnancy women with anemia compared with control

Parameters (mean ± SD)	2nd trimester patients with anemia	Control	*P* value
TP (g/dL)	8.184 ± 1.355	7.910 ± 1.086	0.5312
Alb (g/dL)	3.895 ± 0.704	3.593 ± 0.501	0.1791
Glo (g/dL)	4.184 ± 1.009	4.357 ± 0.350	0.5434
AGR	0.9724 ± 0.2319	0.8260 ± 0.1039	0.0347*
Free amino (mmol/L)	11.79 ± 5.968	7.850 ± 3.170	0.5229
Free amino/TP	1.218 ± 0.3101	1.157 ± 0.3598	0.5965
Total thiol (µmol/L)	956.2 ± 201.1	713.8 ± 87.18	0.0001*
Total thiol/TP	246.4 ± 98.40	90.90 ± 18.54	<0.0001*
Native thiol (µmol/L)	273.1 ± 132.9	365.5 ± 183.5	0.0928
Di sulphid bond (µmol/g)	342.3 ± 92.86	192.7 ± 98.34	0.0001*
Carbonyl (nmol/mL)	11.79 ± 5.968	7.850 ± 3.850	0.0270*
Carbonyl/TP	2.813 ± 1.181	1.018 ± 0.4687	<0.0001*
LH (mg/dL)	>0.2	>0.2	–
FSH (mg/dL)	>0.2	>0.2	–
Cu (µg/dL)	183.7 ± 52.88	123.2 ± 41.28	0.0009*
Fe (µmol/L)	16.23 ± 10.20	16.83 ± 6.635	0.8453
Cd (µg/L)	2.921 ± 3.954	4.691 ± 4.713	0.2746
Pb (µg/L)	5.195 ± 5.121	3.239 ± 4.041	0.2553
As (µg/L)	3.966 ± 2.520	1.825 ± 2.009	0.0157*
IMA	0.881 ± 0.257	0.542 ± 0.0590	0.0001*

Table 4: Levels of all biochemical parameters of the 3rd trimester of pregnancy in women with anemia compared with control

Parameters (mean ± SD)	3rd trimester patients with anemia	Control	*P* value
TP (g/dL)	8.43 ± 0.865	8.313 ± 0.875	0.6971
Alb (g/dL)	4.046 ± 0.573	3.927 ± 0.573	0.5468
AGR	0.9178 ± 0.2000	0.9050 ± 0.1385	0.8331
Glo (g/dL)	4.550 ± 0.907	4.331 ± 0.525	0.3983
Free amino (mmol/L)	8.876 ± 1.474	8.484 ± 3.032	0.6099
Free amino/TP	1.070 ± 0.2101	1.027 ± 0.3861	0.6740
Total thiol (µmol/L)	940.1 ± 233.8	602.4 ± 102.3	0.0004*
Total thiol/TP	112.2 ± 28.52	72.94 ± 12.41	<0.0001*
Native thiol (µmol/L)	459.3 ± 172.2	174.9 ± 66.97	0.0001*
Di sulphid bond (µmol/g)	241.5 ± 114.5	220.1 ± 48.68	0.4890
Carbonyl (nmol/mL)	3.840 ± 2.489	6.833 ± 1.856	0.0004*
Carbonyl/TP	0.4580 ± 0.2993	0.8341 ± 0.2592	0.005*
LH (mg/dL)	>0.2	>0.2	–
FSH (mg/dL)	>0.2	>0.2	–
Cu (µg/dL)	176.7 ± 63.82	220.8 ± 74.1	0.0687*
Fe (µmol/L)	14.60 ± 7.748	14.44 ± 5.060	0.9441
Cd (µg/L)	9.077 ± 4.013	1.649 ± 2.240	0.0004*
Pb (µg/L)	5.659 ± 3.181	1.607 ± 2.220	0.0001*
As (µg/L)	5.132 ± 2.536	2.100 ± 2.100	0.0013*
IMA	0. 985 ± 0.219	0.5257 ± 0.0799	0.0001*

* significant at $P \leq 0.05$.

trimesters in the patients with anemia and control, respectively.

The findings of this study showed that there were nonsignificant differences ($P \geq 0.05$) for Tp, albumin, globulin, AGR, free amino, free amino/Tp, native thiol, carbonyl, carbonyl/TP, LH, FSH, and Cu levels, while there was a significant increase ($P \leq 0.05$) for total thiol, total thiol/Tp, disulfide bond, Fe, Cd, Pb, As, and IMA levels in the 1st trimester of pregnant patients compared to control.

The results showed that there were non-significant differences ($P \geq 0.05$) for TP, albumin, globulin, free amino, free amino/TP, native thiol, LH, FSH, Fe, Cd, and Pb levels, while there were significant ($P \leq 0.05$) increase in AGR, total thiol, total thiol/TP, disulfide bond, carbonyl, carbonyl/TP, Cu, As, and IMA levels in 2nd trimester of pregnant patients compared to control.

The results showed that there were non-significant differences ($P \geq 0.05$) for TP, alb, glo, AGR, free amino, free amino/TP, di sulfide bond, LH, FSH, Cu, and Fe levels, significant ($P \leq 0.05$) increase in total thiol, total thiol/TP, native thiol, Cd, Pb and As levels, while there was significant ($P \leq 0.05$) decrease in carbonyl and carbonyl/TP levels in 3rd trimester of pregnant patients compared to control.

Table 5 summarizes the significant Pearson correlation (R) between the examined variables in the group of pregnant anemia patients.

According to Table 5, there was a favorable significant ($P \leq 0.05$) positive correlation between TP and total thiol in the 1st trimester, while in the 2nd trimester, there was significant ($P \leq 0.05$) positive correlation between TP-alb, TP-glo, carbonyl-Cu, and carbonyl-total thiol, significant ($P \leq 0.05$) negative correlation between carbonyl-Cd and Cd-di sulfide, and finally at 3rd trimester there was significant ($P \leq 0.05$) positive correlation between (TP-alb and IMA-As).

Table 5: Correlation between the parameters that were assessed in pregnancy anemia patients for three trimesters

Parameters		*R*	*P* value
First trimesters			
TP	Total thiol	0.5801	0.0047**
Second trimesters			
TP	Alb	0.7363	0.0002**
TP	Glo	0.6955	0.0007**
Carbonyl	Cd	-0.5170	0.047**
Carbonyl	Cu	0.4651	0.038**
Carbonyl	Total thiol	0.3890	0.050**
Cd	Disulfide	-0.5158	0.0490**
Third trimesters			
TP	Albumin	0.5768	0.0078**
IMA	Arsenic	0.5407	0.0374**

Significant variations ($P \leq 0.05$), R is Pearson correlation

Discussion

The results of TP, alb, and glo levels were non-significantly different in all studied groups compared to the control group. Adjustments in protein metabolism occur within several weeks of conception to maintain maternal homeostasis while accommodating increased

fetal demands and preparing for lactation. Whole-body protein turnover studies suggest that protein turnover in early pregnancy is similar in pregnant and non-pregnant women but a 15% and 25% absolute increase in protein synthesis occurs during the second and third trimesters, respectively.[19] The low levels of albumin may result from the dilution effect of blood components or proteinuria, however, in the third trimester of pregnancy, the decreased level of serum albumin may be associated with increased maternal and infant mortality and morbidity.[20] These results agreed with Hani *et al.*,[21] who studied the protein profile in anemic pregnant. There was a non-significant difference in the free amino group in 1st and 3rd trimesters with anemia and a significant increase in the 2nd trimesters compared to the control. When proteins are exposed to oxidizing agents, the parent amino acid residue is lost, unstable intermediates are formed, and stable products are generated. Each of these events can be used to quantify protein damage. Total free amino group analysis may provide information on the existence of oxidation reactions occurring in poorly understood systems.[22] The results above agreed with Tammo *et al.*,[23] who studied plasma amino acid in cesarean scar pregnancy patients compared with healthy pregnant women.

There was a significant ($P \leq 0.05$) increase in the total thiol in all studied groups compared to the control which may be due to that placenta acts as a source of physiological oxidative stress and a rich source of antioxidants in normal pregnancy.[24] Therefore, the placenta is highly effective in keeping oxidative stress, that is, the balance between oxidant and antioxidant systems during pregnancy. The results of native thiol levels indicated that there were non-significant ($P \geq 0.05$) differences in both 1st and 2nd and a significant increase ($P \leq 0.05$) in the 3rd trimester compared to control, increasing in the native thiol in the 3rd trimester may be due to the increasing in the globulin level which classified under globular proteins containing a lot of sulfate group. This study does not agree with Reem *et al.*[25] which studied total thiol in the pregnancy with anemia and found a decrease in the total thiol level in the pregnancy with anemia.

The levels of carbonyl indicated that there were non-significant ($P \geq 0.05$) differences in the 1st trimester, a significant ($P \leq 0.05$) increase in the 2nd trimester, and a significant ($P \leq 0.05$) decrease in the 3rd trimester compared to control, these results may be due to oxidative stress. Research suggests that anemia during pregnancy tends to raise the pro-oxidant components, which may lead to several issues, including the oxidation of essential body molecules, increasing the danger for both pregnant women and fetuses. The results of this study agreed with Zhu *et al.*,[26] who estimated carbonyl levels in pregnant women with anemia compared to control and found that there were significant ($P \leq 0.05$) decreases in the carbonyl levels in pregnant women with anemia compared to control.

There was a non-significant ($P \geq 0.05$) difference between the LH and FSH in patients' groups compared to the control which may reveal that there was inhibition in the patients and control which may be due to the physiology of pregnancy when the β-HCG stimulated the LH and FSH inhibition.[27]

The results of Cu levels cleared that there was a non-significant ($P \geq 0.05$) in the 1st and 3rd trimesters and a significant ($P \leq 0.05$) increase in the 2nd trimester compared to control. The low levels of serum Cu in pregnancy could be a predictor of some pathological pregnancies (habitual abortion, missed labor, premature rupture of membranes, and spontaneous abortion).[28]

The results of Fe levels indicated that there was a significant ($P \leq 0.05$) increase in 1st trimester and a non-significant ($P \geq 0.05$) difference in the 2nd and 3rd trimester compared to control. Normal women have a decrease in serum Fe during pregnancy because their stores of Fe are depleted due to the fetus's placenta need and require expansion of red cell mass.[29]

The results of Cd and Pb levels indicated that there were significant ($P \leq 0.05$) increases in the 1st and 3rd trimesters and non-significant ($P \geq 0.05$) differences in the 2nd trimester compared to control. Iron deficiency during pregnancy in the 3rd trimester may lead to increased Cd absorption and body burden. Multiparous women exhibit additional increases with increasing age. The results above agreed with Akesson *et al*,[30] who studied Cd level in pregnant women with anemia and then found the Cd level increased in the anemia pregnant. Lead exposure has long been correlated with adverse hematological effects mainly iron deficiency anemia as lead causes interference with both heme biosynthesis and red blood cell survival.[31-34] Hseih *et al.*[35] studied the risk of anemia in both the male and female population of the Pb manufacturing industry.

The arsenic and IMA levels were cleared and there was a significant ($P \leq 0.05$) increase in all patients' groups compared to control. The biological mechanisms of As involved in population environmental arsenic-induced anemia are largely unknown, but several experiments on animal and human studies of hematological indicators provide clues. It was observed that arsenic induces oxidative damage to human erythrocytes producing anemia-related changes including alterations in shape, deformability, agreeability, and osmotic fragility.[36] Studies in human populations highly exposed to arsenic in drinking water also reported structural and functional hemoglobin and erythrocyte alterations due to oxidative stress, and as a result, diminished oxygen binding affinity Mondal *et al.*,[37] and premature cell death Biswas *et al.*,[38] alterations likely to lead to clinical anemia. The high levels of IMA in the anemia group might be attributed to hypoxia due to low hemoglobin levels. Iron is an oxidant element and oral iron supplementation may be associated with

oxidative stress and may increase IMA levels by changing the albumin molecule. IMA can be demonstrative of the severity of anemia since it was correlated with hemoglobin in the anemia group.[39]

Table 5 showed the significant correlation coefficient of pregnancy for the three trimesters and the results indicated that the most effective correlation that could be used to evaluate the trimester is between (TP – total thiol) in 1st trimester, while in the 2nd trimester, there was significant ($P \leq 0.05$) positive correlation between (TP-alb, TP-glo, carbonyl-Cu, and carbonyl-total thiol, significant ($P \leq 0.05$) negative correlation between carbonyl-Cd and Cd-disulfide, and finally at 3rd trimester there were significant ($P \leq 0.05$) positive correlation between TP-alb and IMA-As.

Conclusions

According to the results, Cu levels increased only in the 2nd trimester, Fe only in the 1st trimester, Cd and Pb only in the 1st and 3rd trimesters, while the levels increased in all trimesters of pregnancy, these results could be due to the common exposures to these elements, which may increase the risk of an effect on the fetus. The results also showed that there were changes in some oxidation protein markers that could affect maternal and fetal health. The correlation results showed that it is important to evaluate the relation between TP-total thiol in the 1st trimester, TP-alb, TP-glo, carbonyl-Cu, carbonyl-total thiol, carbonyl-Cd, and Cd-disulfide in the 2nd trimester and TP-albumin and IMA-As in the 3rd trimester of pregnancy in women with anemia.

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Conflicts of interest

There are no conflicts of interest.

References

1. Xu B, Han YW. Oral bacteria, oral health, and adverse pregnancy outcomes. Periodontology 2022;89:181-9.
2. Prins JR, Schoots MH, Wessels JI, Campmans-Kuijpers MJ, Navis GJ, van Goor H, *et al*. The influence of the dietary exposome on oxidative stress in pregnancy complications. Mol Aspects Med 2022;87:101098.
3. Sebastiani G, Navarro-Tapia E, Almeida-Toledano L, Serra-Delgado M, Paltrinieri AL, García-Algar O, *et al*. Effects of antioxidant intake on fetal development and maternal/neonatal health during pregnancy. Antioxidants (Basel, Switzerland) 2022;11:648.
4. Marín R, Chiarello DI, Abad C, Rojas D, Toledo F, Sobrevia L. Oxidative stress and mitochondrial dysfunction in early-onset and late-onset preeclampsia. Biochim Biophys Acta Mol Basis Dis 2020;1866:165961.
5. Akagawa M. Protein carbonylation: Molecular mechanisms, biological implications, and analytical approaches. Free Radic Res 2021;55:307-20.
6. Heety AL, Lina FD, Hasan OM, Emad A, Al-Heety MS. Heavy metal pollution and ecological risk assessment in soils adjacent to electrical generators in Ramadi City, Iraq. Iraqi J Sci 2021:1077-87.
7. Gomes RR, Dutta D, Hasan FM, Saha A, Newaz MM. Correlation between demographic variables, treatment modalities and outcome in acute copper sulphate poisoning in a tertiary care hospital in Bangladesh. Toxicol Open Access 2022;8:2.
8. Zhao D, Wang P, Zhao FJ. Dietary cadmium exposure, risks to human health and mitigation strategies. Crit Rev Environ Sci Technol 2023;53:939-63.
9. Classics Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
10. Müller K, Brunnberg L. Determination of plasma albumin concentration in healthy and diseased turtles: A comparison of protein electrophoresis and the bromocresol green dye-binding method. Vet Clin Pathol 2010;39:79-82.
11. Zaia DA, Barreto WJ, Santos NJ, Endo AS. Spectrophotometric method for the simultaneous determination of proteins and amino acids with p-benzoquinone. Anal Chim Acta 1993;277:89-95.
12. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
13. Hu ML, Louie S, Cross CE, Motchnik P, Halliwell B. Antioxidant protection against hypochlorous acid in human plasma. J Lab Clin Med 1993;121:257-62.
14. Riddles PW, Blakeley RL, Zerner B. Ellman's reagent: 5,5′-dithiobis (2-nitrobenzoic acid)—a reexamination. Anal Biochem 1979;94:75-81.
15. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. In Methods in Enzymology 1990 (Vol. 186, pp. 464-478). Academic Press.
16. Dervisoglu P, Oner T. Usefulness of ischemia-modified albumin for assessment of the effects of small ventricular septal defects on the pulmonary vascular bed. Cardiol Young 2021;31:1207-12.
17. Teresa M, Vasconcelos SD, Tavares HM. Trace element concentrations in blood and hair of young apprentices of a technical-professional school. Sci Total Environ 1997;205: 189-99.
18. Tripathi RM, Raghunath R, Mahapatra S, Sadasivan S. Blood lead and its effect on Cd, Cu, Zn, Fe and hemoglobin levels of children. Sci Total Environ 2001;277:161-8.
19. Mousa A, Naqash A, Lim S. Macronutrient and micronutrient intake during pregnancy: An overview of recent evidence. Nutrients 2019;11:443.
20. Sufrin S, Nessa A, Islam MT, Das RK, Rahman MH. Study on serum albumin in third trimester of pregnancy. Mymensingh Med J 2015;24:464-6.
21. Ahmed HA. Anemia and hypoproteinemia in Sudanese pregnant women during the second and third trimester. Saudi J Biomed Res 2021;6:221-5.
22. Hawkins CL, Morgan PE, Davies MJ. Quantification of protein modification by oxidants. Free Radic Biol Med 2009;46:965-88.
23. Tammo O, Uyanikoglu H, Koyuncu I. Evaluation of plasma free amino acid and carnitine levels in patients with cesarean scar pregnancy. Combinatorial Chem High Throughput Screen 2021;24:1436-45.
24. Erkenekli K, Sanhal CY, Yucel A, Bicer CK, Erel O, Uygur D. Thiol/disulfide homeostasis in patients with idiopathic recurrent pregnancy loss assessed by a novel assay: Report of a preliminary study. J Obstet Gynaecol Res 2016;42:136-41.
25. Reem M, Israa G. Purification and characterization of ceruloplasmin in the sera of women with pregnancy complications. Master Thesis, Kirkuk University. 2021.
26. Zhu Q, Qian Y, Yang Y, Wu W, Xie J, Wei D. Effects of carbonyl iron powder on iron deficiency anemia and its subchronic toxicity. J Food Drug Anal 2016;24:746-53.
27. Bazer FW. Endocrinology of Pregnancy. Springer Science & Business Media 2012;9.
28. Vukelić J, Kapamadžija A, Petrović D, Grujić Z, Novakov-Mikić A, Kopitović V, *et al*. Variations of serum copper values in pregnancy. Srp Arh Celok Lek 2012;140:42-6.

29. Maitra S, Mukthapuram A, Huligol G, Sreelatha G, Vishwanath H. Increased serum ferritin and iron levels in preeclampsia. IOSR 2019;5:50-2.
30. Åkesson A, Berglund M, Schütz A, Bjellerup P, Bremme K, Vahter M. Cadmium exposure in pregnancy and lactation in relation to iron status. Am J Public Health 2002;92:284-7.
31. Abadin H, Ashizawa A, Stevens YW, Llados F, Diamond G, Sage G, *et al*. Toxicological profile for lead.
32. Scholl TO. Maternal iron status: Relation to fetal growth, length of gestation, and iron endowment of the neonate. Nutr Rev 2011;69:S23-9.
33. Hegazy AA, Zaher MM, Abd El-Hafez MA, Morsy AA, Saleh RA. Relation between anemia and blood levels of lead, copper, zinc and iron among children. BMC Res Notes 2010;3:1-9.
34. Tetens I. European Food Safety Authority: EFSA: Panel on Dietetic Products, Nutrition, and Allergies (NDA); scientific opinion on dietary reference values for water. EFSA J 2010;8:1459.
35. Hsieh NH, Chung SH, Chen SC, Chen WY, Cheng YH, Lin YJ, *et al*. Anemia risk in relation to lead exposure in lead-related manufacturing. BMC Public Health 2017;17:1-2.
36. Bollini A, Huarte M, Hernández G, Bazzoni G, Piehl L, Mengarelli G, *et al*. Arsenic intoxication, a hemorheologic view. Clin Hemorheol Microcirc 2010;44:3-17.
37. Mondal B, Chatterjee D, Bhattacharyya M. Structure–function alteration of hemoglobin in arsenicosis patients: A probable pathway to exert toxicity. J Appl Toxicol 2012;32: 581-9.
38. Biswas D, Banerjee M, Sen G, Das JK, Banerjee A, Sau TJ, *et al*. Mechanism of erythrocyte death in human population exposed to arsenic through drinking water. Toxicol Appl Pharmacol 2008;230:57-66.
39. Bilgili S, Bozkaya G, Tütüncüler FK, Akşit M, Yavuz M. Investigation of ischemia modified albumin levels in iron deficiency anemia. Turkish J Biochem 2017;42:259-63.