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Measurement of Protein Oxidation Levels in Serum of Women with Pregnancy Complications

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Abstract

Pregnancy is a physiological condition start from fertilization to delivery. The mother and the fetus are exposed to serious complications during pregnancy. Variations in the antioxidant protection mechanism characterize this condition. The aim of this study was to estimate serum ceruloplasmin activity (Cp) and some biochemical parameters levels as markers of oxidative stress in serum of women with pregnancy complications compared to women with healthy pregnancies as control group and study the correlation between them. The study included (70) serum samples divided into (45) samples as patients with pregnancy complications which are sub-divided to the following groups: preeclampsia, anemia and Abortion and (25) samples of women with healthy control group. All of the studied groups showed non-significant difference in total protein level and ceruloplasmin activity except abortion group which had a significant decrease when compared to other groups. All studied groups showed non-significant differences in free amino, thiol and carbonyl levels, while abortion group showed significant decrease in carbonyl levels. In addition, all of the studied groups showed a non-significant increase in copper (Cu) levels, except for abortion group, that showed a significant decrease. Finally, all of the studied groups showed a non-significant increase in iron (Fe) levels, except the preeclampsia group, that showed a significant increase as compared to other groups.

Keywords: Pregnancy complications; Preeclampsia; Abortion; Ceruloplasmin; Protein oxidation markers; Cu and Fe

1. Introduction

Pregnancy is a natural physiological condition characterized by metabolic and hormonal changes that help the fetus growth and survival. The metabolic, cardiovascular, renal, respiratory, and gastrointestinal systems of the body undergo physiological alterations [1]. Human gestation lasts about (40) weeks and is divided into three trimesters, start with fertilization and end with birth [2]. Serious pregnancy complications may occur at any time during the pregnancy. Smoking status, socioeconomic status, and other health-related conditions and habits are all factors that influence pregnancy outcomes [3]. Abortion is one of the most common pregnancy complications, defined as a sudden failure of an intrauterine pregnancy [4]. Physiological changes in the blood circulatory system are especially noticeable during pregnancy. Even in a healthy pregnancy, the concentration of hemoglobin (Hb) decreases as the volume of circulating blood increases. Anemia, the most prevalent nutritional deficiency disorder in the world, affects about 20% of pregnant women [5]. World Health Organization (WHO) defined pregnancy anemia as the Hb concentration of <11 g/dl [6]. Preeclampsia is characterized as arterial hypertension (systolic pressure 140 mmHg and/or diastolic pressure 90 mmHg) with proteinuria and may be associated with edema and increased platelet aggregation, and it is progressively diagnosed after the 20th week of pregnancy [7]. Preeclampsia can progress to eclampsia, which is marked by extreme hypertension and convulsions and can lead to coma and death if left untreated [8]. Other serious complications can be considered a life threatening condition for the mother and the fetus involve ectopic pregnancy (implantation of the embryo outside the uterine) [9], molar pregnancy (over growth of chorionic fetal tissues inside the uterine) [10], hemostasis (thrombosis) [11] and gestational diabetes mellitus, which is characterized by hyperglycemia as a result of

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low glucose tolerance caused by pancreatic-cell dysfunction in the background of chronic insulin resistance [12]. In pregnant women, an imbalance of oxidative/antioxidant species can put them at risk for serious complications. Many studies have shown that there is an increase in oxidative stress during pregnancy, and the oxidative stress is increase in pregnancies with complications [13]. Few biomarkers have been used to aid in early disease diagnosis in pregnancy [14]. Protein modification causes oxidative stress in which amino acids are targets for oxidative damage. Carbonyl groups can be formed when side chains are directly oxidized [15]. Protein carbonyl content is the most widely used and most general marker of protein oxidation. [16]. Because of its relative stability and early formation of carbonylated proteins and it has many advantages over the other oxidation markers. Multiple radicals, excited state species, and singlet O_2 produce protein carbonyls (because of secondary reactions). The thiol group of protein-bound cysteine residues is also oxidized, which is a commonly used nonspecific indicator of protein oxidation [[17]. Thiols are a major aspect of the complete redox buffer system, and they help to protect the body from oxidative stress. There are conflicting opinions about how antioxidant activity changes in pregnancy. Antioxidant defense mechanisms are complicated and multifactorial [18]. Thiols with a sulfhydryl (-SH) group, which are oxidized by oxidants to form reversible disulfide bridges, are the target of reactive oxygen species (ROS). The disulfide bridges that have formed can be reduced to thiol groups [19]. The Cp has also been considered as a plasma antioxidant due to its ability to scavenge toxic substances like superoxide and H_2O_2, thereby reducing oxidative damage of lipids, proteins and DNA. Ceruloplasmin has a copper-dependent oxidase activity, which is related to the oxidation of ferrous ions (Fe2+) to ferric ions (Fe3+), so its transport in the plasma is estimated in conjunction with transferrin, which can only carry Fe in the ferric state [20]. During pregnancy, progesterone and estrogen levels are elevated and could cause a rise in the levels of serum Cp [21]. This study aimed to estimate serum Cp activity and some biochemical parameters levels including total protein (TP), free amino, free amino/ TP, thiol, thiol/ TP, carbonyl, carbonyl/ TP, Cu, Cu/CP and Fe in the serum of pregnant women with complications compared to women with healthy pregnancy.

2. Experimental

Seventy women were chosen in this study, they were divided to (25) serum samples of women with healthy pregnancy as control group with age ranges between (20 and 40) years and (45) samples of women with pregnancy complications which sub-divided to the following groups: preeclampsia (n =15), anemia (n= 15) and Abortion (n=15) with the same age ranges as control group from 3rd to 9th month pregnancy in all groups. Those patients visited "Kirkuk general hospital" in Kirkuk, Iraq, between October 2019 and March 2020, and were diagnosed by doctors. Blood samples were collected (2–3 mL) by venipuncture in glass tubes inside Gel Tube for separated using a disposable syringe. After (15) minutes at room temperature, the tubes were centrifuged for (10) minutes at (1500xg). The buffy coat was removed and Serum samples were stored at (−20°C) until used.

Ceruloplasmin activity was determined by the method of Menden *et al*. [22] using p-phenylene diamine as substrate. The activity of Cp is calculated according to the following equation: Cp activity (mg/L) = $\Delta A/0.68 \times 1000$, where: (Extinction coefficient) $\varepsilon = 0.68\ M^{-1}\ cm^{-1}$. Lowry *et al* [23]3] method was used to assess total serum protein concentration by using BSA as a standard protein. The free amino group spectrophotometric determination was carried out using the Zaia *et al* [24] process and the protein free amino content was also estimated (free amino concentration (mmol/ml)/total protein (mg/ml) . The concentrations of thiol groups were estimated according to the method of Ellman method [25], which modified by Riddles *et al* [26] using the equation: A = Ɛ. C. l, where: $\varepsilon = 14{,}100\ M^{-1}\ cm^{-1}$. Thiol/total protein ratio was also estimated by dividing thiol concentration (µmol/ml) by total protein (mg/ml). The protein carbonyl content was assayed by the method of Levine *et al*. [27] as in the equation: A = Ɛ. C. I, where: $\varepsilon_{370} = 22{,}000\ M^{-1}\ cm^{-1}$. Carbonyl/total protein ratio was also estimated (carbonyl (nmol/ml)/total protein (mg/ml)). Cu and Fe concentrations were estimated using the kits supplied from ITA and BIOLABO companies, respectively. Finally, copper/ceruloplasmin ratio was estimated using the equation: copper (µmol/L) × 0.132/cerulolpasmin (g/L) [28].

3. Statistical analysis

Statistical analysis was done using GraphPad Prism Version 8 (GraphPad Software, San Diego, CA, USA). Values were expressed as (mean ± standard

error [SE]). The comparison of (mean ± SE) was performed using the ANOVA test. Statistical significance was defined as ($P \leq 0.05$) and the correlation between the parameters.

4. Results and discussion

Table (1) represents the TP and the activity and specific activity of Cp as (mean ± SE) for all studied groups .

Table (1): Total protein, activity and specific activity of ceruloplasmin in serum for all studied groups.

Parameters	Healthy controls (n=25) mean ± SE	Complications (n=45) mean ± SE	Preeclampsia (n=15) mean ± SE	Anemia (n=15) mean ± SE	Abortion (n=15) mean ± SE
Total protein (TP) (mg/ml)	51.03±1.850[a]	52.26 ± 1.362[a]	50.33± 1.612[a]	50.31± 2.558[a]	57.28± 2.667[a]
Cp (mg/l)	366.6± 18.2[a]	358.14±16.1[a]	442.8±31.3[a]	372.2±24.4[a]	273.3±16.9[b] *P* value ≤0.05
Specific activity of Cp (U/mg)	0.492±0.140[a]	0.481±0.03[a]	0.593±0.04[a]	0.52±0.05[a]	0.329±0.03[b] *P* value ≤0.05

The different letters referred to significant differences, while similar letters referred to non – significant differences between the compared groups, P≤0.05 significant and P≥0.05 non- significant.

The results showed that there was non-significant difference *(P* value≥ 0.05) in TP concentration; Cp (activity and specific activity) in all studied groups except women with abortion cleared a significant decrease in the activity and specific activity of Cp compared to other groups. Table (2) showed the levels of some biochemical and protein oxidation parameters in all studied groups.

Table (2): The levels of some biochemical and protein oxidation parameters in serum for all studied groups.

Parameters	Healthy controls (n=25) mean ± SE	Complications (n=50) mean ± SE	Preeclampsia (n=15) mean ± SE	Anemia (n=15) mean ± SE	Abortion (n=15) mean ± SE
Free amino (mmol/l)	39.01±3.38[a]	46.36±2.24[a]	41.67±3.43[a]	47.50±3.31[a]	43.91±3.97[a]
Free amino/TP x10^3(mmol/mg)	0.82±9.49[a]	0.93±5.35[a]	0.85±8.19[a]	0.97±7.67[a]	0.78±7.43[a]
Thiol (µM)	24.20±1.64[a]	22.91±0.87[a]	23.30±1.74[a]	19.73±1.299[a]	24.29±1.68[a]
Thiol/TP x 10^3 (µmol/mg)	0.5±4.14[a]	0.45±2.15[a]	0.47±4.1[a]	0.41±3.83[a]	0.43±3.61[a]
Carbonyl (nM)	54.51±3.47[a]	52.77±2.57[a]	57.51±4.63[a]	56.76±3.85[a]	37.76±1.64[b] *P*value≤0.05
Carbonyl/TP x 10^3 (nmol/mg)	1.03±8.44[a]	0.92±4.47[a]	0.97±6.88[a]	1±6.39[a]	0.64±3.53[b] *P*value≤0.05
Copper (µg/dl)	158.2±16.61[a]	178.4±12.97[a]	209.2±14.95[a]	178.5±25.05[a]	103.5±3.69[b,c] *P*value≤0.05
Fe (µg/dl)	46±7.60[a]	68.78±7.40[a]	96.3±14.75[b] *P* value≤ 0.05	49.2±17.34[a]	72.3±6.39[a]
Cu/CP ratio	13.63±1.34[a]	10.82±0.94[a]	10.17±0.83[a]	11.37±2.34[a]	8.23±0.52[a]

- The different letters referred to significant differences, while similar letters referred to non – significant differences between the compared groups, P≤0.05 significant and P≥0.05 non- significant.

The results showed that there was non-significant ($P \geq 0.05$) difference in the free amino, free amino / TP, thiol and thiol/TP levels in all studied groups. The results of carbonyl and carbonyl/TP levels showed non-significant ($P \geq 0.05$) difference in all studied groups except women with abortion showed significant decrease ($P \leq 0.05$) compared to other groups. The results also indicated a non-significant ($P \geq 0.05$) increase in Cu levels in all studied groups except abortion women showed and significant decrease ($P \leq 0.05$) in Cu levels. Finally the levels of Fe indicated that there were non-significant increase in all studied groups except pregnant women with preeclampsia showed a significant increase compared to other groups. Table (3) summarized the Correlation coefficient between the studied parameters in (pregnant women with complications) patients.

The correlation results indicated that there was significant positive correlation between CP/ Free amino (0.53/ 0.04 r/p) in preeclamptic women, TP/Carbonyl (0.60/ 0.018 r/p) in pregnant women with anemia, and Free amino/Cu (0.50/ 0.05 r/p) in women with abortion and significant negative correlation between Thiol/Fe (-0.50/ 0.05 r/p) in women with abortion.

Table (3): Correlation coefficient between the studied parameters in patients' groups.

	r/*P*		
Parameters	**preeclampsia**	**Anemia**	**Abortion**
Cp/TP	0.11/ 0.70	-0.36/ 0.19	0.023 / 0.94
Cp/free amino	0.53/ 0.04*	0.014/ 0.96	-0.45/ 0.09
Cp/thiol	-0.2/ 0.48	-0.20/ 0.47	-0.075/ 0.79
Cp/carbonyl	-0.27/ 0.34	-0.056/ 0.84	-0.23/ 0.40
Cp/copper	0.39/ 0.15	-0.42/ 0.12	-0.051/ 0.86
Cp/iron	-0.26/ 0.35	0.17/ 0.54	0.45/ 0.09
TP/free amino	-0.10/ 0.71	0.19/ 0.50	0.21/ 0.44
TP/thiol	-0.13/ 0.64	-0.15/ 0.59	0.11/ 0.69
TP/carbonyl	0.086/ 0.76	0.60/ 0.018*	0.23/ 0.40
TP/copper	-0.48/ 0.07	-0.020/ 0.94	-0.079/ 0.78
TP/iron	0.095/ 0.74	-0.19/ 0.49	0.18/ 0.52
Free amino/thiol	-0.19/ 0.49	0.28/ 0.31	-0.23/ 0.41
Free amino/carbonyl	-0.11/ 0.71	0.027/ 0.92	-0.10/ 0.71
Free amino/copper	0.30/ 0.27	0.28/ 0.31	0.50/ 0.05
Free amino/iron	-0.45/ 0.09	0.24/ 0.39	-0.095/ 0.74
Thiol/carbonyl	-0.41/ 0.13	-0.48/ 0.07	0.41/ 0.13
Thiol/copper	0.18/ 0.54	0.075/ 0.79	-0.39/ 0.14
Thiol/iron	0.16/ 0.57	0.046/ 0.87	-0.50/ 0.05
Carbonyl/copper	-0.39/ 0.14	-0.29/ 0.30	0.081/ 0.78
Carbonyl/iron	-0.16/ 0.58	-0.32/ 0.25	-0.43/ 0.11
Copper/iron	-0.38/ 0.17	0.096/ 0.73	0.34/ 0.21

* P≤0.05 significant. r/P: Coefficient of correlation/significance.

During pregnancy dramatic changes occurring in the developing fetus and in the mother. Significant alterations in maternal biochemistry are parts of that process and represent the normal physiology of pregnancy. Pregnancy exposes to many types of complications that may be linked to an increase in oxidative stress, which is often related to the development of multiple pathologies during pregnancy. In pregnancy, oxidative stress is thought to be a risk factor [29].

Ceruloplasmin activity and copper levels rise significantly during pregnancy due to elevated estrogen levels, move across the placenta through passive transfer, and aggregation of Cu primarily during the second and third trimesters, where it is stored as metallothionein in the liver [30]. Estrogens act by regulating Cp synthesis at the transcriptional level, they stimulate the production of mRNA coding for the protein Cp [31]. Ceruloplasmin is a ferroxidase that is synthesized in hepatocytes and excreted into the plasma after 6 Cu atoms are incorporated [32]. Cp is an acute phase reactant, it carries about (70%) of the total copper in the plasma while about (15%) carried by albumin [33], so it could be considered as a sensitive indicator for evaluating the course of pregnancy and placental function [34]. In this study the results of Cp are significantly lower in women with abortion than in women with healthy pregnancy which may indicate that the antioxidants activities are decreased in this type of pregnancy complication [35]. The results above agreed with [36], [37], [38], and may indicate that the activity of Cp and Cu value seem to consider as an index of feto-placental function in early pregnancy [36]. Cu/Cp ratio calculation has been suggested as one of the better measures of Cu status during pregnancy [31], the results of this study indicated that there was non-significant decrease in the ratio of Cu/Cp which agreed with the results obtained by Louro M O et al study [39]. While a rise in serum copper accelerates the rate of ceruloplasmin protein synthesis and release, the decrease in specific oxidase activity of circulating ceruloplasmin will be an indication of the degree of depletion of the mother's Cu deposits to meet the fetus' requirements. Because of their abundance and fast rates of reaction with a wide range of molecules. 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. Through quantifying the loss of individual amino acids, total free amino group analysis may provide

information on the existence of oxidation reactions occurring in poorly understood systems [17]. Determination of the serum thiol level as a measure of oxidative stress in this study showed that thiol levels were decreased in abortion group compared with control group which agreed with the results of Bandyopadhyay et al [40]. Several studies have indicated that the placenta as a main source for ROS [41], [42]. Placental dysfunction caused by oxidative stress can be a common cause of the multifactorial and polygenic etiologies of abortion [43]. Protein carbonyl levels were higher in women with healthy pregnancy than in aborted women which may be considered as sensitive biomarkers of ROS [18]. Normal women have a decrease in serum Fe during pregnancy because their stores of Fe are depleted due to fetoplacental need and required expansion of red cells mass. However, serum Fe levels are elevated in preeclampsia, according to several previously reported research [44], [45], and this study results of Fe level cleared that there were significant increase in serum Fe levels in preeclampsia compared to women with healthy pregnancy. Excess Fe may be assumed as causative factor in the oxidative stress, which could be included in preeclampsia pathogenesis. However, Fe status of pregnant women with preeclampsia should be determined before giving Fe supplements as these may cause more harm than benefit [46]. The correlation results indicated that Cp activity increase in pregnant women with preeclampsia and the antioxidant effect of Cp on oxidative stress has a positive correlation which led to increase the free amino in preeclampsia women. Strong inter-correlations were found among TP with carbonyl group in anemia pregnant women, while women with abortion appeared the presence of an increase in free amino with Cu and finally inverse correlation found between the Fe with thiol level in abortion pregnant women. The correlation results of this study may be indicating that these above parameters which correlate each other might be beneficial for the evaluation of these pregnant complications.

4. Conclusions

The results of this study showed that the oxidative proteins markers have a role in pregnancy complications compared to healthy controls, which might play an active role in the progression of disease. The findings above may support an association between these oxidation proteins and pregnancy complicated with abortion. The stronger response noticed in serum Cp, carbonyl, Cu and Fe from patients with the change in the level of proteins which suggest that these oxidative protein marker contents may be useful in evaluating the disease pathogenesis. The positive correlation results between Cp/Free amino in preeclamptic women, TP/Carbonyl in pregnant women with anemia and Free amino/Cu in women with abortion and significant negative correlation between Thiol/Fe the women with abortion may be important for evaluation and diagnosis of pregnancy complications patients.

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