STUDY OF OXIDANT-ANTIOXIDANT STATUS IN RECURRENT SPONTANEOUS ABORTION

Prof. Dr. Jamal Abdul-Barry*, Assistant Prof. Dr. Sajda A. Al-Rubai*,
Qutaiba A. Qasim**

ABSTRACT

Objective: To evaluate the association of oxidative stress and impaired antioxidant defense system with the occurrence of recurrent spontaneous abortion (RSA) .

Study design : Case –control study consisting of 100 pregnant women with a history of RSA (study group) and 100 healthy pregnant women (control group) , their age ranging (15-45yr.) and both groups are classified into many subgroups according to maternal age , gestational age , parity and number of previous abortions . Fasting blood samples were collected and tested for measurement of serum vitamin C , vitamin E , albumin , oxidative stress biomarker malondialdehyde (MDA) and some essential trace elements (zinc and copper) by using spectrophotometric methods .Student t-test , chi-square and correlation analysis were used for statistical analysis .

Results : Serum level of vitamin C , vitamin E , Cu and Zn were significantly lower ($p<0.05$) and serum level of MDA was significantly higher ($p<0.05$) in study group as compared with control group but no significant difference of serum albumin ($p>0.05$) between the two studied groups .Also no significant differences ($p>0.05$) of all measured biochemical parameters among different age , gestational age, parity and no. of abortions subgroups of patients (study group) except serum albumin, Zinc and Cu/Zn ratio were significantly changed in the last parity subgroup ($>P_5$) as compared with (P_1) for albumin level and with other parity subgroups of the same study group for Zinc and Cu/Zn ratio . While there was a significant increase ($P<0.05$) of MDA level in the (≥ 7 abortions) as compared with other no. of abortions subgroups of the study group .

The linear regression analysis demonstrated a significant ($r=0.61$, 0.85 , 0.3 , 0.73 , 0.47 , $p<0.05$) negative correlation for vit.C , vit. E , Zn , Cu and Cu/Zn ratio respectively and significant ($r= 0.83$, $p<0.05$) positive correlation for MDA values with no. of previous abortions of study group . Also there is a significant ($r= 0.18$, 0.19 , 0.25 , 0.2 , $p<0.05$) negative correlation for vit.C , vit.E , Cu and Cu/Zn ratio respectively and significant ($r= 0.2$, $p<0.05$) positive correlation for MDA values with the age of pregnant females of both control and study groups . while there is a significant positive correlation ($r=0.16$, 0.18, 0.19 , $p<0.05$) of serum vit.C , vit.E and Zn respectively and a significant negative correlation ($r=0.17$, $p<0.05$) of serum MDA with parity in both control and study groups .

Conclusions : Our data demonstrate that elevation of lipid peroxides (MDA) together with impaired antioxidant defense and status of trace elements and the presence of possible interrelationship between those parameters may be related at least partly to the pathogenesis of RSA .

Key words : Recurrent abortion , lipid peroxidation , oxidative stress, antioxidant , trace elements .

---

* Depart. of Biochemistry, College of Medicine, University of Basra, Iraq
** Depart. of Gyne. and Obstet., College of Medicine, University of Basra, Iraq
*** Depart. of Pharmaceutics, College of Pharmacy, University of Basra, Iraq

INTRODUCTION :

Habitual abortion, recurrent miscarriage or recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses usually before the 24th week of gestation or a fetal weight less than 500 gram[1] . about 1% of all women will suffer from RSA [2] . The currently well-established causes together account for only about 40% of the cases include chromosomal abnormalities , maternal diseases including poorly-controlled diabetes mellitus , uncontrolled thyroid disease, severe systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) ; poor maternal lifestyle habits (including alcohol consumption ,with a substantial proportion of cases (60%) classified as "unknown" or "unexplained" [3] . The physiological hypoxia of the first trimester gestational sac may protect the developing fetus against the deleterious and teratogenic effects of oxygen free radicals (OFRs). Independent of the cause of the miscarriage, the excessive entry of maternal blood into the intervillous space has two effects: (i) a direct mechanical effect on the villous tissue which becomes progressively enmeshed inside large intervillous blood thrombi (ii) and a widespread and indirect O_2-mediated trophoblastic damage and increased apoptosis [4,5, 6]. Overall, the consequences are placental degeneration with complete loss of syncytiotrophoblast function and detachment of the placenta from the uterine wall. This mechanism is common to all miscarriages, with the time at which it occurs in the first trimester depending on the etiology[7]. When cells use oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process [8] . At low or moderate levels, ROS and RNS exert beneficial effects on cellular responses and immune function but at high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures [9]. Oxidative stress plays a major part in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases[9]. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements [10 , 11]. A phenomenon called oxidative stress , is occurred When free radicals and oxidants produced in excess, a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) [12,13,14]. Biological free radicals are thus highly unstable molecules that have electrons available to react with various organic substrates such as lipids, proteins, DNA [15] . The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention [13]. The antioxidant process can function in one of two ways: chain-breaking or prevention[16]. For the chain-breaking, when a radical releases or steals an electron, a second radical is formed. The last one exerts the same action on another molecule and continues until either the free radical formed is stabilized by a chain-breaking antioxidant (vitamin C, E, carotenoids , etc), or it simply disintegrates into an inoffensive product[16]. The present study was designed to evaluate the association between the changes in the serum level of antioxidant vitamins(vitamin C , vitamin E) , trace minerals (copper , zinc and Cu/Zn ratio) and changes in serum level of oxidative stress biomarker malondialdehyde (MDA) with the occurrence of recurrent spontaneous abortion (RSA) .

MATERIALS & METHODS

This study was conducted prospectively as a case-control one at Basra Maternity and Children Hospital and private clinic from December 2008 till the end of December 2009 (over one year period) . . It was included a total number of 200 subjects (100 healthy pregnant women [control group] and 100 pregnant women with a history of RSA [study group]) . Their age ranged from 15-45 years with a gestational age between 5-24 weeks and the exclusion criteria for the two studied groups include : Any pregnant woman who have a gestational age more than 24 weeks or having any medical or obstetrical problem like diabetes , cardiovascular diseases , pre-eclampsia , hydatidiform mole , malabsorption and cancer or using an antioxidants supplements like vitamin C , E, and minerals like zinc , copper . Also all studied pt.s were sent for serological testing of Ig M and Ig G antibody titre of toxoplasma , cytomegalovirus (CMV) and anticardiolipin antibody titer and those with positive result were excluded .All subjects gave informed written consent . Ten milliter of venous blood samples were taken from each patient fasting overnight .Serum was separated by centrifugation and samples were processed immediately and the separated serum (not haemolyzed) was divided into three aliquots in plain tubes as following : The first aliquot for vitamin C estimation in which one ml. of serum was treated immediately with 1.5 ml. of metaphosphoric acid . centrifuged for one minute at 3000 rpm and the supernatant was kept at -4C and analyzed within 2 wks .The second aliquot of serum used for testing of serum malondialdehyde within 24 hr. The third aliquot of serum was freezed at -4C and used for estimation of vit. E , zinc , copper , and albumin . Ascorbic acid was determined by reduction of the colored dye 2,6-dichlorophenolindophenol from a blue to a colorless form . the amount of decolorization was determined photometricaly at 520 nm[17]. Measurement of serum vitamin E concentration depends on the Emmerie-Engel reaction in which the tocopherols reduce ferric iron to ferrous iron , which is then reacted with α,α-dipyridyl to give a red-orange color with absorbance at 520 nm[18]. Bromocresol green reagent (RGT) forms with albumin in citrate buffer a colored complex . the absorbance of this complex is proportional to the albumin conc. in the sample [19,20]. zinc reacts with the chromogen present in the reagent forming a colored compound with color intensity is proportional to the zinc conc. present in the sample[21,22]. The cupric ions react with the chromogen Di-Br-PAESA forming a blue compound , which intensity is proportional to the copper conc. Present in the sample[23,24]**.** Malondialdehyde (MDA) , formed from the break down of polyunsaturated fatty acids , serves as a convenient index of determining the extent of the peroxidation reaction . The thiobarbituric acid assay of Buege and Aust (1978) was used to measure the MDA at 535 nm [25] . The data were expressed as mean ± standard error of the mean (SE) and analyzed by using of Microsoft excel 2007 software . The age , parity comparison between groups analyzed by using of chi- sequare test . The changes in oxidative stress biomarker MDA was analyzed by using of student t-test .Correlation and regression analysis was done for all parameters in both groups and for all statistical analysis , the level of significance was evaluated at $p<0.05$.

RESULTS :

The basic clinical characteristics and their subgroups of all studied pregnant females are expressed in :

Table (1) . All subjects were classified according to their age (yr.) , parity , gestational age (weeks) and number of abortions .No significant differences were observed in maternal age and gestational age ($P>0.05$). however , there were significant differences in parity and number of abortions ($P<0.05$) .

Table (2) shows the mean values of the biochemical parameters measured in both control and study subjects . Serum levels of antioxidant vitamins(C and E)as well as trace elements (Zn , Cu and Cu/Zn ratio) were significantly lower in pregnant females with previous recurrent abortions as compared with control group ($p<0.05$), while the serum level of MDA was significantly increased in patients in comparison to controls ($p<0.05$) .There were no significant difference ($p>0.05$) in the serum level of albumin between study and control subjects .

Table(3) shows that serum Zinc level was significantly decreased in the ($>P_5$) parity subgroup in comparison to the nil parity , P_1 and P_2-P_5 parity subgroups of study group . Also this table refers to a significant increase of Cu/Zn ratio of the ($>P_5$) subgroup as compared with other parity subgroups of the same study group .

Table(4) reveals that serum MDA conc. of the third subgroup of $pt._s$ (no. of abortions ≥ 7) was significantly increased ($p<0.05$) as compared to the first and second subgroups of $pt._s$. However , there is no significant difference in the serum MDA conc. between first and second subgroups of $pt._s$ ($p>0.05$) .

Table(5) The correlation analysis among age , gestational age and all measured biochemical parameters (vitamin C , vitamin E , albumin , MDA , zinc , copper and Cu/Zn ratio) in all studied pregnant females were performed and presented in Table (5) .The serum vitamin C showed significant positive correlation ($r =0.67$, 0.27, 0.55 , $p<0.05$) with serum vitamin E , zinc and copper respectively . While there is a significant negative correlation of serum vitamin C ($r =0.6$, 0.18 , $p<0.05$) with MDA and age respectively . Also serum vitamin E showed a significant negative correlation ($r =0.86$, 0.19 , $p<0.05$) with serum MDA and age respectively while there is a significant positive correlation of serum vitamin E ($r=0.34$, 0.77 , $p<0.05$) with zinc and copper respectively . Serum MDA showed a significant negative correlation ($r=0.34$, 0.75 , $p<0.05$) with serum zinc and copper while there is a significant positive correlation ($r=0.2$, $p<0.05$) of serum MDA with age . Serum zinc showed significant positive correlation with serum copper ($r=0.31$, $p<0.05$) and serum copper showed significant negative correlation with age ($r=0.25$, $p<0.05$) . There is a significant positive correlation of Cu/Zn ratio ($r=0.32$, 0.48 , 0.67 , $p<0.05$) with serum levels of vitamin C , E and copper respectively. while a significant negative correlation of Cu/Zn ratio ($r=0.46$, 0.38 , 0.2 , $p<0.05$) with serum levels of MDA , Zinc and age respectively . **Table(6)** The correlation analysis among parity , no. of abortions and all studied biochemical parameters (vitamin C , vitamin E , albumin , MDA , zinc , copper and Cu/Zn ratio) in all studied pregnant females are performed and presented in Table (6) **.** Both serum vitamin C and vitamin E showed significant positive correlation with parity ($r=0.16$, 0.18 , $p<0.05$ respectively) while showed significant negative correlation with no. of abortions ($r=0.61$, 0.85 , $p<0.05$ respectively) . Serum MDA showed significant positive correlation with no. of abortions ($r=0.83$, $p<0.05$) while there is a significant negative correlation of serum MDA with parity ($r=0.17$, $p<0.05$) . Both serum zinc and serum copper showed significant negative correlation with no. of abortions ($r=0.3$, 0.73 , $p<0.05$ respectively) . Also serum zinc showed significant positive correlation with parity ($r=0.19$, $p<0.05$) . The no. of abortions showed significant positive correlation with the age of patients ($r=0.21$, $p<0.05$) . There is a significant negative correlation ($r=0.47$, $p<0.05$) of Cu/Zn ratio with the number of abortions of patients while a non significant negative correlation ($r=0.03$, 0.06 , $p>0.05$) of this ratio with parity and serum albumin of all studied pregnant females.

DISCUSSION

Table(2) shows that circulating levels of oxidative stress biomarker (MDA) was significantly higher in patients as

compared to control group ($p<0.05$). Our results show that systemic oxidative stress, of which lipid peroxidation represents a major manifestation, plays an important role in habitual abortion . These results were consistent with those reported by Simsek *et al.* (1998) [26] , Jauniaux *et al.* (2000) [27] , and Agarwal *et al* (2005) [28] . Since, MDA is a byproduct of lipid peroxidation, thus an elevation in MDA levels may reflects an overproduction of lipid peroxides and/or impaired antioxidant defense mechanism. Our results demonstrate a significant positive correlation between serum MDA and number of abortions as shown in **Table (6)** which support the relationship between lipid peroxidation status and the occurrence of repeated miscarriages .

Increased levels of antioxidants have been documented in normal pregnancy [29] , whereas loss of antioxidant defenses have been observed in patients with recurrent abortion as a result of their increased consumption[30] and this is consistent with the results of this study . These results are consistent with those reported by Simsek *et al.* (1998)[26] and vural *et al*(2000) [30].

These findings also indicate that there are ascorbate-dependent tocopherol regenerating mechanisms in plasma of recurrent aborters and increased lipid peroxidation causes consumption of those vitamins . The significant negative correlation between serum level of antioxidant vitamins and serum level of MDA indicates that serum antioxidants decreases with increasing MDA value as shown in **Table (5)** and **Table (6)** , this is possibly due to increased its utilization during free radicals scavenging . These findings were consistent with observation of other worker [31] . The significant negative correlation between serum antioxidant vitamins level and maternal age indicates that with advancing age , serum antioxidants decreases as shown in **Table (5)** and **Table (6)** . This change could be due to depletion of those antioxidant vitamins as a result of increased free radicals production which is supported by the positive correlation between age and MDA level . There is a significant negative correlation between serum antioxidants level and number of previous repeated miscarriages as shown in **Table(6)** which support the importance of antioxidants in prevention the occurrence of consecutive pregnancy losses that caused by elevated lipid peroxidation status , while there is a significant positive correlation between serum antioxidants level and parity . As shown in **Table (2)** , serum albumin concentrations expressed no changes between study and control groups was due to method used for its measurement (bromocresol green method) was depend on the measurement of concentration of total serum albumin (native and denatured) but not only the native albumin which has the antioxidant capacity . The results about serum albumin level of the present study are consistent with those reported by vural *et al* 2000 [30] .while are inagreement with those reported by Ryuji Bito *et al* 2005 [32] which indicates that free radical species attack plasma albumin and that the denatured albumins caused by the reaction are rapidly eliminated from the circulating blood, resulting in subsequently decreased concentrations of total albumin. As shown in **Table (2)** , the results revealed that there was a significant reduction in the serum levels of zinc in pregnant women with history of recurrent spontaneous abortions in comparison to the healthy pregnant control females , these results are consistent with those reported by Jameson S. 1993 [33] , Also our results indicates a significant positive correlation between serum zinc level and parity as shown in **Table(6)** . This may explain the importance of zinc nutriture during pregnancy which may affect the pregnancy outcome and live birth rate . This is supported by the negative correlation between serum zinc level and number of abortions as shown in **Table (6)** so that increasing serum zinc level associated with decreasing the number of abortions . The present study explain the significant negative correlation of serum zinc values with serum lipid peroxides (MDA) as

shown in **Table (5)** which is consistent with the results of other studies [34] . It has been reported that the increase in serum copper during pregnancy , (this is consistent with the results of this study) , is mainly in bound form due to increase in the carrier proteins, ceruloplasmin ; in response to stimulation by elevated levels of maternal estrogens [35] or as a compensatory mechanism to counteract anemia in anemic pregnant mothers because it has ferroxidase like activity [36] . Superoxide dismutase (SOD) activity was decreased in Cu deficient embryos as compared to controls. Collectively, these data suggest that oxidative stress, secondary to an impaired oxidant defense system, is a mechanism contributing to Cu deficiency-induced teratogenesis [37] . As shown in **Table (2)** ,we revealed that there was a significant reduction in the serum levels of copper in pregnant women with history of recurrent spontaneous abortions in comparison to the healthy pregnant control females , these results are consistent with those reported by Priyali Pathak *et al* 2003 [38] . There was a significant negative correlation between serum copper values and maternal age as shown in **Table (5)** , this indicates decreasing serum copper with advancing age . While serum copper was not significantly correlated with parity and gestational age as shown in **Table (6)** and **Table (5)** respectively . Also we found that serum copper was negatively correlated with the number of repeated abortions of patients as shown in **Table (6)** and this may indicate the importance of copper nutriture during pregnancy and its effect on pregnancy outcome which is evidenced by other workers [37] . There is a significant negative correlation of serum copper level with serum lipid peroxides (MDA) as shown in **Table (5)** and this is explained by the role of copper as important antioxidant cofactor of the antioxidant enzyme Zn/Cu superoxide dismutase (Zn/Cu SOD) which combating oxidative stress by helping to neutralize free radicals that would otherwise cause severe cellular damage [39] . In conclusion , the present study had pointed out that recurrent miscarriage might be caused by increased oxidative stress level and decreased antioxidant activity causing reactive oxygen species induced damage to cellular macromolecules like lipids, proteins and DNA , also the patients with higher number of recurrent miscarriages were having significantly higher oxidative stress value , lower antioxidant activity and elderly rather than young women as compared with control healthy women . Furthermore, advancing maternal age was associated with increasing oxidative stress and decreasing antioxidant activity .

Table (1) : The basic clinical Characteristics of all studied pregnant females.

Variables		Control Group			Study Group (patients)		
		n=100	%		n=100	%	
Age (yr.)	15 – 25 years	42	42		36	36	
	26 – 35 years	46	46		52	52	
	36 – 45 years	12	12		12	12	
Parity	Nil	22	22		15	15	*a
	P_1	17	17		26	26	*a
	P_2 - P_5	47	47		57	57	*a
	$>P_5$	14	14		2	2	*a
Gestational Age of Pregnancy (Weeks)	1st Trimester (5-13 weeks)	42	42		55	55	
	2ndTrimester (14-24weeks)	58	58		45	45	
Number of abortions	0	100	100		0	0	*a
	3 and 4	0	0		69	69	*a
	5 and 6	0	0		23	23	*a
	≥ 7	0	0		8	8	*a

Values are expressed as mean ± standard error of the mean .
* = Significant at p< 0.05 .
a = as compared with control group .

Table (2) : The estimated biochemical parameters of all studied pregnant females in both Control and study groups.

Parameters	Control Group n=100				Study Group (pt.) n=100			
Vitamin C (mg/dl)	1.69	±	0.11		0.24	±	0.01	a*
Vitamin E (mg/L)	17.96	±	0.23		5.82	±	0.11	a*
Albumin (g/dl)	4.73	±	0.06		4.65	±	0.06	
MDA (µmole/L)	0.27	±	0.01		1.05	±	0.02	a*
Zinc (µmole/L)	9.39	±	0.28		7.40	±	0.27	a*
Copper (µmole/L)	3.12	±	0.08		1.28	±	0.04	a*
Copper /Zinc Ratio	0.38	±	0.02		0.20	±	0.01	a*

Values were expressed as mean ± standard error of the mean .
* = Significant at p< 0.05 . , a = as compared with control group

Table (3) : Effect of parity on serum level of trace elements (zinc and copper) and Cu/Zn ratio of all studied pregnant females in both control and study groups

Parameters	Control Group n=100										Study Group (patients) n=100											
	n	Zinc (µmole/liter)			Copper (µmole/liter)			Copper/zinc ratio			n	Zinc (µmole/liter)				Copper (µmole/liter)				Copper/zinc ratio			
Nil parity	22	8.81	±	0.63	3.46	±	0.17	0.45	±	0.05	15	6.27	±	0.60	a*	1.21	±	0.11	a*	0.22	±	0.02	a*
P_1	17	9.51	±	0.53	3.11	±	0.21	0.35	±	0.03	26	7.02	±	0.43	a*	1.18	±	0.08	a*	0.19	±	0.01	a*
P_2-P_5	47	9.20	±	0.44	3.02	±	0.11*	0.39	±	0.03	57	7.48	±	0.28	a*	1.34	±	0.05	a*	0.19	±	0.01	a*
>P_5	14	10.79	±	0.69	2.94	±	0.28	0.28	±	0.03	2	4.25	±	0.25	abc*	1.30	±	0.10	a*	0.31	±	0.04	bc*

Values are expressed as mean ± standard error of the mean .
*** = Significant at p< 0.05 .**
a = as compared with control group .
b = as compared with nil parity subgroup of study group .
c= as compared with P_1 and P2-P5 parity subgroup of study group .

Table(4) : Effect of no. of repeated abortions on serum level of MDA of all studied pregnant females in both control and study groups .

Parameters	Control Group n=100			Study Group n=100								
	0			(3 and 4 abortions)				(5 and 6 abortions)				≥ 7 abortions		
	n=100			n=69				n=23				n=8		
MDA (µmole/liter)	0.27	±	0.01	1.04	±	0.02	a*	1.01	±	0.05	a*	1.24	±	0.07ab*

Values were expressed as mean ± standard error of the mean .
* = Significant at p< 0.05 .
a = as compared with control group .
b = as compared with (3 and 4 abortions) and (5 and 6 abortions) values .

Table (5): correlation coefficients (r) among age , gestational age and all studied biochemical parameters (vitamin C , vitamin E , albumin , MDA , zinc , copper , and Cu/Zn ratio)

Parameters	Vitamin C	Vitamin E	Albumin	MDA	Zinc	Copper	Cu / Zn Ratio	Age	Gestational Age
Vitamin C	**1.00**	**0.67***	**0.00**	**-0.60***	**0.27***	**0.55***	**0.32***	**-0.18***	**0.06**
Vitamin E	**0.67***	**1.00**	**0.05**	**-0.86***	**0.34***	**0.77***	**0.48***	**-0.19***	**0.07**
Albumin	**0.00**	**0.05**	**1.00**	**-0.10**	**0.14**	**0.09**	**-0.06**	**-0.05**	**-0.04**
MDA	**-0.60***	**-0.86***	**-0.10**	**1.00**	**-0.34***	**-0.75***	**-0.46***	**0.20***	**-0.04**
Zinc	**0.27***	**0.34***	**0.14**	**-0.34***	**1.00**	**0.31***	**-0.38***	**-0.04**	**-0.12**
Copper	**0.55***	**0.77***	**0.09**	**-0.75***	**0.31***	**1.00**	**0.67***	**-0.25***	**0.07**
Cu / Zn Ratio	0.32*	0.48*	-0.06	-0.46*	-0.38*	0.67*	**1.00**	-0.20*	0.12
Age	**-0.18***	**-0.19***	**-0.05**	**0.20***	**-0.04**	**-0.25***	**-0.20***	**1.00**	**-0.13**
Gestational Age	**0.06**	**0.07**	**-0.04**	**-0.04**	**-0.12**	**0.07**	**0.12**	**-0.13**	**1.00**

*= correlation is significant at ($p<0.05$) .

Table(6): correlation coefficients(r) among parity , no. of abortions and all studied biochemical parameters (vitamin C , vitamin E , albumin , MDA , zinc , copper and Cu/Zn ratio) .

Parameters	Vitamin C	Vitamin E	Albumin	MDA	Zinc	Copper	Cu / Zn ratio	Parity	Number of abortions
Vitamin C	**1.00**	**0.67***	**0.00**	**-0.60***	**0.27***	**0.55***	**0.32***	**0.16***	**-0.61***
Vitamin E	**0.67***	**1.00**	**0.05**	**-0.86***	**0.34***	**0.77***	**0.48***	**0.18***	**-0.85***
Albumin	**0.00**	**0.05**	**1.00**	**-0.10**	**0.14**	**0.09**	**-0.06**	**0.06**	**-0.05**
MDA	**-0.60***	**-0.86***	**-0.10**	**1.00**	**-0.34***	**-0.75***	**-0.46***	**-0.17***	**0.83***
Zinc	**0.27***	**0.34***	**0.14**	**-0.34***	**1.00**	**0.31***	**-0.38***	**0.19***	**-0.30***
Copper	**0.55***	**0.77***	**0.09**	**-0.75***	**0.31***	**1.00**	**0.67***	**0.09**	**-0.73***
Cu / Zn Ratio	0.32*	0.48*	-0.06	-0.46*	-0.38*	0.67*	**1.00**	-0.03	-0.47*
Parity	**0.16***	**0.18***	**0.06**	**-0.17***	**0.19***	**0.09**	-0.03	**1.00**	**-0.14**
Number of	**-0.61***	**-0.85***	**-0.05**	**0.83***	**-0.30***	**-0.73***	-0.47*	**-0.14**	**1.00**

*= significant correlation at ($p<0.05$) .

REFERENCES :

1. Raj Rai , recurrent miscarriage , Dewhursts textbook of gynaecology and obstetrics 2007 ; 7th edition : 100-105 .
2. Stirrat G , Recurrent miscarriage. Lancet , 1990; 336:673–675 .
3. Clifford K, Watson H, Grajewaski BA, An informative protocol for the investigation of recurrent miscarriage, Hum. Reprod. 1994; 9:1328-1332.
4. Kokawa K, Shikone T and Nakano R , Apoptosis in human chorionic villi and decidua during normal embryonic development and spontaneous abortion in the first trimester. Placenta (1998) ; 19:21–26.
5. Hempstock J, Jauniaux E, Greenwold N and Burton GJ , The contribution of placental oxidative stress to early pregnancy failure. Hum Pathol (2003) ; 34:1265–1275.
6. Jauniaux E, Hempstock J, Greenwold N and Burton GJ, Trophoblastic oxidative stress in relation to temporal and regional differences in maternal placental blood flow in normal and abnormal early pregnancies. Am J Pathol (2003b) ; 162:115–125 .
7. Eric Jauniaux, Lucilla Poston and Graham J.Burton , Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution , Human Reproduction Update 2006,Vol.12, No.6 pp. 747–755.
8. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine.4th ed. Oxford, UK: Clarendon Press; 2007 .
9. Valko M, Leibfritz D, Moncola J, Cronin MD, *et al.* Free radicals and antioxidants in normal physiological functions and human disease. Review. Int J Biochem Cell Biol 2007;39:44-84.
10. Valko M, Rhodes CJ, Moncol J, Izakovic M, *et al.* Free radicals, metals and antioxidants in oxidative stress-induced cancer. Mini-review .Chem Biol Interact. 2006;160: 1–40 .
11. Chatterjee M, Saluja R, Kanneganti S, *et al*. biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats .Cell Mol Biol. 2007;53:84-93 .
12. Droge W. Free radicals in the physiological control of cell function .Review. Physiol Rev. 2002;82:47-95.
13. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Review. Crit Rev Food Sci Nutr 2004;44:275-95.
14. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007;87:315-424.
15. Genestra M. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Review. Cell Signal. 2007;19:1807-19 .
16. Young I, Woodside J. Antioxidants in health and disease. J Clin Pathol. 2001;54:176–86.
17. Toro G , Aackermann PG. practical clinical chemistry /first edition . Boston :Little , Brown and company , 1975;634-636 .
18. Varley H , Gowenlock AH, Bell M. Practical Clinical Biochemistry : Hormones , vitamins , drugs , and poisons . 5th edition . London . William Heinemann medical books Ltd. 1976;(2):223-256 .
19. Rodkey, F.L, Estimation of serum albumin (BCG-method), Clin. Chem. 10, 606(1964).
20. Doumas, B.T. *et al*., Colorimetric test for Albumin, Clin. Chem. Acta 31, 87(1971) .

21. Pasquinelli F. , diagnostica e tecniche di laboratorio, (1984); Rossini editrice: page [1103 –1104]
22. Tetsuo makino , chimica clinica acta (1991) ; 197:209 – 220.
23. Pasquinelli F. , diagnostica e tecniche di laboratorio, [1984]; Rossini editrice: page [1099 – 1102]
24. Akita Abe ,Sumico yiamashita , Clin. Chem. [1989];35[4]:197,552-554
25. Buege JA . Aust SD . Microsomal lipid peroxidation method enzymol 1978 ; as 52: 302-303 .
26. Simsek M, Naziroglu M, Simsek H, *et al*. Blood plasma levels of lipoperoxides, glutathione peroxidase, beta carotene, vitamin A and E in women with habitual abortion. Cell Biochem Funct 1998;16:227–231 .
27. Jauniaux E, Gulbis B and Burton GJ, The human first trimester gestational sac limits rather than facilities oxygen transfer to the foetus: a review. Placenta-Trophoblast Res (2003a) ; 24:S86–S93.
28. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2005; 3:28 .
29. Cranfield LM, Gollan JL, White AG, *et al*. Serum antioxidant activity in normal and abnormal subjects. Ann Clin Biochem 1979;16:299–306.
30. Vural P, Akgul C, Yildirim A, Canbaz M. Antioxidant defence in recurrent abortion. Clin Chim Acta 2000;295:169–177.
31. SB Patil , MV Kodliwadmath , Sheela M Kodliwadmath ,. Lipid peroxidation and nonenzymatic antioxidants in normal pregnancy , J Obstet Gynecol India Vol. 56, No. 5 : September/October 2006 Pg 399-401.
32. Ryuji Bito, Sayaka Hino, Atsushi Baba, Miharu Tanaka, Haruka Watabe and Hiroaki Kawabata. Degradation of oxidative stress-induced denatured albumin in rat liver endothelial cells, Am J Physiol Cell Physiol 2005; 289:531-542.
33. Jameson S. Zinc status in pregnancy: the effect of zinc therapy on perinatal mortality, prematurity, and placental ablation. Ann N Y Acad Sci 1993;678:178–92 .
34. A. Mezzetti *et al* , Copper/Zinc Ratio and systemic oxidant load: Effect of aging and aging-related degenerative diseases . Free Radical Biology & Medicine, 1998; Vol. 25, No. 6: pp. 676–681 .
35. Henkin, I.R., Marshall, J.R. and Meret, S. Materno fetal metabolism of copper and zinc at term. Am. J. Obstet. Gynecol. 1971; 110:131-34.
36. Sharma, D.C., Ajmera, P., Sharma, S. and Sharma, P. Association between serum iron and copper in pregnant anemic vegetarian women. SDMH Jour. (1999) ; 23 (1): 37-39.
37. Keen CL, Uriu-Hare JY, Hawk SN, Jankowski MA, Daston GP, Kwik-Uribe CL, Rucker RB. Effect of copper deficiency on prenatal development and pregnancy outcome. Am J Clin Nutr 1998; 67(suppl 5): 1003S–1011S.
38. Priyali Pathak *et al*, copper nutriture amongst pregnant women in a rural area of India , Eastern Journal of Medicine (2003) ; 8(1) :15-17 .
39. Carl Burtis , Edward Ashwood , Tietz textbook of clinical chemistry, second edition , trace elements ; 1994 ;2:1335- 1339 .

دراسة حالة الجهد المؤكسد و المواد المقاومة للتأكسد لدى النساء الحوامل اللواتي يعانين من إسقاط الحمل العفوي المتواتر

الأستاذ الدكتور جمال عبد الباري الدوهان*
الأستاذ المساعد الدكتورة ساجدة عبدالرضا الربيعي **
الصيدلاني قتيبه عبدالكريم قاسم ***

خلاصة الدراسة

الهدف من الدراسة :
تقييم ترافق زيادة الجهد المؤكسد و نقصان مستوى المواد المقاومة للتأكسد (الفيتامينات والمعادن) مع حدوث مرض إسقاط الحمل المتواتر.
طريقة إجراء الدراسة :
اخترنا مجموعتين من النساء الحوامل : المجموعة الأولى (مجموعة السيطرة) تتألف من مئة أمراه حامل لا تعاني من أية إسقاطات سابقه ، و المجموعة الثانية (مجموعة الدراسة) التي تضم مئة أمراه حامل تعاني من الإسقاط المتواتر. تتراوح أعمارهن بين (١٥-٤٥ سنه) و قد قسمت كل من هاتين المجموعتين إلى عدة مجاميع تحتية حسب العمر ، عمر الحمل ، عدد الأبناء و عدد الإسقاطات المتواترة (بالنسبة لمجموعة الدراسة فقط) . ثم قمنا بسحب عينات الدم ، فصل و تجميع مصل الدم و استخدامه لغرض قياس كل من فيتامين ج ، فيتامين ه ، ألبومين ، مالون ثنائي الالديهايد (مقياس الجهد المؤكسد) و بعض العناصر النادرة مثل الزنك و النحاس باستعمال طرق العمل القياسية .
نتائج الدراسة :
أظهرت نتائج الدراسة وجود زيادة معنوية في ($p<0.05$) في مستوى مقياس الجهد المؤكسد(مالون ثنائي الالديهايد) مع نقصان معنوي ($p<0.05$) في مستوى المواد المقاومة للتأكسد (فيتامين ج ، فيتامين ه ، الزنك و النحاس) لدى النساء الحوامل في مجموعة الدراسة عند مقارنتها مع مثيلاتها في مجموعة السيطرة بينما لوحظ عدم وجود تغير معنوي ($p>0.05$) في مستوى الألبومين بين المجموعتين .
اظهر تحليل الانحدار الخطي ارتباطا معنويا سالبا ($r=0.61, 0.85, 0.3, 0.73, 0.47, p<0.05$) لمستويات فيتامين ج ، فيتامين ه ، الزنك ، النحاس و نسبة النحاس إلى الزنك على التوالي و ارتباطا معنويا موجبا ($r=0.83, p<0.05$) لمستوى مالون ثنائي الالديهايد مع عدد إسقاطات الحمل المتواترة لدى النساء الحوامل في مجموعة الدراسة . كما دلت الدراسة أيضا على وجود ارتباطا معنويا سالبا ($r=0.18, 0.19, 0.25, 0.2, p<0.05$) لمستويات فيتامين ج ، فيتامين ه ، النحاس و نسبة النحاس إلى الزنك على التوالي و ارتباطا معنويا موجبا ($r=0.2, p<0.05$) لمستوى مالون ثنائي الالديهايد مع أعمار النساء الحوامل في كلا المجموعتين .
كما بينت الدراسة عدم وجود ارتباط معنوي لمستويات فيتامين ج ، فيتامين ه، مالون ثنائي الالديهايد، ألبومين ، الزنك ، النحاس و نسبة النحاس إلى الزنك مع عمر الحمل في كلا المجموعتين . كذلك دلت النتائج على وجود ارتباطا معنويا موجبا ($r=0.16, 0.18, 0.19$, $p<0.05$) لمستويات فيتامين ج ، فيتامين ه، والزنك على التوالي و ارتباطا معنويا سالبا ($r=0.17, p<0.05$) لمستوى مالون ثنائي الالديهايد مع عدد الأبناء في كلا المجموعتين .
استنتاجات الدراسة :
إن الجهد المؤكسد الذي تسببه زيادة العوامل المؤكسدة مع قلة مستويات مضادات التأكسد قد يسبب إسقاط الحمل العفوي المتواتر عن طريق التلف الذي يحدثه في الكثير من مكونات الخلايا مثل الأحماض الدهنية غير المشبعة (PUFA) في أغشية الخلايا ، الأحماض النووية (DNA) و البروتينات الموجودة في الخلايا و بالتالي يؤدي إلى موت تلك الخلايا نتيجة الاضطراب الوظيفي الحاصل في مكوناتها و على هذا الأساس قد يكون الجهد المؤكسد و الضرر الذي تسببه العوامل المؤكسدة المختلفة لمكونات خلايا الأنسجة كالمشيمة هو الحل لأحجية الإسقاط العفوي المتواتر مجهول العلة .

---

* قسم الكيمياء الحياتية، كلية الطب، جامعة البصرة، العراق
** قسم النسائية و الولادة، كلية الطب،جامعة البصرة، العراق
***قسم الصيدلانيات ، كلية الصيدلة ، جامعة البصرة، العراق