

## Evaluation of Lipid Peroxidation ,Level of Selenium and Enzymatic Antioxidant Activity in Woman During Pregnancy and Abortion at First Trimester

1Rana M. Hameed

1Department of Biochemistry .College of Medicine ,University of Kerbela. Iraq

**Keyword** :glutathione peroxidase • lipid per oxidation •selenium, , miscarriage ,antioxidants

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### Abstracted:

**Background:**Spontaneous abortion, or miscarriage, is defined as the loss of a clinically recognized pregnancy that occurs before 20 weeks of gestation; early miscarriage occurs prior to week 12 while late spontaneous abortion occurs between weeks 12 and 20.abortion is the termination of pregnancy by the removal or expulsion from the uterus of a fetus or embryo .Human chorionic gonadotropin or human chorionic gonadotrophin (HCG.) is a glycoprotein hormone produced during pregnancy that is made by the developing embryo after conception and later by the syncytiotrophoblast (part of the placenta). the trace mineral selenium is an essential component of the selenoproteins,.Malondialdehyde (MDA) is a major breakdown product split off from lipid peroxidation and can be used to assess the degree of lipidperoxidation . Glutathione and Glutathione peroxidase (G-P<sub>X</sub>) and superoxide dismutase (SOD) are the main enzymes responsible for the detoxification of superoxide anion and required for normal health and reproduction. Total antioxidant activity( TAA )its compounds play an important role as a scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that can lead to pathological conditions like miscarriage

**Aim:** was to assess whether serum MDA, serum selenium, serum HCG, serum GSH and G-P<sub>X</sub>, serum total antioxidant TAA , and serum SOD levels altered during early abortions at first trimester and compare the results with healthy pregnant as controls

**Material/Methods:** A group of pregnant women at first trimester gestation age with spontaneous abortion (n=40) and a control group of healthy pregnancies with similar characteristics (n=25) were included. Serum MDA levels, serum selenium, serum HCG, serum GSH and G-P<sub>X</sub>, serum total antioxidant TAA , and serum SOD levels were determined and compared among the groups. **Results:** Characteristics, including maternal age, parity, gestational age, serum total protein, serum albumin, and serum lipid profile, were similar across the groups. Abortion in first trimester was associated with increased mean serum MDA, GsH levels and the selenium serum concentrations in women with miscarriage were significantly lower as compared to those in women with normal pregnancy and The glutathione peroxidase activity ,SOD ,TAA, HCG were reduced significantly in women with miscarriage. **Conclusions:** Increased lipid peroxidation and inhibition of SOD,G-Px ,TAA activity and reduced selenium concentration might be involved in abortions and expulsion of fetoplacental material out of the uterine cavity and may play an important role in the aetiology of spontaneous abortion.

تقييم أكسدة الدهون، مستوى السيلينيوم وفعالية مضادات الأكسدة الإنزيمية في المرأة أثناء الحمل والإجهاض في الثلث الأول

رنا مجيد حميد فرح الكيمياء الحياتية / كلية الطب / جامعة كربلاء

**الخلاصة:**

الإجهاض التلقائي أو فقدان الحمل يعرف على أنه فقدان سريري للحمل يحدث قبل الأسبوع العاشر من عمر

الحمل ويكون نوعين: إجهاض مبكر يحدث خلال فترة الأسبوع الثاني عشر بينما الإجهاض المتأخر يحدث بين الأسبوع (12-20) والإجهاض هو نهاية للحمل من خلال إزالة أو طرد للجنين من الرحم . موجهة الغدد التناسلية موجهة للغدد هو هرمون بروتين سكري يصنع خلال فترة الحمل من قبل الجنين بعد الحمل في وقت لاحق من الأرومة (جزء من المشيمة). ومعدن السيلينيوم هو عنصر أساسي من البروتينات الحاوية عليها النهائي لتكسير الدهون ويستعمل لتقييم درجة أكسدة الدهون، المألون ثنائي الالديهيد الأنزيمات الرئيسية المسؤولة عن انزيمات مضادات الأكسدة وتعتبر هذه الأنزيمات ضرورية. مجموع نشاط ,إزالة السموم من الايون السالب مضادات الأكسدة مركبات تلعب دور مهم في اعتراض الجذور الحرة فهي بذلك تثبط ميكانيكيات الأكسدة التي ممكن أن تقود إلى حالات مرضية مثل الإجهاض . الهدف: هو تقييم ما إذا كان مستوى السيلينيوم ، هرمون الحمل وانزيمات مضادات الأكسدة ومستوى مضادات الأكسدة الكلي تتغير خلال عمليات الإجهاض في وقت مبكر في الأشهر الثلاثة الأولى ومقارنة النتائج مع العوامل الأصحاء / طرق : شملت مجموعة من النساء في الثلث الأول من الحمل تعرضوا للإجهاض التلقائي وعددهن (40) ومجموعة الأصحاء وهن من النساء ذوات الحمل الطبيعي وعددهن 25 مع خصائص مشابهة. تم تحديد مستويات المتغيرات المقاسة في هذه الدراسة ومقارنتها مع بعضها. وكانت الخصائص، بما في ذلك سن الأمهات، والمساواة، وعمر الحمل، البروتين الكلي في الدم، ومستوى الألبومين ومحتوى الدهون ، متماثلة في المجموعتين : النتائج. ارتبط وتركيز GSH، ومستويات MDA الإجهاض في الأشهر الثلاثة الأولى مع زيادة معنوية في مستوى معدل السيلينيوم في النساء اللاتي يعانين الإجهاض بالمقارنة مع تلك القيم الموجودة في العوامل الصحية . ومستوى فكانت تقل معنويا في النساء التي تعاني الإجهاض بالاستنتاجات: زيادة أكسدة الدهون G-Px, SOD, TAA, HCG وتثبيط فعالية أنزيمات مضادات الأكسدة وقلة تركيز عنصر السيلينيوم ربما يكون المسؤول عن حدوث الإجهاض وطرد الجنين من تجويف الرحم ويمكن أن تلعب دورا هاما في التنبؤ للإجهاض التلقائي

**مفتاح الكلمات :** المألون ثنائي الالديهيد,كلوتاتايون بيروكسيديز,سوبر اوكسايد دسميوتيز,اكسدة الدهون,السيلينيوم, الاجهاض,مضادات الاكسدة,نظام مضادات الاكسدة, الاجهاض العفوي .

## Introduction :

Free radicals are reactive molecules with an unpaired electron and they are continuously produced in cells deliberately or as accidental by-products of metabolic routes <sup>[1]</sup>. A series of oxidation-reduction reactions in the metabolic transformation of dietary proteins, carbohydrates, and fats takes place in the mitochondria of animal cells. These reactions are called oxidative phosphorylation <sup>[2]</sup>. The end products are oxygen and its derivatives such as superoxide and hydroxyl radicals <sup>[1]</sup>. A sophisticated defense mechanism against these reactive oxygen species exerts its effects on balancing the harmful consequences of oxidative stress <sup>[3]</sup>. Superoxide dismutase (SOD) is found in the cytoplasm of aerobic cells and converts superoxide anion to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). On the other hand, glutathione peroxidase (G-P<sub>X</sub>) has an important function in the detoxification of H<sub>2</sub>O<sub>2</sub> into water and nonreactive oxygen molecule <sup>[4]</sup>. Malondialdehyde (MDA) is a major breakdown product split off from lipid peroxides and can be used to assess the degree of lipid peroxidation <sup>[5]</sup>. Oxidative attack on essential cell components by reactive oxygen species as a result of a disturbed oxidant-antioxidant system balance is recognized in the pathogenesis of placental deficiency syndromes such as preeclampsia and fetal growth restriction <sup>[6-11]</sup>. Early pregnancy failure might also be associated with oxidative stress throughout the placenta, leading to apoptosis and decrease in type IV collagen <sup>[12,13]</sup>. Although the relationship between spontaneous abortions and some circulating markers of oxidative stress has been evaluated in some animal and human studies. In our study we investigated serum MDA, serum SOD, and serum G-P<sub>X</sub> levels and other factors in women experiencing spontaneous abortions with vaginal bleeding and cervical dilatation at first three months of gestation. Various studies have demonstrated that during pregnancy, the serum selenium concentrations, and the activity of glutathione peroxidase in serum decline in a linear fashion from the first trimester to parturition, with the lowest levels at delivery <sup>(14)</sup>. It has also been demonstrated that the requirement of selenium is increased during pregnancy as a result of transport to the growing fetus. In veterinary practice, idiopathic miscarriage has been shown to be

associated with selenium deficiency. In the last few years some studies suggested that miscarriages in women might be related to selenium deficiency<sup>(15-17)</sup>.

## Methods

The subjects were recruited from the department of obstetrics and gynecology between 1st April 2011 and 31 December 2011. The analysis of blood samples was carried out in the department of biochemistry. Our study group consisted of 40 pregnant women with a clinical diagnosis of spontaneous abortion at first three months of gestational age. Another 25 women with an ongoing healthy pregnancy at first trimester served as our control group. Maternal age ranged from 20 to 35 years (mean 26.97 years). Gestational age was determined according to the best obstetric criteria, including either last menstrual period in the absence of oligomenorrhea or initial crown-rump length measurements by transvaginal ultrasonography. Blood was drawn from the antecubital vein after the completed spontaneous abortion. Samples were immediately centrifuged at  $1500 \times g$  for 10 min. The separated serum samples were stored at  $-8^{\circ} C$ .

**Assay for lipid peroxidation:** the level of malondialdehyde was determined by modified procedure described by (Guidet B. and Shah S.V., 1989) Principle based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA<sub>2</sub> product that absorbs at 532 nm<sup>(18)</sup>.

**Assay of selenium levels (ng/ml) and Glutathione peroxidase activity (U/L):** The serum selenium level were measured by spectrophotometrically (SHIMADZU 1601/UV) at room

temperature by using 3,3-diaminobenzidine hydrochloride dye<sup>(19)</sup>.

**Assay of superoxide dismutase SOD (U/ml):** assay by Marklund S. (1974); involvement of superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase<sup>(20)</sup>.

**Assay of Glutathione GSH (mg/dl):** GSH was measured by used 5,5-dithiobis(2-nitrobenzoic acid) is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound the absorbance of the reduced chromogen is measured at 412 nm<sup>(21)</sup>.

**Assay of total antioxidant activity TAA (mmol/L):** TAA was measured by D. Koracevic, (2001) method for the measurement of antioxidant activity in human fluids<sup>(22)</sup>.

**Assay of chorionic gonadotrophin (HCG) (mIU/ml)** from (RANDOX-UK) kits was measured with (spectra UV-UIS dual beam-8 auto cell UVS 2800) (Lambdamed, INC-USA).

**Statistical analysis:** All statistical analyses in studies were performed using SPSS version 15.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability  $P < 0.05$  = significant,  $P > 0.05$  = non-significant. Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the differences between variables of differentiated groups.

## Results:

The results of the present study indicate that physiological state of pregnancy stimulates a rise in the lipid peroxidase as determined by the serum MDA levels. Enzymatic antioxidant activities in the form of glutathione, glutathione peroxidase, remain suppressed during conditions of both pregnancy and abortion and decreased

level of serum superoxide dismutase SOD activity and we observed a significant decline in total antioxidant activity in abortion women compared with normal pregnant women. The serum selenium levels and serum human chorionic gonadotrophin (HCG) were significantly lower in women with abortion as compared to the pregnant women

Table (1-A) shows the the means, SD, minimum, maximum of all the variables in theabortion women at first trimester of gestational age and Table (1-B) in normal pregnant women at the same gestational age

Table 2 shows the statistical comparison (P value) when the various variables were compared with each other with respect to all the parameters under study.

Table (1-A) shows the the means, SD, minimum, maximum of all the variables in theabortion women

Variable	Mean	SD	MIN.	MAX.
HCG	39875	5694.37	22400	48505
Se	60.9924	3.582245	55.81	70.01
MDA	4.9715	1.188836	3.100	6.890
TAA	1.42500	0.155349	1.2100	1.6500
GSH	47.6180	1.074259	45.300	49.800
SOD	3.18000	0.195619	2.88000	3.6800
G-Px	185.5000	5.252594	177.00	197.00
Age	26.975	4.022294	20.00	35.000

Table (1-B) shows the the means, SD, minimum, maximum of all the variables in thepregnant women as controls

Variable	Mean	SD	MIN.	MAX
HCG	42669	5633.135	33900	52410
Se	63.4785	3.799220	53.19	73.00
MDA	2.5868	0.5582	1.92000	3.88
TAA	1.6476	0.0829	1.48000	1.8
GSH	41.8688	3.5370	35.8000	46.85
SOD	3.842500	0.215165	3.4500	4.2500
G-Px	235.0800	3.2521	229.000	240
Age	26.600	4.2031	20.0000	36

Table 2 show thestatistical comparisonwhen the various variables were compared with each other.

Vira.	HCG	Se	MDA	TAA	GSH	SOD	G-P <sub>x</sub>
HC G	1	0.284*	0.5477***	0.5301***	0.4178**	0.1227	0.5627***
Se	0.2848*	1	0.6160***	0.6555***	0.5996***	0.3986*	0.6668***
MD A	0.5477**	0.6160***	1	0.8101***	0.6666**	0.4507**	0.8894***
TAA	0.5301***	0.6555***	0.8101***	1	0.5945***	0.5701***	0.8983***
GSH	0.4178**	0.5996***	0.6666***	0.5945***	1	0.3523*	0.7871***
SOD	0.12273	0.3986*	0.4507**	0.5701***	0.3523*	1	0.5020**
GP <sub>x</sub>	0.5672***	0.6668***	0.8894***	0.8983***	0.7871***	0.5020**	1

\*correlation is significant at p<0.05 compared with control

\*\* Correlation is significant at p<0.001 compared with control

\*\*\* Correlation is significant at p<0.0001 compared with control

## DISCUSSION:

Selenium, as an integral component of the enzyme glutathione peroxidase (glutathione-Px), plays an important role during pregnancy. We have shown previously that the selenium concentrations in serum, and the serum activity of glutathione-Px are significantly lower in women at abortion, compared with normal-pregnant women.<sup>23-24</sup> Similar findings have been published by others<sup>25-26</sup>. We have shown<sup>24</sup> an almost linear decline in these parameters from the first trimester to parturition. Lowest values were found at first trimester. (Smith and Picciano)<sup>27</sup> have demonstrated that during pregnancy the requirement for selenium is increased as a result of the transport of this element to the growing fetus. It is believed that deficiency of selenium in humans and animals may be the cause of some health complications<sup>28-29</sup>. In the past few years some authors<sup>30-32</sup> have suggested that miscarriage is related to selenium deficiency. In farm animals selenium deficiency may be the cause of infertility, abortion and placental retention.<sup>33-35</sup> Theoretically, two mechanisms have been proposed to explain the cause of miscarriage related to selenium deficiency – the loss of antioxidant qualities attributed to selenium leading to damage to the biological membranes and DNA and reduced antithrombin III activity due to selenium deficiency<sup>36</sup>.

Selenium is incorporated into the active site of glutathione-Px<sup>37-38</sup>. In our study, serum glutathione Px levels were found to be significantly lower in abortion woman when compared to those in the normal pregnant at the same gestational age (first trimester). Glutathione-Px catalyses the reduction of H<sub>2</sub>O<sub>2</sub> and organic hydroperoxides, thus preventing lipid peroxidation of cell membranes and acting as a free radical scavenger<sup>39</sup>. Oxidative stress, of which lipid peroxidation represents a major manifestation, is involved aetiologically in a variety of clinical conditions including pregnancy and miscarriage<sup>40</sup>. In addition these authors found that glutathione and lipid peroxide levels in the plasma of aborted women were significantly higher than in healthy controls, the increased concentration of glutathione participates in the elimination of free radicals which are higher in aborted women<sup>40-41</sup>. Glutathione is the most important non-enzymatic thiol of the cells and functions as an essential redox compound<sup>45</sup>. It is well known that glutathione, as an effective reductant, plays an important role in a variety of detoxification processes. It readily neutralizes the hydroxyl radicals, which are considered a major source of free radical damage<sup>46</sup>. Serum MDA levels were reported to be higher in abortion women during the first trimester compared with normal pregnant controls<sup>[47,48]</sup>. Abortions have been associated with further increase in lipid peroxidation<sup>[49-52]</sup>. Our results are in agreement with those reported previously. Sane et al.<sup>49</sup> reported elevated serum MDA levels in women with either induced or spontaneous abortions compared with controls. These data might indicate that lipid peroxidation increases in abortions, probably due to termination of pregnancy. Therefore, lipid peroxidation might be a factor for accelerating the uterine evacuation during abortions rather than a cause of abortion itself. Similar to our results, low SOD activity in serum were observed during spontaneous abortions<sup>[40,53]</sup>. Superoxide dismutase<sup>54</sup>, which plays a major role in catalysing the transformation of superoxide radical anion to less harmful H<sub>2</sub>O<sub>2</sub><sup>55</sup>. Detailed investigations on SOD enzyme activity and occurrence of miscarriages are needed to clarify this issue. SOD activity might be important for corpus luteum activity, embryonic development, and maintenance of early pregnancy. In the gestational corpus luteum, theca interna cells stain heavily for SOD activity<sup>[56]</sup>. SOD probably works in the ovary against the inhibitory actions of peroxide on gonadotropic hormone action, steroidogenesis, and loss of follicular function<sup>[57]</sup>. The

production of superoxide radicals was also found to increase in regressing rat corpus luteum, indicating low SOD activity<sup>[58]</sup>. These data might signify that SOD is required for the maintenance of the corpus luteum and early gestation. SOD activity was reported to parallel serum progesterone concentrations in early pregnancy<sup>[59,60]</sup>. Progesterone induces decidualization of the endometrium, and there is now some evidence that decidualization induces SOD expression, as well<sup>[61]</sup>. In addition, SOD seems to be crucial for embryonic development. Their results suggested that high oxygen concentrations were harmful for in vitro embryonic development and that this effect could be prevented by culturing embryos under low-oxygen conditions and in the presence of SOD<sup>[62]</sup>.<sup>[40]</sup> found decreased total SOD activity in spontaneous abortions with vaginal bleeding they speculated that oxidative stress in terms of reduced SOD activity might be involved in the termination of spontaneous abortions via stimulating prostaglandin synthesis<sup>[50]</sup>. Moreover, pregnancies that went successfully to term were reported to be associated with increased plasma levels of SOD early in the first trimester<sup>[53]</sup>. Hence SOD activity might be an important factor for the maintenance of fertility and early pregnancy. Over the past few years, interest has been generated in the antioxidant status in normal pregnancy and pregnancy related diseases. Studies have documented a significant rise of antioxidant activity during normal pregnancy<sup>63</sup>. The antioxidant status acts as a physiological barrier against free radical attack. Free radicals have been shown to be associated with DNA changes, which may lead to mutagenesis, cell death and even tumor formation. Human chorionic gonadotropin interacts with the LHCG receptor and promotes the maintenance of the corpus luteum during the beginning of pregnancy, causing it to secrete the hormone progesterone. Progesterone enriches the uterus with a thick lining of blood vessels and capillaries so that it can sustain the growing fetus. Due to its highly-negative charge, HCG may repel the immune cells of the mother, protecting the fetus during the first trimester. It has also been hypothesized that HCG may be a placental link for the development of local maternal immunotolerance. For example, HCG-treated endometrial cells induce an increase in T cell apoptosis (dissolution of T-cells). These results suggest that HCG may be a link in the development of peritrophoblastic immune tolerance, and may facilitate the trophoblast invasion, which is known to expedite fetal development in the endometrium.<sup>[64]</sup> It has also been suggested that HCG levels are linked to the severity of morning sickness in pregnant women.<sup>65</sup>

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