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Original Article

Estradiol Hormone, Vitamin D, and Oxidative Stress Status in Women with Habitual Abortion

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Abstract
Habitual abortion (HA) is well-defined as 3 or more sequential pregnancy losses before twenty weeks of pregnancy and affects about >1% of couples trying to get a baby. It remains a multifaceted fertility trial, frequently connected to oxidative stress (OS), which is implicated in the pathophysiology of frequent reproductive problems, including abortion, infertility, fetal growth restriction, and preterm labor. The disproportion concerning the creation of reactive oxygen species (ROS) and the capacity of the body system to neutralize their destructive effects on lipid, protein, and nucleic acid is termed as OS. Subsequently, it is crucial for regulating various physiological purposes; this disproportion is closely related to the expansion and sequence of numerous illnesses. The aimed of this research was to evaluate estradiol (E2), vitamin D (VD), and oxidative stress markers (OSMs): malondialdehydee (MDA), peroxynitrite ($ONOO^-$), and glutathione (GSH) in women with habitual abortion in addition to explore whether vitamin D deficiency and hormonal dysregulation contribute to a disrupted redox state, which may impair placental function and trigger pregnancy cessation as well as recurrent habitual abortion. This study comprised 96 women participants, consisting of 42 healthy pregnant volunteers as controls and 54 women with habitual abortion, who were divided into subcategories according to their maternal and gestational age. About the measured results in the present work, an important reduction of serum estradiol, vitamin D, and glutathione was shown in women with HA, consistent with healthy pregnant women, while a significant elevation was observed in the oxidant markers MDA and $ONOO^-$. The effect of maternal age among women with habitual abortion showed a significant drop of VD, and GSH with increasing maternal age, whereas oxidant markers showed a significant rise, as well as a non-significant alteration in estradiol was noticed concerning the 2 age groups. Finally, no significant effect of gestational age on all parameters measured in this study was observed in women of HA, except E2, which showed a significant rise in the 2nd trimester of habitually aborted women.
Keyword. Habitual abortion, Estradiol, Vitamin D, Oxidative stress, Malondialdehydee, Peroxynitrite, Glutathione.

Introduction

Habitual abortion, also referred to as Recurrent Spontaneous Abortion (RSA), Recurrent Miscarriage (RM), and Recurrent Pregnancy Loss (RPL), is well-defined as the inability to achieve 3 or more consecutive recognized conceptions before twenty weeks from the last menstrual period [1, 2], and is measured as an important reproductive fitness factor; subsequently, it affects a great quantity of pregnancies. The identified risk factors of HA contain congenital and acquired uterine aberrations, malfunctioning endometrial receptivity, leiomyoma, hyper- or hypothyroidism, diabetes mellitus, and semen DNA destruction, environmental factors, and unexplained factors [3-5]. Habitual abortion is still a multifaceted generative problem, frequently connected to OS, which is concerned with the pathology of many reproductive problems, including infertility, Preeclampsia, fetal growth restriction, preterm labor, and early pregnancy loss [6,7].

Oxidative species, particularly reactive oxygen species (ROS) and nitrogen species (RNS), are highly reactive molecules and serve dual roles in human physiology [8,9]. There are a number of factors causative to OS produced intrinsically from typical cell metabolisms, and extrinsically, from ecological influences (i .e., Ultraviolet, pollutants, ionizing radiations, and heavy metals) [9,10]. The human physiology has a combined antioxidant organization including non-enzymatic and enzymatic antioxidants. Catalase and GSH peroxidase are the primary enzymatic antioxidants. Non-enzymatic antioxidant, include vitamins and GSH [9, 11]. An inequity between lipids, the production of free radicals, and the human body's ability to counter their destructive effects on nucleic acids, lipids, and proteins is identified as OS. Meanwhile, it is vital for regulatory of numerous living purposes; this inequity is closely linked with the progress and sequence of numerous illnesses [12, 13].

Estradiol-E2 is a steroidal sex hormone related to the estrogen cluster. It is mainly formed by the ovaries of femal patients; however, lesser quantities are also manufactured in the adrenal glands and throughout gestation, by the placenta (14). Four types of estrogen hormones, estrone (E1), 17β-estradiol (E2), estriol (E3), and estetrol (E4), have been recognized in females. E2 is the most powerful and plentiful estrogen throughout female reproductive ages, although additional types, such as E1 and E3, show roles in post menopause female [15]. It is vital for numerous biological purposes, as well as adaptable in promoting reproductive system development, the menstrual cycle, maintaining bone density, and promoting cardiovascular and brain health [16,17].

Vitamin D (VD), a lipid-soluble vitamin, is essential for the regulation of a sequence of biological purposes, including calcium homeostasis, bone health, the immune system, reproductive health, and the prevention of chronic diseases and

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oxidative stress [18,19]. Vitamin D is primarily manufactured in human skin by the action of ultraviolet B (UVB) radiation and acquired through a specific diet [20, 21].

Materials and Methods

This research was performed on 96 women of reproductive age. Fifty-four (54) women with habitual abortion HA (suffering more than 3 consecutive losses of pregnancy before 20 weeks of gestation), and forty-two (42) apparently healthy pregnant women HP as controls. All women were of fertility age, and the pregnancy age for the HA and HP groups was < 20 weeks of gestation. Women were further categorized separated according to maternal age (25- 34 years old) and (35- 44 years old), and subdivided depending on their gestational age (1st and 2nd trimester groups). All data were recorded for each woman, including name, age, occupation, smoking habit, alcohol consumption, family history, and any medication consumption. Women with a history of medical diseases like (diabetes mellitus, thyroid, renal, cardiovascular, or any chronic diseases), smoking habit, alcohol consumption, and drug abuse were excluded from the study. The detection of HA is built on the medical history, clinical examination by an obstetric ultrasound, and the gynecology doctors.

Approximately five milliliters of venous blood was collected from all women participating in this study, immediately transferred into a non-heparinized gel separator tube, then placed in a 37 °C incubator for ten minutes to allow coagulation in the sample before being centrifuged for 15 min. at 3000 round/minute to ensure separation of blood sera. The collected blood sera were used for the evaluation of estradiol-E2, vitamin D, and oxidative stress markers (MDA, $ONOO^-$, and GSH). Estradiol-E_2 level is assessed using ELFA (enzyme-linked fluorescence assay). Serum human vitamin D is measured by an ELISA kit according to the factory-made procedure (Bioassay laboratory technology). MDA level was estimated by a modified method described by Muslih *et al.* 2002, using the thiobarbituric acid (TBA) method to quantify the MDA [22]. Vanuffelene *et al.,* 1998 established an altered method for assessing $ONOO^-$ concentration [23]. Finally, GSH levels were checked using the dithiobisnitrobenzoic acid (DTNB) method [24].

Statistical Analysis

The results were evaluated statistically by a t-test. The result was expressed as means with standard deviations (SD). A significance level set at $p \leq 0.05$ (25).

Results

In this work, the compares of the mean number of estradiol (E2), vitamin D (VD), and oxidative stress markers (OSMs) between women with habitual abortion HA and healthy pregnant women HP as shown in Table (1), the results exposed a significantly elevation of sera MDA, and $ONOO^-$ levels associated with HA (7.18±1.42), and (69.3±14.3) respectively compared to healthy pregnant women (6.50±1.01), and (62.0±15.9) respectively. Contrariwise, significant reduction was noticed in E2, VD, and GSH among women with HA (271±214), (14.64±1.76), and (5.891±0.922), respectively, in contrast to HP (668±498), (20.06±2.94), and (6.40±1.10), respectively.

According to the effect of maternal age on E2, VD, and OSMs among HA women, as established in (Table 2), the results revealed that the levels of MDA and $ONOO^-$ are significantly elevated with increasing maternal age among HA women; conversely, VD and non-enzymatic antioxidant GSH decrease significantly, whereas the estradiol level showed no significant difference with increasing maternal age. Last of all, the effect of gestational age on E2, VD, and OSMs among women in the HA group were revealed in (Table 3); the outcomes showed no significant effect of gestational age on VD and OSMs within women of HA, except that estradiol showed a significant rise in the 2nd trimester of habitually aborted women.

Table 1. Estradiol, vitamin D and oxidative stress markers in women with HA and the control group

Parameters	Mean±S.D		T-value	P-value
	Control group (No.=42)	HA group (No.=54)		
Estradiol (pg/ml)	668±498	271±214	5.28	0.0000
Vitamin D (ng/ml)	20.06±2.94	14.64±1.76	11.22	0.0000
MDA (µmol/L)	6.50±1.01	7.18±1.42	-2.63	0.010
$ONOO^-$ (µmol/L)	62.0±15.9	69.3±14.3	-2.37	0.020
GSH (µmol/L)	6.40±1.10	5.891±0.922	2.44	0.016

Table 2 Effect of maternal age on estradiol, vitamin D, and oxidative stress markers in HA group

Parameters	Mean±S.D		T-value	P-value
	(25-34) yr (No.=22)	(35-44) yr (No.=32)		
Estradiol (pg/ml)	319±209	238±215	1.38	0.17
Vitamin D (ng/ml)	15.25±1.76	14.21±1.65	2.22	0.031
MDA (µmol/L)	6.63±1.39	7.55±1.33	-2.44	0.018

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$ONOO^-$ (µmol/L)	63.5±14.2	73.3±13.2	-2.61	0.012
GSH (µmol/L)	6.231±0.958	5.657±0.832	2.34	0.023

Table 3 Effect of gestational age on estradiol, vitamin D, and oxidative stress markers in HA group

Parameters	Mean±S.D		T-value	P-value
	1st trimester (No.=34)	2nd trimester (No.=20)		
Estradiol (pg/ml)	110.6±14.9	543.6±59.3	-40.73	0.0000
Vitamin D (ng/ml)	14.97±1.85	14.07±1.46	1.87	0.067
MDA (µmol/L)	6.90±1.24	7.65±1.60	-1.92	0.060
$ONOO^-$ (µmol/L)	66.6±12.4	73.9±16.4	-1.85	0.070
GSH (µmol/L)	6.069±0.843	5.586±0.992	1.90	0.062

Significant at ($p \leq 0.05$)

Discussion

The present work focused to evaluate sera estradiol- E2, vitamin D (VD), and OS in female patients with habitual abortion and healthy gravid females, showing a significant elevation of MDA and NOO in HA women, whereas E2, VD, and GSH presented a significant decline. These findings are in agreement with Zhang *et al*.[26] and Ghneim *et al*.,[27], who have provided an indication of the incidence of an imbalance between antioxidant heights and ROS production OS, which might be accountable for the initiation and development of compulsive progressions linked to habitual abortion. OS leads to cell injury and tenderness; it has been implicated in disordered blood movement of the placenta, leading to placental and fetal cell death, and exacerbating inflammatory responses, all of which can contribute to gravidity loss. Raised concentration of ROS might be related to weakened placental function, weakened fetal growth, and augmented danger of recurrent pregnancy loss. Moreover, OS can modify motherly immune reactions, actually leading to complications in the immune system during pregnancy. Exaggerated maternal immune reaction and OS status are considered the main mechanisms for recurrent pregnancy loss [26].

One of estradiol's main purposes in gravidity is to adapt the uterine situation. It works together with progesterone to preserve the endometrial lining, confirming it remains dense and highly vascularized to support the growth of the embryo. This is vital for the embedding of the inseminated egg and inhibition of initial abortion [28]. Estrogens have antioxidant properties through their capacity to bind to estrogen receptors and to upregulate the expression of antioxidant enzymes by intracellular signaling pathways. [29]. Have previous readings shown that serum E2 concentrations are mostly lower in women with miscarriage [30, 31]. Moreover, vitamin D shows crucial role in human health and disease [32]. It is playing an important antioxidant role outside its traditional purposes in calcium metabolism and bone well-being. Vitamin D exhibits strong antioxidant properties [33]. Vitamin D can lessen ROS production by preventing NADPH oxidase action, improve mitochondrial role, and upregulate the antioxidant protection systems, for example, glutathione production [34, 35].

The influence of maternal and gestational age among HA women highlights a significant elevation of serum MDA, $ONOO^-$ with progression of maternal age, while VD, GSH were dropping. These findings are in agreement with Yogesh *et al*. [36]. However, the gestational age showed a non-significant effect on VD and OSMs, except that E2 showed a significant elevation in the 2nd trimester of habitually aborted women.

Conclusion

Estradiol, vitamin D, and oxidative stress markers are deeply interconnected in maintaining early pregnancy and are critical for a healthy progression of pregnancy. Deficiencies in estradiol, vitamin D, and glutathione antioxidant, alongside elevated levels of oxidants, are strongly linked to heightened oxidative damage, which is a primary driver in the pathology of habitual abortion and early recurrent pregnancy loss.

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Conflict of interest

The author confirms there is no conflict of interest.

Adherence to Ethical Standards

The approval number and date Reference code: PREC-26-1-50 Date Monday, June 6, 2026**.** Make sure that contact with the committee is through pharmacy.ethics@uomosul.edu.iq.

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