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Oxidative Stress and Biochemical Evaluation of PAPP-A and Vitamin D in Recurrent Pregnancy Loss

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Abstract:

This study aimed to evaluate the role of oxidative stress and selected biochemical markers in women with recurrent miscarriage (RM). Specifically, it assessed serum levels of glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), vitamin D3 (VD3), and pregnancy-associated plasma protein A (PAPP-A). A total of 100 serum samples were obtained and classified into four groups: 30 women with a history of RM (with recent loss within six months), 30 pregnant women at risk of miscarriage, 20 healthy pregnant women, and 20 non-pregnant controls with no miscarriage history. All participants underwent biochemical testing to determine the levels of antioxidant enzymes and vitamin D3, while PAPP-A levels were measured only in pregnant groups. The findings revealed a significant increase in CAT (166.88 ± 37.03) and MDA (4.02 ± 0.26) levels, alongside a notable reduction in GPx (57.89 ± 14.24) and vitamin D3 (20.37 ± 7.19) in the RM group compared to controls, with statistical significance at $p \leq 0.05$. However, PAPP-A levels showed no significant difference between pregnant women with or without miscarriage history, likely due to biological variability and gestational age differences. These results indicate that oxidative stress and vitamin D deficiency may play a key role in the pathophysiology of recurrent miscarriage. The study supports the value of early detection and correction of these imbalances as part of preventive strategies for high-risk pregnancies. Further longitudinal studies with larger cohorts are recommended to validate these biomarkers and explore their utility in clinical risk prediction and targeted intervention.

Keywords: Oxidative stress, Recurrent miscarriage, Vitamin D, Pregnancy-associated plasma protein A (PAPP-A), Biomarkers, Antioxidants.

الإجهاد التأكسدي والتقييم البيوكيميائي لـ PAPP-A وفيتامين D في الإجهاض المتكرر

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مستخلص:

تهدف هذه الدراسة إلى تقييم دور الإجهاد التأكسدي وبعض المؤشرات الكيميائية الحيوية المختارة لدى النساء المصابات بالإجهاض المتكرر (RM). وقد ركزت بشكل خاص على قياس مستويات مصل الدم لكل من إنزيم الجلوتاثيون بيروكسيداز (GPx)، الكاتالاز (CAT)، الجلوتاثيون (GSH)، المالوندايالديهايد (MDA)، فيتامين D3 (VD3)، وبروتين البلازما المرتبط بالحمل A (PAPP-A). تم جمع ما مجموعه 100 عينة مصل وتصنيفها إلى أربع مجموعات: 30 امرأة لديهن تاريخ من الإجهاض المتكرر (مع فقدان حديث خلال الأشهر الستة الماضية)، 30 امرأة حامل معرضة لخطر الإجهاض، 20 امرأة حامل سليمة، و20 امرأة غير حامل ليس لديهن تاريخ للإجهاض. خضعت جميع المشاركات لاختبارات كيميائية حيوية لتحديد مستويات الإنزيمات المضادة للأكسدة وفيتامين D3، بينما تم قياس مستويات PAPP-A فقط في المجموعات الحاملات.

أظهرت النتائج زيادة معنوية في مستويات الكاتالاز (166.88 ± 37.03) والمالوندايالديهايد (4.02 ± 0.26)، إلى جانب انخفاض ملحوظ في مستويات الجلوتاثيون بيروكسيداز (57.89 ± 14.24) وفيتامين D3 (20.37 ± 7.19) في مجموعة الإجهاض المتكرر مقارنةً بمجموعة الضبط، مع دلالة إحصائية عند مستوى $p \leq 0.05$. في المقابل، لم تُظهر مستويات PAPP-A فرقًا معنويًا بين النساء الحوامل مع أو بدون تاريخ للإجهاض، ويرجع ذلك على الأرجح إلى التباين البيولوجي واختلافات العمر الحملي.

تشير هذه النتائج إلى أن الإجهاد التأكسدي ونقص فيتامين D قد يلعبان دورًا رئيسيًا في الفيزيولوجيا المرضية للإجهاض المتكرر. وتدعم الدراسة أهمية الاكتشاف المبكر وتصحيح هذه الاختلالات كجزء من الاستراتيجيات الوقائية للحوامل المعرضات للخطر. توصي الدراسة بإجراء المزيد من الدراسات الطولية على مجموعات أكبر للتحقق من صحة هذه المؤشرات واستكشاف فائدتها في التنبؤ بالمخاطر السريرية والتدخل العلاجي الموجه.

الكلمات المفتاحية: الإجهاد التأكسدي، الإجهاض المتكرر، فيتامين D، بروتين البلازما المرتبط بالحمل A، المؤشرات الحيوية، مضادات الأكسدة.

1. Introduction

Recurrent pregnancy loss (RPL) is considered one of the challenging clinical issues in the field of obstetrics and gynecology. It is defined as the spontaneous loss of two or more consecutive pregnancies before the 20th week of gestation. Epidemiological estimates indicate that this condition affects approximately 1–2% of women of reproductive age, making it a significant source of both psychological and physical distress[1]. It also represents a major medical challenge in the field of reproductive healthcare. Despite continuous advancements in diagnostic methods, more than 50 percent of recurrent pregnancy loss cases are classified as unexplained, meaning that no clear causes can be identified after standard evaluations[2]. This highlights the need to expand research efforts to better understand the hidden and influential factors involved. Among the most prominent mechanisms that have gained increasing scientific attention in recent years is the hypothesis of oxidative imbalance within the uterine environment, manifested as what is known as oxidative stress[3]. This type of physiological stress occurs as a result of an imbalance between the production of reactive free radicals, particularly reactive oxygen species (ROS), and the capacity of antioxidant defense systems to neutralize and eliminate them[4]. In this context, biomarkers such as catalase (CAT), glutathione peroxidase (GPx), the non-enzymatic antioxidant glutathione (GSH), and the lipid peroxidation product malondialdehyde (MDA) serve as valuable diagnostic and analytical tools for assessing the oxidative status of the body[5]. Available evidence indicates that elevated levels of reactive oxygen species (ROS) and the decline in the efficiency of antioxidant systems directly contribute to detrimental changes in the endometrial lining, adversely affecting embryo implantation and pregnancy maintenance[6]. These oxidative reactions also negatively impact the placental vasculature and the molecular signaling essential for fetal development, potentially leading to recurrent pregnancy loss even in the absence of evident chromosomal or anatomical abnormalities[7]. Furthermore, vi-

tamin D is considered a vital element that has shown an increasing association with pregnancy maintenance. Although primarily a steroid hormone, its role extends beyond regulating calcium and phosphate balance to include modulation of the immune system through vitamin D receptors (VDR) present in reproductive tissues, the placenta, and immune cells[8].

Numerous studies have demonstrated that deficiency of the active form of vitamin D in serum, or dysregulation of its receptor gene expression, may be associated with an increased risk of pregnancy loss. This association is attributed either to disruption of immune balance at the maternal-fetal interface or to adverse effects on embryo implantation and placental development[9]. Moreover, pregnancy-associated plasma protein A (PAPP-A) is an important biomarker secreted by placental trophoblast cells. It plays a regulatory role in fetal growth by modulating the availability of insulin-like growth factors (IGFs)[10]. Although PAPP-A is widely used as a marker in early screening for Down syndrome and preeclampsia, its role in assessing the risk of recurrent miscarriage remains a subject of scientific debate, particularly due to the temporal variability of its levels throughout different stages of pregnancy and the influence of confounding factors such as gestational age and sample characteristics[11]. Building upon the existing knowledge gap surrounding the biochemical mechanisms underlying recurrent pregnancy loss, this study aims to elucidate the potential relationship between oxidative stress-related biomarkers (CAT, GPx, GSH, MDA), vitamin D levels, and serum PAPP-A concentrations in women with a history of recurrent miscarriage compared to women with normal pregnancies and non-pregnant women serving as controls. Given these insights, the present study aims to evaluate the relationship between oxidative stress markers (CAT, GPx, GSH, MDA), serum vitamin D levels, and PAPP-A concentrations in women with recurrent miscarriage. By comparing these parameters across groups of pregnant and non-pregnant women, this research seeks to clarify the potential diagnostic value of these biomarkers in identifying at-risk pregnancies and guiding fu-

ture therapeutic strategies.

2. Methodology

This study was conducted at gynecology clinics in Tikrit, Iraq, from October 2024 to March 2025. A total of 100 participants, aged 20–40 years, were divided into four groups:

- Group 1: 30 women with recurrent miscarriage (RM) with the most recent loss within six months.
- Group 2: 30 pregnant women at risk of miscarriage.
- Group 3: 20 healthy pregnant women (reference group).
- Group 4: 20 non-pregnant women with no history of miscarriage (control group).

2.1 Sample Collection:

Venous blood (5 mL) was drawn from each participant using sterile syringes and collected into serum separator tubes (gel tubes). Samples were allowed to clot at room temperature for 10 minutes, followed by centrifugation at 3000 rpm for 15 minutes to isolate serum. Aliquots of serum were stored at –20°C until biochemical analysis to prevent enzymatic degradation.

2.2 Biochemical Analyses:

Catalase activity was determined spectrophotometrically based on Aebi's method (1984), by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800).

- Fresh 30 mM H_2O_2 solution was prepared in 50 mM phosphate buffer (pH 7.0).
- 0.1 mL of serum was mixed with 2.9 mL of substrate solution, and the decrease in absorbance was recorded over 1 minute.
- Enzyme activity was expressed in µmol/min/ml.

2.3 Glutathione Peroxidase (GPx) Activity:

GPx activity was measured using the Paglia and Valentine (1967) method, based on NADPH oxidation at 340 nm in the presence of glutathione (GSH) and glutathione reductase.

- Reaction mixture included GSH, NADPH, and glutathione reductase in phosphate buffer (50 mM, pH 7.0).
- 0.2 mL of serum was added, and

absorbance was recorded every 15 seconds for 3 minutes.
- Results were expressed as mmol/min/ml.

2.4 Malondialdehyde (MDA) Levels:

Lipid peroxidation marker MDA was quantified by the thiobarbituric acid reactive substances (TBARS) assay according to Buege and Aust (1978).
- 0.5 mL of serum was mixed with 2.5 mL of 10% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid (TBA).
- Samples were heated at 95°C for 15 minutes, cooled, and centrifuged at 3000 rpm for 10 minutes.
- Absorbance of the supernatant was measured at 532 nm, and MDA concentration was calculated in nmol/mL

2.5 Pregnancy-Associated Plasma Protein-A (PAPP-A):

PAPP-A was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Thermo Fisher Scientific, USA) following the manufacturer's instructions.
- Standards with known concentrations were used to construct a calibration curve.
- Absorbance was measured at 450 nm using an ELISA microplate reader (BioTek ELx800).

2.6 Vitamin D3 Levels:

Serum 25-hydroxyvitamin D3 [25(OH)D3] was determined using a direct competitive chemiluminescent immunoassay (CLIA) on the LIAISON® system (DiaSorin, Italy).

Internal quality control was performed using high and low concentration reference samples.

2.7 Statistical Analysis

The results were statistically analyzed using Minitab software version 17. Analysis of variance (ANOVA) was performed to assess correlations between the biochemical markers and to compare the mean values across the study groups. Duncan's Multiple Range Test was applied for post hoc comparisons at significance levels of 0.01 and 0.05.

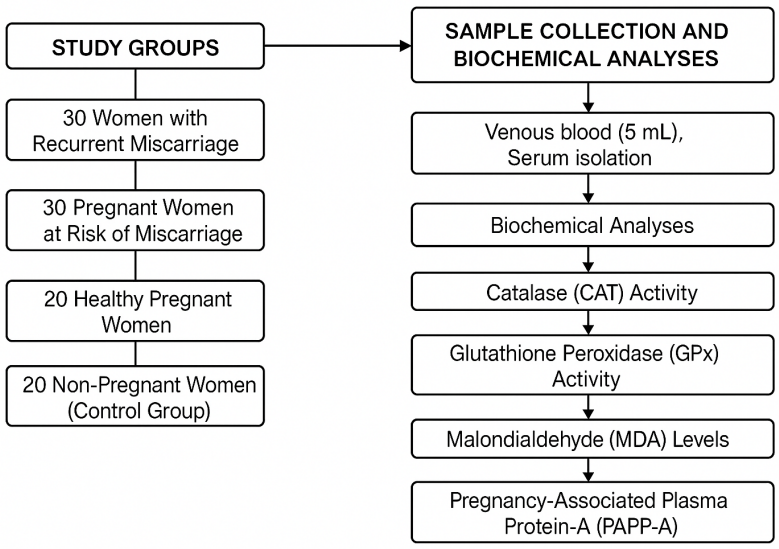

Figure 1: Study Groups and Analysis Workflow

3. Results

The biochemical analysis revealed significant differences between women with recurrent miscarriage (RM) and the other study groups. Catalase (CAT) activity was markedly elevated in the RM group (166.88 ± 37.03 µmol/ml) compared to healthy pregnant women (70.73 ± 17.25 µmol/ml) and non-pregnant controls (116.53 ± 26.31 µmol/ml), with statistical significance at $p \leq 0.05$. This elevation likely represents a compensatory antioxidant response to increased oxidative stress in RM patients.

Conversely, glutathione peroxidase (GPx) activity was significantly reduced in the RM group (57.89 ± 14.24 Mmol/ml) relative to non-pregnant controls (81.80 ± 17.50 Mmol/ml) and healthy pregnant women (98.90 ± 20.60 Mmol/ml). This decrease indicates a weakened antioxidant defense system, potentially contributing to cellular damage within the uterine environment.

Malondialdehyde (MDA), a marker of lipid peroxidation, was notably elevated in RM patients (4.02 ± 0.26 moles/ml) compared to the non-pregnant control group (3.37 ± 0.38 moles/ml), reflecting enhanced oxidative membrane damage.

Serum vitamin D3 levels were also

significantly lower in the RM group (20.37 ± 7.19 ng/ml) than in non-pregnant controls (42.95 ± 15.73 ng/ml) and healthy pregnant women (30.25 ± 11.44 ng/ml), with strong statistical significance ($p \leq 0.05$). This deficiency underscores the potential role of vitamin D3 in modulating immune tolerance and maintaining pregnancy.

Pregnancy-associated plasma protein-A (PAPP-A) levels, however, did not show significant differences between pregnant women with or without a history of miscarriage, possibly due to gestational age variability and inter-individual biological differences. As shown in Table 1.

Table 1: Comparative Analysis of GPx, CAT, GSH, MDA, Vitamin D, and PAPP-A in Miscarriage and Control Groups

parameter	Abortion N=30	Pregnant with abortion N=30	pregnant N=20	Control N=20	P Value
Catalase µmol/ml	166.88±37.03	106.28±29.05	70.73±17.25	116.53±26.31	P≤0.05
GPX Mmol/ml	57.89±14.24	50.21±16.50	98.90±20.60	81.80±17.50	P≤0.05
GSH µmol /l	567.5±45.20	638.3±48.61	552.4±52.40	563.2±46.21	P≤0.05
MDA moles /ml	4.0205±0.2628	3.9482±0.3576	3.9892±0.3467	3.3794±0.3876	P≤0.05
VitD3 ng/ml	20.37 ± 7.19	21.03 ± 6.89	30.25 ± 11.44	42.95 ± 15.73	P≤0.05
PAPP-A pg/ml		4490 ± 72.3	4486 ± 72.8		NS

4. Discussion

The results presented in the above table indicate that the antioxidant enzyme catalase (CAT) levels were elevated in patients with recurrent miscarriage compared to healthy controls. This increase in enzyme activity among the affected patients aligns with findings reported in previous studies, the elevated levels of catalase can be explained as supporting the compensatory mechanism against oxidative damage[12]. As shown in Table (1), the presence of an abnormal oxidative response or possible disturbances in oxygen metabolism. This reflects a disruption in the redox balance, leading to an increase in catalase levels as part of a defensive mechanism aimed at protecting cells

from the harmful effects of oxidative stress[1], [12]. When comparing the group of pregnant women at risk of miscarriage with the healthy control group, no statistically significant difference was observed. This finding is consistent with the results reported in Study[13]. The body increases the secretion of antioxidant enzymes, such as catalase, as a compensatory mechanism in response to oxidative stress. This suggests that the body activates certain antioxidant enzymes to mitigate oxidative damage, reflecting an adaptive response to the physiological stress associated with miscarriage[12]. An elevated level of catalase was also observed in pregnant women with a history of previous miscarriage compared to those with normal pregnancies, which is consistent with the findings reported in Study[14]. This can be interpreted as a decline in individual antioxidant defenses to suppress oxidative stress during pregnancy. Even in cases where antioxidant levels appear elevated during pregnancy, the overall outcome may still reflect a reduced total antioxidant capacity[15]. The decrease in catalase levels observed in the normal pregnancy group compared to the non-pregnant healthy controls is consistent with the findings reported in both referenced studies[16], [17]. For the same reason mentioned earlier, the study indicated the potential decline in overall antioxidant capacity during pregnancy despite variable individual antioxidant levels[15]. A significant difference was observed between the miscarriage group and the group of pregnant women with a history of previous miscarriage, suggesting that antioxidant defense mechanisms may be compromised in patients experiencing spontaneous miscarriage[14]. A highly significant difference was also observed when comparing the miscarriage group with the normal pregnancy group, which is consistent with the findings of Study , likely due to the same previously mentioned reasons related to impaired antioxidant defense mechanisms[12].

As for GPx, a decrease in its levels was observed in the miscarriage group compared to the healthy control group, which is consistent with the findings reported in the referenced study[18]. Oxidative stress associated with pre-

eclampsia may result from diminished antioxidant defense pathways, particularly those involving glutathione peroxidase (GPx), and could be linked to reduced selenium availability. A decrease in GPx activity may lead to increased production of toxic lipid peroxides, which contribute to endothelial dysfunction and the elevated blood pressure characteristic of preeclampsia[19]. It was found that the enzyme activity and concentration in the pregnant group with miscarriage were decreased compared to those with normal pregnancies and the healthy control group, consistent with the findings of the referenced study[20], [21]. During normal pregnancy, reactive oxygen species (ROS) activity is suppressed by balancing oxidative stress with antioxidant levels, including GPx. When antioxidant defenses are insufficient or their levels are inadequate, this imbalance can lead to recurrent miscarriage (RM)[22]. Similarly, when comparing the normal pregnancy group with the healthy control group, no significant difference was observed, which is consistent with the findings of the referenced study[23]. When comparing GPx levels between the miscarriage group and the pregnant group with miscarriage, no statistically significant differences were observed.

The decreased activity of glutathione peroxidase (GSH-Px) may significantly contribute to the occurrence of spontaneous miscarriage. This suggests that reduced antioxidant enzyme activity can be a risk factor for miscarriage, highlighting the potential role of oxidative stress in this condition[18]. When comparing GPx levels between the miscarriage group and the normal pregnancy group, a significant difference was observed, consistent with studies (67) and (68), due to the previously mentioned reasons related to oxidative stress imbalance[14], [18]. As for glutathione (GSH), a non-enzymatic intracellular antioxidant, comparison of its levels between the miscarriage group and both the normal pregnancy and healthy control groups revealed no statistically significant differences[24]. The result of the comparison between the miscarriage group and the normal pregnancy group is consistent with the findings of the referenced study[25], [26]. The stability of GSH levels in pa-

tients with recurrent miscarriage may indicate a compensatory response to counteract oxidative stress. However, if this response is insufficient, it may lead to the accumulation of oxidized glutathione (GSSG), thereby exacerbating oxidative stress and contributing to the occurrence of recurrent miscarriage. A significant difference was observed in GSH levels when comparing the pregnant group at risk of miscarriage with both the healthy control group and the normal pregnancy group. This finding may be interpreted as a reduction in individual antioxidant capacity to suppress oxidative stress during pregnancy. Even in cases where certain antioxidant levels appear elevated, the overall antioxidant capacity may still be diminished, reflecting a compromised defense system against oxidative stress[15]. A statistically significant difference was observed when comparing the pregnant group with a history of miscarriage to the miscarriage group, suggesting that the body may be attempting to produce more GSH to combat the excess reactive oxygen species. This increase is considered a compensatory response. However, despite elevated antioxidant levels during pregnancy, the overall antioxidant capacity may ultimately be reduced, indicating a potentially insufficient defense against oxidative stress[15].

The absence of a statistically significant difference in GSH levels between the normal pregnancy group and the control group suggests that the body maintains GSH levels through its continuous regeneration by the enzyme glutathione reductase. This mechanism prevents excessive conversion of GSH to its oxidized form (GSSG), thereby preserving redox balance during normal pregnancy. This is because normal pregnancy is accompanied by physiological adaptations and immuno-hormonal modulation that enhance antioxidant defenses to maintain a stable, non-oxidative intrauterine environment. This balance is attributed to the precise regulation between the production of reactive oxygen species (ROS) and the body's antioxidant capacity.

Malondialdehyde (a marker of lipid peroxidation, LPO), is a byproduct generated by reactive oxygen species (ROS). It can disrupt the integrity of the phospholipid bilayer, leading to the

dysfunction of various membrane-associated enzymes and receptors. This disruption ultimately increases tissue permeability and contributes to cellular damage[27]. When comparing the miscarriage group and the pregnant group with a history of miscarriage to the healthy control group, a marked increase in malondialdehyde (MDA) levels was observed among patients with recurrent miscarriage. This elevation indicates enhanced lipid peroxidation and is suggestive of increased oxidative stress compared to the healthy controls[28], [29], [30], [31]. According to previous studies, elevated levels of malondialdehyde (MDA) induce oxidative damage to cells, thereby contributing to miscarriage. Reactive oxygen species (ROS) are derived from molecular oxygen through the addition of an electron and represent a class of oxygen free radicals. These species are unable to diffuse across lipid membranes, causing them to remain confined to the site of their production, where they exert localized cellular damage[32]. Superoxide anion (SOA) is the primary generator of reactive oxygen species (ROS), initiating a cascade that leads to oxidative damage resulting from increased ROS levels. Pregnancy is characterized as an inflammatory state in which leukocytes exhibit changes similar to those observed in sepsis. Notably, increased production of reactive oxygen species (ROS) by leukocytes has been demonstrated, as evidenced by significantly elevated spontaneous chemiluminescence levels in granulocytes from patients with recurrent miscarriage compared to healthy pregnant controls[33]. During the progression of a healthy pregnancy, these levels are considered normal, as pregnancy is a physiologically stressful condition involving alterations in various metabolic and physiological functions. This stress leads to the generation of free radicals that target lipids, resulting in lipid peroxidation[34], [35]. Due to increased energy metabolism—since pregnancy requires higher energy to support fetal and placental growth—there is an elevated production of free radicals. Additionally, the placenta itself is an active source of free radicals, especially during the second and third trimesters of pregnancy. This contributes to fluctuations in the antioxidant

balance. Although the body possesses antioxidant defense mechanisms, pregnancy may induce a temporary imbalance, leading to the accumulation of oxidative stress byproducts such as malondialdehyde (MDA), a stable compound used to measure oxidative damage[17], [36]. Similarly, no significant differences were observed when comparing the miscarriage group with the normal pregnancy group and the pregnant group at risk of miscarriage.

When comparing vitamin D levels between the miscarriage group and the healthy control group, a clear decrease was observed in the patient group, consistent with the findings of the referenced study[37], limited exposure to sunlight[38], poor nutrition and skin pigmentation[39], and pancreatic or liver disorders can lead to a deficiency of the enzymes necessary for fat digestion, thereby impairing the absorption of vitamin D, which is primarily absorbed in the small intestine and is fat-dependent[40].

Autoimmune diseases such as Hashimoto's thyroiditis and hypothyroidism are associated with reduced vitamin D levels[41]. Additionally, decreased vitamin D levels have been observed in antiphospholipid syndrome (APS). Vitamin D inhibits the expression of tissue factor (TF), a key initiator of blood coagulation. Therefore, vitamin D deficiency may lead to increased thrombotic activity in APS, which in turn contributes to miscarriage[42]. When compared to the normal pregnancy group, a clear reduction in vitamin D levels was observed in the patient group, which is consistent with the findings reported in the referenced study[43]. T cells are essential for preventing autoimmune responses and thus play a critical role in maintaining a healthy pregnancy. In cases of miscarriage, regulatory T cells (Tregs) exhibit altered proportions and impaired function, leading to reduced maternal immune tolerance toward the fetus[44]. When comparing the normal pregnancy group with the healthy control group, a significant difference was observed, which is in agreement with the findings of the referenced study[45], [46], [47]. Free (unbound) vitamin D levels decrease significantly during pregnancy, primarily due to the rise in vitamin D-binding protein

(DBP) levels. DBP binds to vitamin D in the bloodstream, thereby reducing the biologically available fraction accessible to tissues[48]. Vitamin D levels in the blood tend to decline as pregnancy progresses, particularly during the second and third trimesters, due to the increased demand for calcium to support fetal development[49]. When comparing the pregnant group at risk of miscarriage with the normal pregnancy group, a statistically significant difference in vitamin D levels was observed, which is consistent with the findings of the referenced study. An important finding has emerged regarding pregnancy complications, particularly miscarriage, which accounts for approximately 17% of clinically recognized pregnancies ending in loss associated with vitamin D deficiency. Pregnant women with low vitamin D levels are at increased risk due to elevated expression of CYP27B1 and VDR in seminal fluid and placental tissue. The placenta is responsible for converting $25(OH)D_3$ to its active form, $1,25(OH)_2D_3$. Vitamin D plays a crucial role in trophoblast invasion and the remodeling of placental spiral arteries—processes that are adversely affected in cases of miscarriage[50], [51]. When comparing the miscarriage group with the normal pregnancy group (30.25 ± 11.44 b), a clear difference was observed, which is consistent with the findings of the referenced study[52], [53]. Vitamin D deficiency may lead to an imbalance in immune cell regulation, thereby increasing the risk of miscarriage. For example, studies have shown that women with vitamin D deficiency exhibit reduced levels of both vitamin D and its receptors in uterine tissues. This negatively affects the immune response at the maternal-fetal interface and contributes to a heightened risk of recurrent miscarriage[54]. When comparing PAPP-A levels between the group of pregnant women with a history of miscarriage and the normal pregnancy group, no statistically significant difference or notable effect on miscarriage was observed. PAPP-A levels in maternal blood generally increase with advancing gestational age, reaching their peak concentrations in maternal serum, followed by a rapid decline after delivery[55]. The results are consistent with the findings of the referenced

study[56]. Given that miscarriage is a multifactorial condition, PAPP-A levels in a subsequent pregnancy may remain unaffected if PAPP-A was not the underlying cause of the previous miscarriage[57]. PAPP-A is primarily used during the first trimester of pregnancy to assess the risk of Down syndrome and certain complications such as preeclampsia and fetal growth restriction. However, its value as a predictor of miscarriage remains scientifically inconclusive, this is likely because the study samples included two pregnant groups for comparison, among which some participants were in the second trimester. This variability affects the statistical values, indicating that PAPP-A is neither the sole nor the most reliable marker for predicting miscarriage[58]. Some studies indicate that other markers, such as β-hCG or uterine artery Doppler measurements, may be more strongly associated with the risk of early miscarriage than PAPP-A[59], and the lack of a significant difference may be attributed to insufficient sample size, low statistical power, or inadequate homogeneity in confounding factors such as age, body mass index, and medical history[60].

5. Conclusions

This study demonstrates that oxidative stress markers and vitamin D deficiency are closely associated with recurrent miscarriage, suggesting that disturbances in redox balance and immune regulation may play a crucial role in pregnancy loss. The significant elevation in catalase and MDA levels, along with the reduction in GPx and vitamin D3, reflects a compromised antioxidant defense system in affected women. Although PAPP-A did not show significant variation between groups, its role remains uncertain due to gestational timing and biological variability. These findings support the need for early biochemical monitoring in high-risk pregnancies and point to the potential benefit of correcting vitamin D deficiency and oxidative imbalance as a preventive strategy. Future research should focus on larger, longitudinal studies to validate these biomarkers and explore the clinical utility of combining them with other diagnostic tools for more accurate prediction and prevention of miscarriage.

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