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RESEARCH ARTICLE

Di tyrosine Levels in Placental Tissue: A Gestational Age-dependent Quantitative Analysis Using Anti-Di tyrosine Antibodies

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Abstract

Di tyrosine (DT), a marker of protein oxidative modification, is formed during oxidative stress, which is particularly involved in pregnancy-related pathology. However, the dynamics of DT expression at different stages of gestation remain poorly understood. This study is regarding the correlations between gestational age and the expression of DT in placental tissue. The authors studied placental tissues taken from 90 pregnant women who gave birth prematurely, at term or after term. The samples were processed by immunohistochemistry using anti-DT monoclonal antibodies. The results showed structural differences and expression of DT between groups. The assessment of DT could thus provide clinical insight into placental function and pregnancy complications related to oxidative stress.

1. Introduction

Di tyrosine (DT), a species generated through tyrosine oxidation, acts as a biomarker of oxidative stress, indicating the presence of reactive oxygen (ROS) or reactive nitrogen species (RNS) in biological systems. Di tyrosine, one of the main components of tyrosine oxidation products, consists of two tyrosine molecules cross-linked by carbon–carbon bonds; it is structurally stable and resistant to protease and acid hydrolysis processes. Oxidative stress has a crucial role during pregnancy, in both normal fetal development and the pathogenesis of several maternal–fetal disorders. However, the association between DT levels and gestational age is critical in understanding oxidative stress dynamics during gestation [1]. In normal pregnancies, amniotic fluid DT levels rise progressively with gestational age. This elevation indicates a physiological increase in oxidative stress with fetal development. In contrast, pregnancies affected by pregnancy-induced hypertension, gestational diabetes mellitus, fetal growth restriction, and chromosomal abnormalities, including Down syndrome and trisomy 18, have significantly decreased DT concentrations relative to normal pregnancies. These results suggest a possible alteration of oxidative processes in such pathological conditions [2].

Furthermore, maternal exposure to environmental disruptors influence oxidative stress markers such as DT in mothers and neonates. These exposures may induce sexually dimorphic alterations in oxidative stress, which can compromise fetal development and pregnancy outcomes. Together, these studies highlight the need for assessing DT levels as a marker of oxidative stress in pregnancy.

Oxidative stress and its role in placental disorders have drawn significant attention in recent years; accordingly, DT has been suggested as a potential biomarker of oxidative stress in various conditions, even in placental tissues. Hence, through evaluating DT levels, this study aims to assess its utility as a marker of oxidative insult and its link to adverse pregnancy outcomes.

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2. Materials and Methods (Design of Study and Collection of Samples)

A case control study was conducted in 90 pregnant women recruited from Al-Hakeem and Al-Imamayn Al-Kadhimiyayn Medical City Hospitals in Baghdad. The study sample consisted of 30 pregnant women in each of the three groups. Group 1 enrolled women who had preterm birth, Group 2 enrolled women who had term delivery, and Group 3 consisted of women with post-term birth. Participants were on an average 29 years . Anthropometric measures were taken, including the body mass index (BMI), gestational age at delivery. Women who had gestational diabetes (abnormal blood glucose along with a history of gestational diabetes), had an abnormal renal function profile, or had hypertension were all excluded. Specimens of placental tissue were taken from the mid-placenta, halfway between the umbilical cord and the periphery of the placenta, for histological and immunohistochemical analyses.

The tissue was prepared by paraffin embedding devices using the routine protocols [3]. The process consisted of fixation using 10% formalin, dehydration using ethanol alcohol, clearing with xylene, impregnation, and embedding with molten paraffin. Sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (H&E) to visualize histological structures.

2.1. Immunohistochemical (IHC) Analysis for Di tyrosine

A monoclonal antibody was utilized for IHC to localize DT within the placental tissues. We used a micro polymer detection system (the Expose Mouse and Rabbit Specific HRP/DAB detection IHC Kit, Abcam, UK), which is thought to provide better penetration through the tissue. IHC procedure started with deparaffinization antigen retrieval, , and rehydration by stepwise ethanol. Antigen retrieval was carried out by heating in citrate buffer (pH 6.0). Hydrogen peroxide (H_2O_2) and protein blockers were used to block nonspecific binding.

2.1.1. Primary Antibody Application

This included incubation of anti-DT monoclonal antibody at 37°C for 1 h, followed by detection using HRP-conjugate and DAB substrate. H_2O_2 worked as a substrate. HRP reacted with H_2O_2 to release an oxygen atom. DAB was the chromogen which modified the molecule to produce a colored product, leading to brown-colored precipitates at antigen locations.

2.1.2. Counterstaining and Mounting

After contrasting staining with Mayer's hematoxylin, the samples were dehydrated, cleared, and mounted. The images were taken using a DSC-TX5 digital microscope camera and analyzed for selected fields of tissue with Aperio Image software. The Aperio Image Scope V12 positive pixel count at the algorithm program was used to quantify the amount of a specific color in a tissue section. This system has a set of default input parameters, which has been configured for brown color quantification in the three intensity ranges, namely weak positive, positive and strong positive. SPSS v.22 was used for statistical analysis.

3. Results

General morphological arrangements of placental tissue revealed the presence of different-sized villi with a highly branched villus system heavily embedded in a profusely vascularized connective tissue with a wide area of intercellular space. Preterm villi were less compact and had a uniform outline (Figures 1A 1B). The outer layer is syncytio trophoblast and inner layer is the cytotrophoblast. Within the villi, many small blood vessels were observed, and a few syncytial knots were identified in some villi. The villus core consisted of connective tissue matrix with fetal blood vessels.

The placentogenic part of term pregnancy revealed highly branched, larger villi with a large villus core with few arterioles and venules encumbered by an exiguous kind of connective tissue stroma, which involved most of the villi, and the intervillous space was reduced. Most of the villi exhibited capillaries at their surface (Figure 2A). The trophoblastic bilayer villi had two different types of cells; the trophoblast and syncytioblast cells had a highly basophilic cytoplasm appearance. The syncytial knots are very much more than in preterm. These are large cells positioned on the basal membrane with large, pale vacuolated cytoplasm. The syncytial trophoblastic cells present as a dark layer of different thickness, with several small dark nuclei consisting of basophilic cytoplasm (Figure 2B).

Post-term placental examinations revealed a severe reduction in the size of villi and irregular outlines, and only a few large villi were seen. The degenerative ones were observed at the center, and fibrin deposition was seen. Blood vessels were virtually absent due to massive fibrin deposition in the intervillous space, resulting in the entrapment of numerous villi into a single mass of fibrin-rich tissue (Figure 3A). The degenerative cells with fibrin transformed the basophilic core of the villi into an acidophilic layer, between cytotrophoblast and syncytio trophoblast, through degeneration (Figure 3B).

3.1. Immunohistochemical Study of Anti- Monoclonal Antibody

Monoclonal Ab exhibited very limited cellular reactivity among term and preterm groups. Villi demonstrated cytoplasmic clarity with very slight reaction in a restricted field, while syncytial knots, blood vessels, and the vascular core of villi were observed to show clarity (Figures 4 and 5).

There was a clear response of the anti-DT antibody in the blood lakes and in the vicinity of large blood vessels within the villi among the post-term women group. As for the syncytio trophoblast, the apical cytoplasm contained a very narrow and thin rim of brown granules.

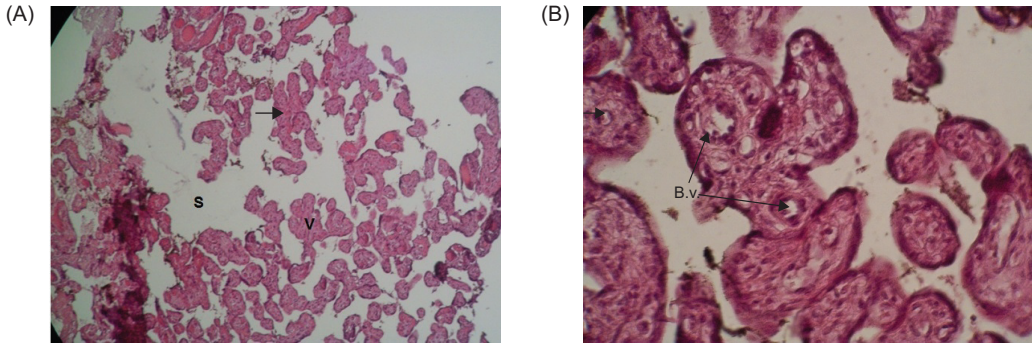

Figure (1): Cross-section through placental tissue in preterm group showing (A) small chorionic villi (V) in different shapes with wide intervillous space (S). 4X; (B) A bilayer villous wall with numerous blood vessels (B.V). 20X, H&E, preterm group.

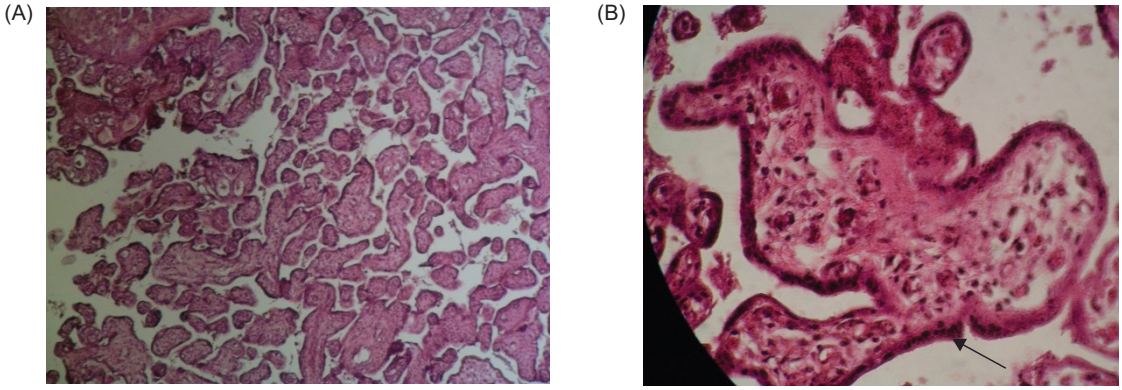

Figure (2): (A) Term pregnancy placental tissue showing highly branched large villi and diminished intervillous spaces 4X; (B) Higher magnification of the term pregnancy villi showing the clear double layers (→)and wide vascular core, a wide villus core 40X. H&E, term group.

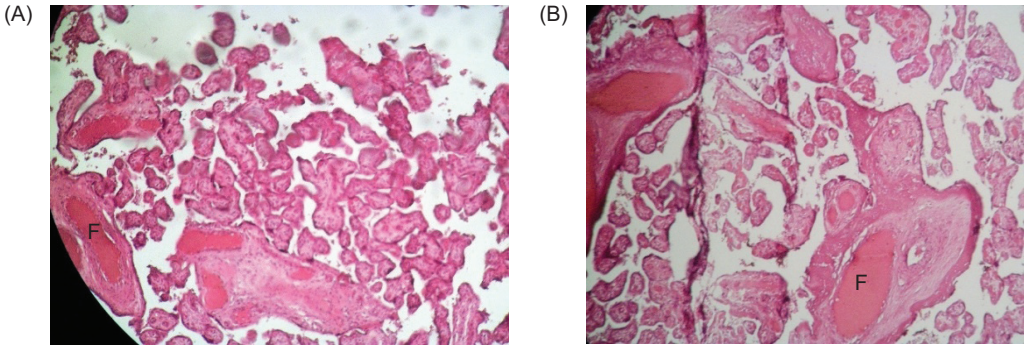

Figure (3): Cross-section in placental tissue of post-term pregnancy showing (A) small villi and irregular outline and fibrin deposition (F) H&E, 4X; (B) A higher magnification of A showing the fibrinoid in the core of villi (F), H&E, X10, and X20.

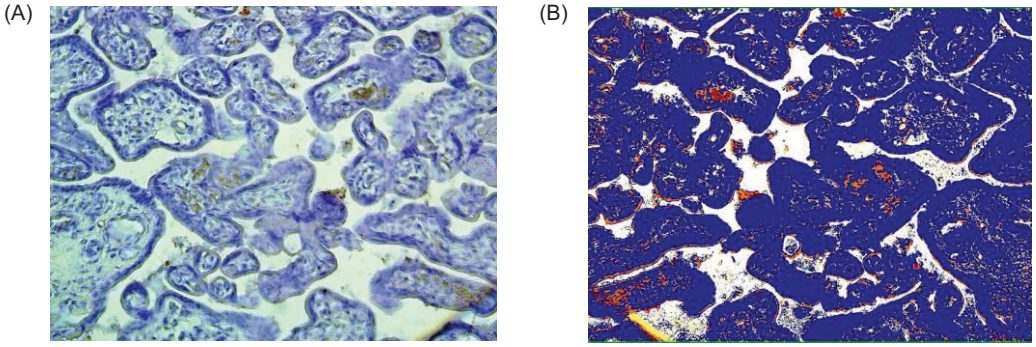

Figure (4): Cross-section of preterm placental tissue showing anti-Di tyrosine antibody reaction in the blood lakes and villi, anti-DT, 20X (A) showing the weak reaction of anti-DT as brown color; (B) is the same figure analyzed by the Aperio software.

Extreme responses in the blood lakes were also observed in addition to syncytio trophoblast severe cytoplasm reaction (Figures 6–9).

4. Discussion

The placenta is a temporary organ that is essential for the exchange of nutrients and gases between mother and fetus, and optimal availability of tyrosine is needed for its normal growth [4]. Gestational age may affect maternal concentrations of tyrosine, and good placentation is essential for health maintenance [5]. With the demands for certain nutrients altering as pregnancy develops, research into how tyrosine is linked to placental environment development at the various stages of pregnancy is an important area of study [6]. This study sheds light on the complex interplay between mother and fetus, providing insight into the vital role of tyrosine not just as a biochemical player but also as a prospective target for clinical intervention in conditions impacting gestational health.

Oxidative DT cross-linking is one of the factors that provides stability and rigidity to ECM proteins, the integrity of which is a pivotal property of placental tissue. Thus, strengthening collagen and elastin networks might be critical for placental development and function [7]. Each nutrient influences fetal development through biochemical processes that can only be understood for its relevance

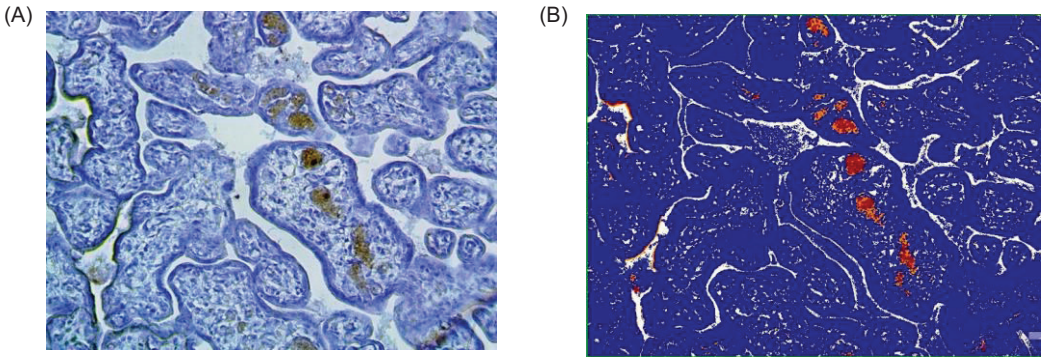

Figure (5): Cross-section of preterm placental tissue showing anti-Di tyrosine antibody reaction in the blood vessels and villi, anti-DT, 20X.

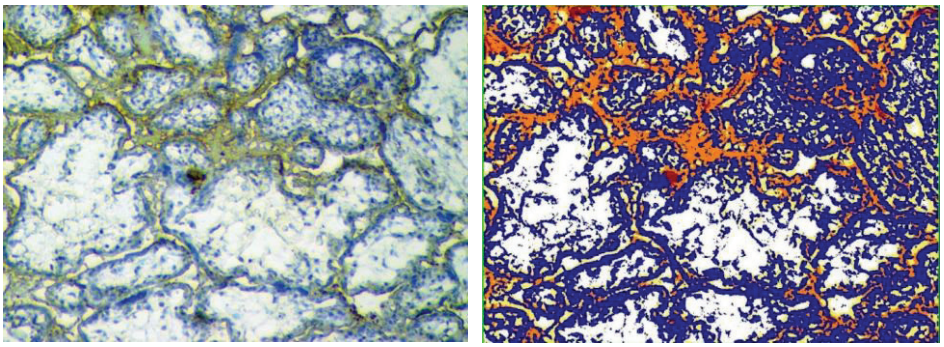

Figure (6): Cross-section of the term placental tissue showing anti-Di tyrosine antibody reaction between villi. Anti-DT, 20X.

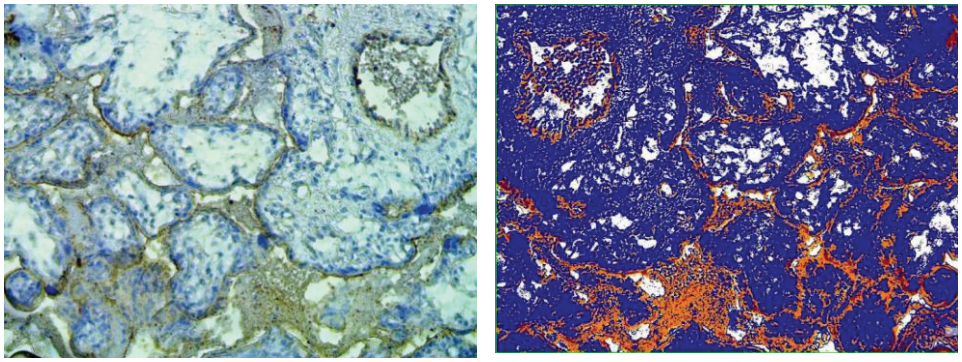

Figure (7): Cross-section of the term placental tissue showing anti-Di tyrosine antibody reaction in the syncytio trophoblast with a very narrow rim of brownish granules in the apical cytoplasm. Anti-DT, 20X.

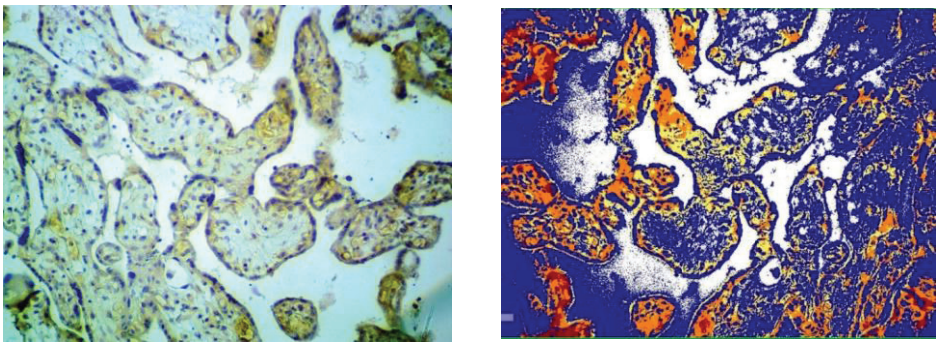

Figure (8): Cross-section of term placental tissue showing anti- antibody reaction in the syncytio trophoblast with a narrow rim of brownish granules in the apical cytoplasm. Anti-DT, 20X.

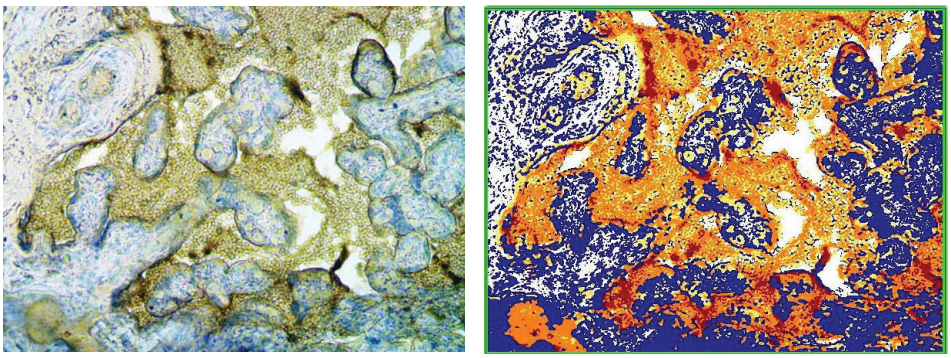

Figure (9): Cross-section of preterm placental tissue showing anti-Di tyrosine antibody reaction in the syncytio trophoblast with a very narrow rim of brownish granules in the apical cytoplasm. Anti-DT, 20X.

to pregnancy. Tyrosine is mainly derived from the diet or synthesized from phenylalanine via phenylalanine hydroxylase [8]. Tyrosine transport and metabolism in the placenta is modulated by amino acid transporters and enzyme activity [9].

Di tyrosine, a cross-linking amino acid synthesized via oxidative polymerization of two tyrosines, is widely regarded as a marker of protein oxidation and oxidative stress [10]. The role of DT in placental tissues throughout different gestational periods (term, preterm, and post-term) indicates oxidative stress status and possible implications for fetal development and pregnancy outcomes. Studying the contribution of DT to placental health could lead to new therapeutic targets for pregnancy disorders. Therapies such as the use of antioxidants that are capable of reducing DT levels may help prevent oxidative damage in high-risk pregnancies [11].

4.1. Relationship between Di tyrosine and Gestational Age

Di tyrosine, a noncanonical amino acid, is known to have crucial roles in a number of biochemical and physiological processes in the placenta, thereby providing a deeper understanding of the complexities in its levels between gestational ages. Tyrosine serves as a precursor to important neurotransmitters and hormones impacting maternal and fetal health during pregnancy [5]. Normal variation of tyrosine during pregnancy may serve as a biomarker of placental function and pregnancy light. Notably, impaired tyrosine metabolism is associated with diseases such as preeclampsia, a disorder defined by hypertension and potential placental insufficiency. Mechanisms that regulate trophoblast invasion, a critical process in placental development, are analogous to processes governing tumor progression, and thus tyrosine modulation may play a key role in both models [12].

Due to its high metabolic activity and adjacent to maternal and fetal circulation, the placenta is prone to oxidative stress [13]. The presence of increased levels of DT can be indicative of oxidative stress, since it is produced via reactions mediated by peroxidases that utilize reactive oxygen species (ROS) [14]. However, excessive DT formation might give rise to placental dysfunction in the path to preeclampsia and intrauterine growth restriction (IUGR) disorders [4].

Increased placental or maternal circulation levels of DT can be a biomarker for pregnancy-related disorders associated with oxidative stress [14] This biomarker may reflect an increase in protein oxidation, known to be associated with several pregnancy complications, including gestational diabetes mellitus and fetal hypoxia. Di tyrosine expression was reduced in the case of placentas from preterm samples compared with those from term and post-term histologic samples. This finding may be explained by the shorter period of exposure to oxidative

stress and lower levels of oxidized protein accumulation. Nevertheless, some reports have shown that preterm birth was associated with increased oxidative stress, likely due to immature antioxidant defenses[15&16]

In contrast, moderate DT expression was observed in term placentas, indicating a balanced equilibrium between oxidative stress and antioxidant protection at full-term gestation [4]. The results in this study corroborate the hypothesis that placental oxidative stress levels, as measured here, escalate with gestational age, and peak in the post-term placenta, as this may lead to placental aging and dysfunction.

4.2. Immunohistochemical Reaction quantative Measurement of Oxidative Stress in Placenta Specimens

Immunohistochemical analysis using anti-DT antibodies has been used to show oxidative protein modifications in human placenta and reads out as a sensitive analysis for protein oxidation in tissues. Oxidative stress has emerged as a pivotal factor in molecular pathophysiology, and immunohistochemistry has been instrumental in evaluating oxidative damage in multiple tissues, such as the placenta. These antibodies were specific enough to allow localization of oxidized protein species, giving important information relating to the localization of oxidative damage.

Moreover, exploring whether DT expression is related to clinical outcomes, including fetal growth and maternal health, previous studies have suggested that DT might hold clinical value, and could shed light on the clinical meanings behind our findings [16].

5. Conclusion

This study findings show for the first time that DT expression rate is associated with gestational age and is increased in post-term placenta. These findings emphasize the implication of oxidative stress in placental aging and dysfunction and suggest that DT could be a potential biomarker of oxidative damage during pregnancy. Higher concentrations of DT were observed compared to previous research with lower concentrations being added, indicating that it is more likely to be formed at mechanistic levels, which could prove detrimental to maternal and fetal health.

Ethical Approval

All the procedures adopted in studies involving human participants were performed in accordance with the ethical standards of the institutional board (IRB).

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Authors' Contributions

May F.M. Al-Habib: Conceptualized and designed the study, supervised the research process, and contributed to manuscript editing; Zeena Abdul Ilah Al-Sa'adi: Conducted laboratory experiments, handled sample preparation and data acquisition; Laith Hekmat Zaki: Performed data analysis, contributed to interpretation of results, and prepared figures; Rayah Sulaiman Baban: Drafted the initial manuscript, performed literature review, and assisted in revisions. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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References

[1] Tang, X.; Zeng, F.; Wang, J.; Lyu, Y.; Yu, R.; Lu, N.; et al. Dietary di-tyrosine induces oxidative stress and mitochondrial-lipid imbalance in mouse liver viaMiR-144-3p-mediated downregulation of Nrf2. Mol Nutr Food Res, 67:2200674, 2023. https://doi.org/10.1002/mnfr.202200674

[2] Hattori, Y.; Mukaide, T.; Jiang, L.; Kotani, T.; Tsuda, H.; Mano, Y.; et al. Catalytic ferrous iron in amniotic fluid as a predictive marker of human maternal fetal disorders J Clin Biochem Nutr, 56(1):7–63, 2015. https://doi.org/10.3164/jcbn.14-82

[3] Suvarna, K. S.; Layton, C.; Bancroft, J.D. Bancroft's Theory and Practice of Histological Techniques. 8th ed. Elsevier, USA, 2018.

[4] Al-Gubory, K.H.; Fowler P.A.; Garrel, C. Reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int J Biochem Cell Biol, 42(10):1634–1650, 2010. https://doi.org/10.1016/j.biocel.2010.06.001

[5] Gangooly, S.; Murphy, E.M.; Smith, T.; O'Connell, B. Tyrosine metabolism & placental function: insight into fetal development and pregnancy complications. Am J Obstet Gynecol, 224(6):635–647, 2021.

[6] Louwen, F.; Muschol-Steinmetz, C.; Reinhard, J.; Reitter, A.; Yuan, J. From trophoblasts to cancer: a lesson of how invasive properties of malignant cells may develop from cell fusion events. Publisher, 24(1):27–42. https://doi.org/10.1093/humupd/dmy027.2018

[7] Barrera, G.; Pizzimenti, S.; Ciamporcero, E.; Daga, M.; Ullio, C.; Arcaro, A. The impact of oxidative stress on placentation and its implication in the pathogenesis of disease. Placenta, 33(2):77–84, 2012.

[8] Ioana, V.; Victor, B.; CristianGeorge, F.; Pusa Nela, G.; Raluca, A.; CristinaStefania, D.; et al. Oxidative stress and placental pathogenesis: a contemporary overview of potential biomarkers and emerging therapeutics. Int J Mol Sci, 25(22):12195, 2024. https://doi.org/10.3390/ijms252212195

[9] Alahakoon, T.I.; Zhang, W.; Kalionis, B.; Murphy, V.E.; Keogh, R.J. Materials and methods: the vascular

connections of pre-eclampsia and fetal growth restriction. Pregnancy, 2016:1–12, 2016.

[10] Jauniaux, E.; Poston, L.; Burton, G.J. Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. Human Reprod. Update, 12(6):747–755, 2006. https://doi.org/10.1093/humupd/dml016

[11] Sultana, Z.; Maiti, K.; Aitken, J.; Morris, J.; Dedman, L.; Smith, R. Oxidative stress, placental ageingrelated pathologies and adverse pregnancy outcomes. Am J Reprod Immunol, 77(1): e12653, 2017. https://doi.org/10.1111/aji.12653.2017

[12] Ekelund, C.K.; Rode, L.; Tabor, A.; Hyett , J.; McLennan, A. Placental growth factor and adverse obstetric outcomes in a mixed-risk cohort of women screened for preeclampsia in the first trimester of pregnancy. Fetal Diagn Ther, 48(4):304–312, 2021. https://doi.org/10.1159/000514201

[13] Myatt L. , Cui X. Oxidative stress in placenta. Histochemistry & cell biology, 122(4), 2004.

[14] Rejc, J.; Tomsič, M.; Fabjan, T. Potential utility of urinary Di tyrosine as a novel biomarker of oxidative stress in pregnancy complications. J Matern Fetal Neonatal Med, 29(3):405–411, 2016.

[15] Castillo, M.; Martín-Gallán, P.; Cadefau, J.A.; González-Clemente, J.M. Oxidative stress and preterm birth: a review. Redox Biol, 17:62–68, 2018.

[16] Moore, T.A.; Ahmad, I.M.; Zimmerman, M.C. Oxidative stress and preterm birth: an integrative review. Biol Res Nurs, 20(5):497–512, 2018. https://doi.org/10.1177/1099800418791028.2018