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Oxidative Stress Biomarkers in Placenta Accreta Spectrum (PAS): A Placenta Accreta Versus Placenta Previa Non-accreta

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Abstract

The objective of this study was to evaluate the impact of imbalance between oxidants, measured by MDA levels, and antioxidants, evaluated by SOD and CAT activity, as well as TAC levels, on the occurrence and development of placenta accreta spectrum (PAS). This study comprised 44 individuals who were scheduled for elective caesarean section due to a sonographic diagnosis of either placenta previa or placenta previa accreta. A total of ten millilitres of maternal blood was collected before delivery, and the serum was subsequently separated. Sections of placental tissue were extracted and cleansed of blood. The sera and tissues were promptly preserved at a temperature of -80°C. MDA, SOD, CAT, and TAC levels were quantified in maternal serum and placental tissue homogenates using a colorimetric assay. Placenta or hysterectomy samples were submitted for histopathological assessment. Based on the final histopathological diagnosis, the patients were divided into two groups: the placenta accreta group (study group, n=16) and the placenta previa non-accreta group (control group, n=28). In the placenta accreta group, there was a rise in the MDA level and a decline in the SOD, CAT, and TAC levels in both maternal serum and placental tissues, as compared to the placenta previa non-accreta group. Furthermore, a direct relationship was identified between the oxidative stress indicators in the maternal serum and placental tissues of both groups. It can be concluded that the imbalance between the formation of reactive oxygen species (ROS), as indicated by the assessment of malondialdehyde (MDA), and the release of antioxidants, as indicated by the evaluation of superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC), may contribute to the incidence and development of placenta accreta spectrum.

Keywords: Placenta accreta, Oxidative stress, MDA, SOD, CAT, TAC

Full-length article

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1. Introduction

The common oxygen-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS) include superoxide radicals (O2·−), hydroxyl radicals (HO·), hydrogen peroxide (H2O2), peroxynitrite (ONOO−), and nitric oxide (NO) [1]. They are commonly produced in the placenta by prooxidative enzymes such xanthine oxidase (XO) and NADPH oxidase (Nox) and the mitochondrial respiratory chain [2, 4]. An imbalance between the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and their removal by protective antioxidants is known as oxidative stress (OS) [5]. Cellular damage happens when the level of oxidative damage surpasses the capability for repair by antioxidants. In oxidative stress-induced deficits, lipids, particularly polyunsaturated fatty acids with numerous carbon-carbon

double bonds, are the most impacted macromolecules. The generation of unstable lipid radicals (L-) is the outcome of the action of oxidants at this level, which involves extracting a hydrogen. Lipid hydroperoxides (LOOHs), which are more stable molecules, are created when an oxygen molecule is subsequently inserted into the reaction. This causes the production of lipid peroxyl radicals (LOO-), which then take another hydrogen from a different lipid molecule to continue the reaction. Both the lipid hydroperoxides and the lipid peroxyl radicals can go through cyclisation and cleavage processes in this process, which is known as lipid peroxidation, leading to the creation of secondary products [6], [7]. The main and best-studied product of lipid peroxidation, malondialdehyde (MDA) is recognised to have hazardous and mutagenic properties [8]. Enzymatic and non-enzymatic antioxidant mechanisms increase their functions to combat cellular stress as the oxidative stress rises. In the body's first line of defence mechanism against oxidative stress, glutathione peroxidase (GSX/Px), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), carotenoids, flavonoids, ascorbic acid, and alpha tocopherol are important antioxidant enzymes and non-enzymatic antioxidants [9]. Reactive oxygen species (ROS) and oxidative stress have already been shown to rise when placental circulation develops during human pregnancy due to a sharp rise in placental oxygen levels [10], [11]. Furthermore, since this systemic oxidative damage persists until the completion of the pregnancy, it is hypothesised that specific levels of oxidative stress and ROS are essential for the formation of placental vessels throughout pregnancy [12]. However, it has been suggested that placental angiogenesis may be limited by extremely elevated oxidative stress produced by placental hypoxia, which could result in diseases including intrauterine growth restriction (IUGR) and preeclampsia [13], [14]. Increased angiogenesis in placenta accreta has been linked to an imbalance between oxidant and antioxidant systems, as increased oxidative stress has already been demonstrated to contribute to limiting angiogenesis. Pereira et al. [15] suggested that the six transcription factors, namely E26 transformation specific oncogene homolog 1 (Ets-1), Krüppel-like factor 8 (KLF8), nuclear factor kappa-light-chain-enhancer of activated B (NF-κB), NF-E2-related factor 2 (Nrf2), specificity protein 1 (Sp1) and specificity protein 3 (Sp3), along with signal transducer and activator of transcription 3 (STAT-3), may play a role in connecting oxidative stress with trophoblast invasion and vascular development in the placenta. The underlying molecular mechanisms responsible for the development of placenta accreta remain unclear. Additionally, there is a scarcity of biochemical indicators to predict the occurrence of placenta accreta. The main aim of this study was to evaluate the levels of malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), and catalase (CAT) in the serum of pregnant women with placenta accreta spectrum (PAS). Additionally, the study aimed to determine if there is a correlation between the levels of these biomarkers in maternal serum and placental tissues, and if these changes can serve as biochemical predictors for the occurrence of PAS. The consistent and unwanted alterations in indicators of oxidative stress may indicate the underlying pathophysiology of placenta accreta spectrum.

2. Materials and methods

2.1. Chemicals

All chemicals used in the present study were obtained from Sigma–Aldrich, St. Louis, MO, USA, unless furtherly declared.

2.2. Patients

The research article was carried out on a sample of 44 patients who underwent elective caesarean section due to the presence of placenta accreta or placenta previa, as confirmed by ultrasonography. The study was conducted at Minia University Maternity Hospital, located in Minia University, Egypt, during the period from January 2022 to July 2022. The study received clearance from the Institutional Review Board of the Faculty of Medicine, Minia University (approval No. 213-2022). Prior to their involvement in this study, explicit agreement was obtained from all female participants. The clinical and demographic data for each patient were collected, which included age, parity, gravidity, gestational age, and number of prior caesarean sections (Table 1).

2.3. Blood samples

Before the elective caesarean section, a volume of ten millilitres of venous blood was obtained from patients and placed in centrifuge tubes. The tubes were then allowed to coagulate at room temperature for a duration of thirty minutes. The blood samples underwent centrifugation at a speed of 4000 revolutions per minute for a duration of 15 minutes in order to separate the serum samples. The sera were promptly stored in a freezer at a temperature of -80°C until they were utilised for the assessment of oxidative stress indicators.

2.4. Tissue Samples and their homogenisation

Tissue slices obtained from recently excised placenta were rinsed in normal saline to eliminate surplus blood and promptly placed in a -80 ^{o}C freezer for subsequent analysis of oxidative stress indicators in placental tissues. Before analysing the placental oxidative stress indicators, a gram of each placental tissue was mixed thoroughly in 5 ml of phosphate buffered saline. The homogenised sample was subjected to centrifugation at a force of 13,000 xg for a duration of 10 minutes at a temperature of 4°C. The supernatant was then preserved at a temperature of -80°C until the analysis of oxidants and antioxidants biomarkers.

2.5. Oxidative stress biomarkers

The oxidative stress biomarkers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and total antioxidant capacity (TAC) were determined in the patients' sera and their placental tissues. For their assessment, specific colorimetric kits under catalogue numbers: MD 25 29, SD 25 21, CA 25 17 and TA 25 13 for MDA, SOD, CAT, and TAC, respectively were purchased from Biodiagnostic company, Egypt. They were used for measurement of those oxidative biomarkers as described by the manufacturer. MDA assessment is based on the colorimetric estimation of a pink coloured thiobarbituric acid reactive product at 534 nm wavelength. This coloured product results from the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) in acidic medium at temperature of 95°C for 30 minutes [16]. SOD assay relies on

the ability of the superoxide dismutase to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. This is done by measurement of the change in absorbance at 560 nm over 5 minutes following the addition of phenazine methosulphate to the reaction mixture of control and sample [17].

The measurement of catalase (CAT) is firstly based on the reaction of catalase with a given quantity of H_2O_2, and then the catalase inhibitor is used to stop the reaction after a minute. When peroxidase (HRP) is present, the leftover H_2O_2 combines with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to produce a colourful substance that is inversely related to the quantity of catalase in the initial sample [18]. TCA assay is based on the reaction of the antioxidants in the sample with a predetermined volume of exogenously supplied hydrogen peroxide (H_2O_2). Then a portion of the H_2O_2 is removed from the sample by the antioxidants; the remaining H_2O_2 is then colorimetrically detected at 505 nm using an enzymatic reaction that produces a colourful material from 3, 5, dichloro-2-hydroxybenzensulphonate [19].

2.6. Pathological examination

The delivered placenta and the hysterectomy specimen were received intraoperative and were fixed in 10% neutral buffered formalin. Detailed gross examination was performed to assess the invasion of placenta within myometrium and take tissue sections for histological assessment. Histological examination was performed to detect chorionic villi within the myometrium [20]. The presence of placenta accreta was diagnosed by detecting chorionic villi in the myometrium and accordingly FIGO grading was used to classify PAS [21].

2.7. Statistical analysis

In this study, all data were collected and arranged for statistical analyses using IBM SPSS statistics 20 software. The test used was a one-way ANOVA test, followed by the Tukey post-hoc test for multiple comparisons. All values were presented as means ± standard deviation of mean (SD). The differences for the output data were considered significant when $p < 0.05$.

3. RESULTS

3.1. Patients

According to histopathological classification, 16 patients were diagnosed as placenta accreta (study group) and 28 patients as non-accreta (control group) (Fig.1). The two groups were comparable regarding maternal age, number of previous deliveries, number of abortions and gestational age. Number of previous CS was higher in placenta accreta group 3.37± 0.95 (range 2-5) compared to placenta non-accreta group 2.57 ± 1.2 (range 0-5) with a statistically significant difference (p=0.035) (table 1).

The current study revealed that the maternal serum level of MDA was statistically significantly ($p < 0.001$) higher in the placenta accreta group when compared to the placenta previa non-accreta group. In contrast, the maternal serum activities of SOD and CAT as well as the level of TAC were statistically significantly ($p < 0.001$) lower in the placenta accreta group when they were compared to the placenta previa non-accreta group (Table 2). Interestingly similar results to those of the maternal serum were obtained for the placental tissue as the concentration of placental tissue MDA was statistically significantly ($p < 0.001$) higher in the placenta accreta group when compared to the placenta previa non-accreta group. On the other hand, the placental tissue activities of SOD and CAT as well as the concentration of TAC were statistically significantly ($p < 0.001$) lower in the placenta accreta group when they were compared to the placenta previa non-accreta group (Table 3).

The present study showed a statistically significant ($p < 0.001$) positive correlation among the maternal serum levels of MDA, SOD, CAT and TAC and their levels in the placental tissue of both placenta previa non-accreta group (Fig. 2) and placenta accreta group (Fig. 3)

4. Discussion

Placenta accreta is the condition where the placenta attaches itself either completely or partially to the myometrium. Its occurrence has risen to be 3 cases per 1000 deliveries and appears to be correlated with the rising number of caesarean deliveries [5]. Placenta accreta is a severe medical disorder that endangers mortality because of bleeding during childbirth and after giving birth. It requires a comprehensive solution including multiple disciplines. The incidence of maternal mortality and morbidity associated with placenta accreta has been documented to reach up to 7% and 60%, respectively [1].

The recent increase in caesarean sections appears to be linked to the higher occurrence and insufficiency of decidualisation. Additionally, increased angiogenesis and excessive invasiveness of trophoblasts are thought to contribute to the development of placenta accreta. However, the specific molecular mechanisms underlying this condition remain uncertain [4], [5]. The processes of placental angiogenesis and trophoblastic invasion are in equilibrium during pregnancy. Conversely, an imbalance in the levels of angiogenic and antiangiogenic factors, as well as an imbalance in proinvasive and antiinvasive factors, can lead to abnormal uteroplacental vascularisation and consequently improper placental invasion [3].

Pregnancy is a state in which the body is more vulnerable to oxidative stress, which can result in possible harm to tissues [22]. During a typical and uncomplicated pregnancy, there is a rise in the production of reactive oxygen species (ROS) called prooxidants towards the end of pregnancy. However, this increase is counteracted by the higher production of natural antioxidants within the body, such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and total antioxidant capacity (TAC). Additionally, external antioxidants like carotenoids, tocopherols, and ascorbic acid also contribute to maintaining a normal state. The equilibrium is believed to be disrupted during pregnancy with placenta accreta spectrum, as indicated by an elevated production of reactive oxygen species (ROS) by the placenta that was deduced from low total oxidant status (TOS) and reduced levels of antioxidants that was inferred by high total antioxidant status (TAS) [23]. Antioxidants function as scavengers of free radicals and inhibitors of ROS. The placenta is the primary origin of reactive oxygen species (ROS), which significantly contributes to the pathophysiology of pregnancy illnesses such as preeclampsia (PE), intrauterine growth restriction (IUGR) [24], and placenta accreta [23].

Table 1. Patients characteristics in placenta previa non-accreta group (control group) and placenta accreta group (study group)

	Placenta previa non-accreta group (n = 28)	Placenta accreta group (n = 16)	P value
	Mean ± SD (Range)	Mean ± SD (Range)	
Age in years	31.18 ± 5.56 (21- 39)	32.75 ± 3.8 (27-38)	0.313
Number of deliveries	2.96 ± 1.34 (1-5)	3.56 ± 0.89 (2-5)	0.123
Number of abortions	0.86 ± 1.2 (0-5)	1.38 ± 0.95 (0-3)	0.06
Gestational age in weeks	37 ± 0.54 (36-38)	36.69 ± 1.7 (32-38)	0.619
Number of previous CS	2.57 ± 1.2 (0-5)	3.37 ± 0.95 (2-5)	0.035*

* Significant difference at P value < 0.05

Table 2. Maternal serum oxidative stress biomarkers in placenta previa non-accreta group (control group) and placenta accreta group (study group)

Maternal serum Antioxidants	Placenta previa non-accreta group (n = 28)	Placenta accreta group (n = 16)	P value
	Mean ± SD (Range)	Mean ± SD (Range)	
MDA (nmol/ml)	1.09 ± 0.17 (0.92 – 1.48)	2.45 ± 0.12 (2.22 – 2.62)	**<0.001***
SOD (U/ml)	2 ± 0.3 (1.61 – 2.59)	0.9 ± 0.16 (0.68 - 1.14)	**<0.001***
CAT (U/L)	2.4 ± 0.4 (2.03 – 3.25)	1.15 ± 0.18 (0.84 - 1.37)	**<0.001***
TAC (mM/L)	2.8 ± 0.3 (2.49 – 3.43)	1.39 ± 0.15 (1.11 - 1.54)	**<0.001***

* Significant difference at P value < 0.001

Table 3. Placental tissue oxidative stress biomarkers in the placenta previa non-accreta group (control group) and placenta accreta group (study group)

Placental tissue antioxidants	Placenta previa non-accreta group (n = 28)	Placenta accreta group (n = 16)	P value
	Mean ± SD (Range)	Mean ± SD (Range)	
MDA (nmol/g tissue)	5.31 ± 0.56 (4.54 - 6.34)	11.9 ± 0.64 (10.7 - 12.7)	**<0.001***
SOD (U/g tissue)	9.76 ± 1.56 (8.23 - 12.7)	4.84 ± 0.82 (3.42 - 5.89)	**<0.001***
CAT (U/g tissue)	11.6 ± 1.9 (9.85 - 15.3)	5.82 ± 0.95 (4.36 - 7.06)	**<0.001***
TAC (mM/g tissue)	12.8 ± 2.06 (10.8 - 16.8)	6.41 ± 1.07 (4.63 - 7.78)	**<0.001***

* Significant difference at P value < 0.001)

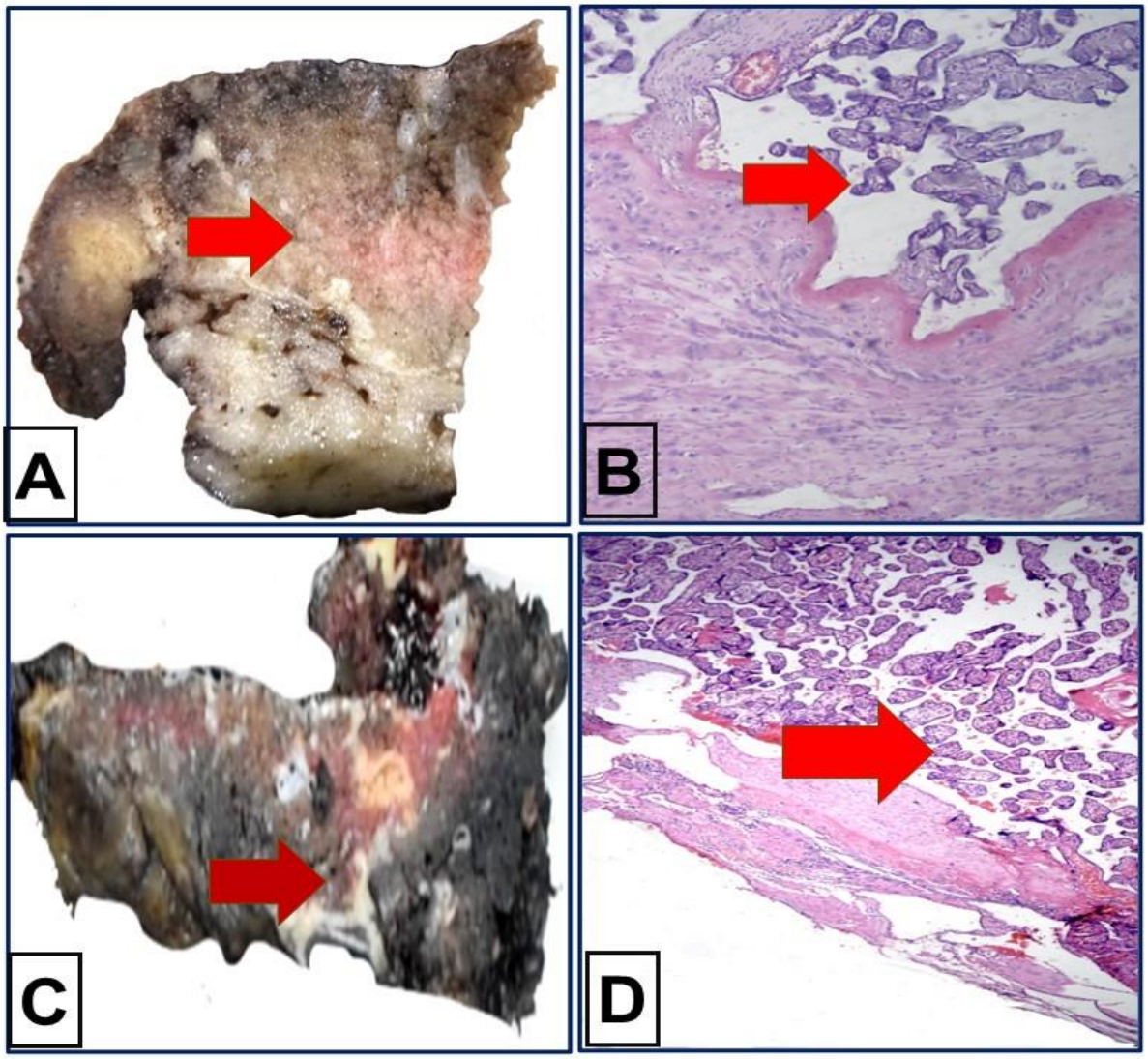

Figure 1. Representative examples of pathology of PAS
(A) A gross feature of PAS grade 2 where the placental tissue invades the superficial myometrial layer (arrow). (B) A histological section stained with haematoxylin and eosin of a PAS grade 2, the chorionic villi invade superficial in myometrium (arrow) 100x. (C) A gross feature of PAS grade 3 where the placental tissue invades the deep myometrial layer (arrow). (D) A histological section stained with haematoxylin and eosin of a PAS grade 3, the chorionic villi invade deep in myometrium (arrow) 100x.

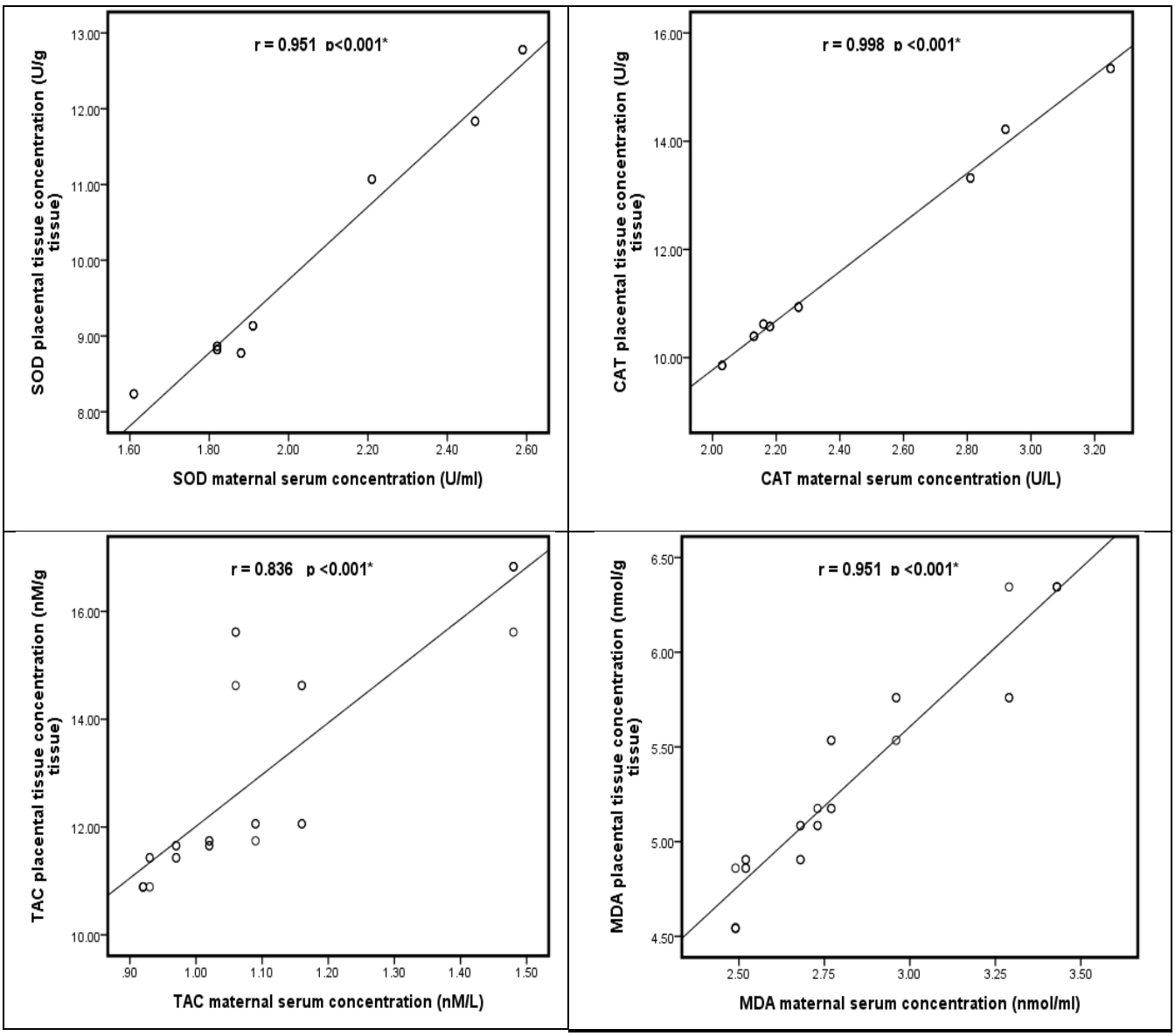

Figure 2. Showing the correlation among maternal serum and placental tissues levels of oxidative stress biomarkers in the placenta previa non-accreta group (control group)

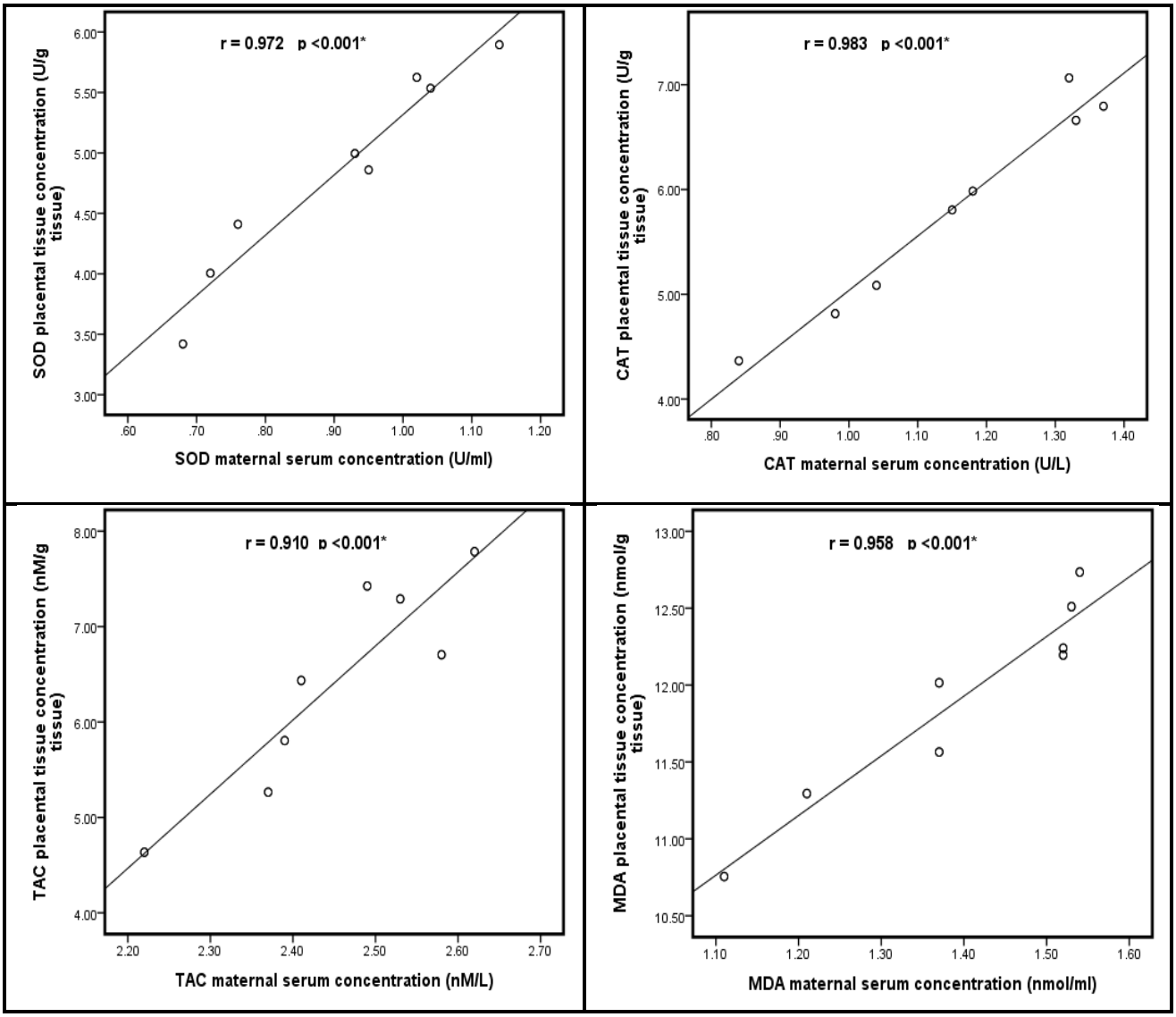

Figure 3. Showing the correlation among maternal serum and placental tissues levels of oxidative stress biomarkers in the placenta accreta group (study group)

Nevertheless, it is probable that the maternal endothelium and leucocytes also play a role in this phenomenon. ROS interacts with polyunsaturated fatty acids in the cellular membrane of the placenta, resulting in the formation of lipid peroxides. During a typical pregnancy, the placental cells and tissues are shielded from harmful lipid peroxides by a range of protective antioxidants [22]. One Elevated levels of nitrotyrosine residues, which are a product of the reaction between superoxide (ROS) and nitric oxide (NO) to form peroxynitrite (ONOO-), are observed in pregnancy-related disorders such as miscarriage, preeclampsia, and intrauterine growth restriction compared to normal pregnancy. This indicates the presence of lipid peroxides and suggests a state of increased pro-oxidants [25]. Malondialdehyde (MDA) is a byproduct formed when polyunsaturated fatty acids of the cell membrane undergo peroxidation. It has been utilised as a biomarker to quantify oxidative stress in diverse biological samples in individuals afflicted by a broad spectrum of disorders including placenta-related illnesses of pregnancy, such as preeclampsia and miscarriage.

Multiple investigations have demonstrated elevated levels of oxidants in pregnancies affected by both preeclampsia and foetal growth retardation. These findings imply that oxidative stress plays a significant role in the development of endothelial dysfunction associated with preeclampsia and foetal growth retardation [26], [27]. Currently, there is a lack of studies that directly compare oxidative stress biomarkers in maternal serum and placental tissues between placenta previa non-accreta and placenta accreta. However, we have formulated a hypothesis that suggests a mechanism similar to that of preeclampsia may have played a role in the development of placenta accreta. Hence, we conducted a study on 44 patients who were scheduled for elective caesarean section due to a sonographic diagnosis of placenta previa or placenta previa accreta. The purpose was to evaluate the significance of oxidative stress biomarkers in distinguishing between these conditions and to understand the involvement of these oxidative factors in the development of placenta accreta. To validate our hypothesis, malondialdehyde (MD) as oxidant biomarker and superoxide dismutase (SOD), catalase (CAT) and total antioxidant

capacity (TAC) as antioxidant biomarkers were assayed in both maternal serum and placental tissues. The results of the present study revealed increased level of MDA in both maternal serum and placental tissue of placenta accreta group when compared to the placenta previa non-accreta group. In contrast, there were decreased levels of SOD, CAT and TAC in both maternal serum and placental tissue of placenta accreta group compared to the placenta previa non-accreta group. These findings answered our hypothesis which stated that the oxidative stress which was manifested by imbalance between oxidants and antioxidants plays an important role in the pathogenesis of placenta accreta.

In a healthy pregnancy, there is a natural rise in lipid peroxidation products found in the mother's blood. This increase is counteracted by the enhanced functioning of antioxidant mechanisms. The levels of GPx in maternal erythrocytes and platelets, as well as the activity of extracellular SOD, gradually rise during pregnancy until the third trimester [28], [29]. Nevertheless, women with preeclampsia lack the antioxidant capacity, which causes an imbalance between the pro-oxidant and antioxidant systems. This imbalance leads to oxidative stress, as indicated by studies [30]–[32]. The placenta is the primary organ that is most susceptible to difficulties during pregnancy. The failure of that organ is the primary factor contributing to the development of obstetric disorders, such as preeclampsia [33]. It is widely recognised that the antioxidant balance is disrupted in preeclamptic pregnancies. This is supported by the fact that there is a surge in the synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the placenta, while the levels of antioxidants, which help remove harmful free radicals and prevent the formation of ROS, are reduced [34], [35]. Furthermore, preeclampsia is linked to reduced superoxide dismutase (SOD) activity [36] and decreased mRNA expression of copper-zinc superoxide dismutase (CuZn-SOD) in isolated trophoblasts in cases of preeclampsia [37]. Multiple investigations have demonstrated reduced CAT and SOD activity, as well as elevated levels of lipid peroxidation by-products (MDA) in the blood plasma of women diagnosed with preeclampsia [38]. Studies have also demonstrated reduced activity of GPx, SOD, and glutathione S-transferase in placentas affected by preeclampsia [38]. Our results were concomitant with the findings of the authors who assayed oxidative stress biomarkers in maternal serum of preeclamptic patients and these results assume to be logic as both preeclampsia and placenta accreta are considered ones of the placenta-related illnesses of pregnancy in which an increase in the MDA as oxidants biomarker and a decrease in SOD, CAT and TAC as antioxidants biomarkers that were exhausted to neutralize the oxidants. Moreover, our findings were confirmed by the presence of a similar pattern of oxidant-antioxidant biomarkers in placental tissues of both placenta previa non-accreta group and placenta accreta group to those in their serum. On contrary, other researchers found a decrement of oxidants and of increment antioxidants in maternal serum of patients with placenta accreta compared to pregnant women with normal placenta [23], [39]. This controversy in our results and those of other authors could be attributed to the sample size and the type of control involved in those studies.

5. Conclusions

The results indicate that the current study found elevated levels of MDA and reduced activities of SOD and CAT, as well as a decrease in TAC levels, in both maternal and placental tissues of pregnant women with placenta previa accreta compared to those with placenta previa non accreta. Furthermore, there were significant positive associations observed between the levels of these oxidative biomarkers in both the maternal serum and placental tissues of pregnant women with placenta accreta and placenta previa non accreta. These findings suggest a disparity between oxidants and antioxidants that could contribute to the development of placenta accreta. The oxidative stress biomarkers show potential as reliable indicators for predicting the occurrence of placenta accreta. In order to provide clarity and comprehensiveness to this theory, it is imperative to conduct a future study that encompasses a substantial sample size of individuals diagnosed with placenta accreta.

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