*Original Research Article*

Evaluating the Impact of Umbilical Cord *Toxoplasma gondii* on Oxidative Stress and its Association with Low Birth Weight among Deliveries at Nkwen District Hospital, Bamenda, Cameroon

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ABSTRACT

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| **Aim:** To determine effect of umbilical cord *T. gondii* on the profile of some oxidative stress biomarkers and the relationship with birth weight.**Methodology:** A total of 97 neonates born with cord *T. gondii* positive (77) and negative (20) were assessed for malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), catalase (CAT) and gluthatione (GSH) in the cord blood by Colorimetric enzymatic assays. Neonate birth weights at birth were also recorded. **Results:** The level of MDA and NO was higher (p<0.0001), and low level of SOD, CAT and GSH (p<0.0001) in infected cord compared to non-infected. The levels of MDA and GSH (p = 0.016 and p = 0.005) were higher, while SOD and CAT (p = 0.004 and p = 0.005) were lower in chronic infection compared to acute infection. Also, a high level of NO was observed, though not significant in chronic infection. In neonate with *T. gondii* positive cord, those born with normal birth showed a high levels of MDA, SOD, CAT, and GSH (p<0.0001) and low level of NO (p<0.0001) compared to those the birth weight is low. The birth weight was negatively correlated with cord blood levels of MDA and NO (p = 0.001), and positively correlated with the levels of CAT, GSH and SOD (p = 0.001). In neonate with *T. gondii* negative cord, there was no difference in MDA, SOD, CAT, and GSH and NO between those with normal and low birth weight. The birth weight was not significantly correlated with MDA, CAT, GSH, SOD and NO in cord blood.**Conclusion:** Umbilical cord *T. gondii* infection may cause oxidative stress of cord blood with MDA, which alongside high NO could lead to low birth weight. This finding contributes to the understanding of the pathophysiology of Umbilical cord *T. gondii* infection in neonate. |

**Keywords:** *Toxoplasma gondii*; Umbilical cord; Oxidative stress biomarkers

1. INTRODUCTION

Pregnancy outcome also called birth outcome is various events that arise to the newborn from 28 weeks (age of viability) to the first weeks of life. From pregnancy to pregnancy and include live birth, those events can be different. The term “adverse pregnancy outcome” therefore represents the health problems that take place to the newborn, the mother or both during pregnancy, labor and delivery, and the postpartum period (Tadese et al., 2022). About 810 women daily die of adverse pregnancy outcome during pregnancy, childbirth, or postpartum period according to the World Health Organization (WHO) reports (Tadese et al., 2021).The causes of pregnancy complications are many among there transplancental infections.

*Toxoplasma gondii* (*T. gondii*) infection is one of the infections identified to have transplacental transmission. In most cases, transplacental infections such as malaria infection are linked to tissue inflammation, low birth, oxidative stress etc. Oxidative stress has been associated in several pregnancy pathologies (Beghin, 2022). In fact, there is an appropriate pro-oxidant/anti-oxidant balance in normal cells. Increasing of pro-oxidant than anti-oxidant, this imbalance result in oxidative stress (OS). Oxidative stress is characterized by elevated levels of free radicals such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) and increase cell membrane lipid peroxidation such as malondialdehyde (MDA). MDA is the lipid peroxide product that the level can be used as oxidative stress index (Zsuzsanna et al., 2021). There various antioxidant systems that counteract the increase of free radicals includes enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH), small molecules like Nitric oxide (NO), ascorbic acid, uric acid, β carotene, α tocopherol, reduced glutathione (Pinheiro and Stika, 2020). Although information exists on the pathophysiological mechanisms implicated in the genesis of low birth associated to transplacental infections, there is none on umbilical cord T. gondii infection and oxidative stress biomarkers and their link to low birth weights. An imbalance between oxidants and antioxidants in women during pregnancy was reported in malaria infection (Omer et al., 2021). Other studies have suggested that host biomarkers may be useful in discriminating women likely to experience poor pregnancy outcomes (Vornic et al., 2021). In this way, since oxidative stress condition is related to fetal infection or transplacental transmission, umbilical cord biomarkers of oxidative stress may be of particular value. This study therefore aims to determine effect of umbilical cord *T. gondii* on the profile of some oxidative stress biomarkers and the relationship with birth weight. The levels of MDA, GSH, CAT, SOD and NO were assessed in cord blood serum of *T. gondii* infected and non-infected umbilical cords and the birth weight was also measured during delivery.

2. material and methods

**2.1 Ethical consideration**

This study was carried out on women recruited from September 2022 to January 2023 at NKwen District Hospital. Ethical Clearance (No:2022/0688H/UBa/IRB) for this study was gotten from the Institutional Review Board of the Faculty of Health Sciences of the University of Bamenda. An authorization to carry out the research was gotten from the regional delegation of public health.

**2.2 Study areas**

The recruitment area for the cohort was the district hospital Nkwen. Bamenda is the capital of the Northwest Region, Cameroon. Bamenda lies between latitude 5° 94′N and 5° 98′N and longitudes 10° 15′E and 10° 18′E. It sits along the Cameroon Volcanic Line with two distinct relief features: A High Lava Plateau of about 1,400 m, and the Lower Plateau, with an average altitude of 1100 m above sea level both separated by a vast escarpment. It has a tropical climate with two seasons, a long rainy season of eight months (March to October) and a short dry season of four months. Toxoplasmosis transmission in Northwest Cameroon is perennial but seasonal and peaks during the rainy season.

**2.3 Study design**

The study was a birth cohort carried out on 97 pregnant women during delivery recruited at Nkwen District Hospital. Demographic information was gotten through obtaining secondary data from the maternity records of the participants. These data included; the ages of the mothers, and the birth weights of the neonates

**2.4 Study population and exclusion criteria**

Population study is comprised of women aged between 18 to 45 years delivered that their babies in Nkwen district hospital. All neonates from mothers HIV positive mothers, mothers that had still births, and mothers that had premature deliveries were excluded. They were also excluded nonresident in the study area, some delivered at home and some did not give their consent to participate in the study and some had other reasons. Participants were excluded because when decided to withdraw their consent and had no baseline data available. Some collected samples were excluded when neonates birth weight was not been recorded and some had sickle cell traits.

**2.5 Collection of umbilical cord blood sample collection**

Cord blood samples were immediately collected following parturition using routine procedures by clamping and cannulating the umbilical blood vessels. Blood samples (2 to 5 ml) were collected in dry tubes and serum was gotten after centrifugation at 3000 g for 10 minutes. The collected serum samples were frozen at −70°C until the day of analysis.

**2.6 Diagnosis of *T.* *gondii***

Test strips (CJ SMART DIAGNOSTICS; Lot No: TGM21050008) were used for the rapid diagnosis of *T. gondii*. Blood sample (1 ml) was placed on the labeled test strips and incubation for 15 minutes at the temperature room to permit the reaction to take place. Test results were read and recorded as IgM positive (IgG+) or IgM positive (IgM+). All patients positive for IgG (IgG+) were taken as latent infection and IgM+ or both positive cases were as acute infection.

**2.7 Measurement of malondialdehyde level**

Malondialdehyde react with thiobarbituric acid (TBA) in a condensation reaction to form a coloured solution that can be measured with colorimetric technique at 530 nm (Wilbur *et al.,* 1949). The serum (200 µL) was introduced in test tubes and 200 µL of distilled water was introduced in blank tubes. Two hundred micro-litters (200 µL) of Tris-KCl buffer (50 mM; KCl, 150 mM; pH 7.4) was added in all tubes alongside with 100 µL of trichloroacetic acid (20%) and 200 µL of thiobarbituric acid (0.67%). After addition of reagents, all tubes were sealed with a glass marble and heated for 15 minutes in water bath at 100ºC. The mixture was cooled in running water and centrifugated at 3000 rpm for 15 minutes. The organic layer was separated, and the absorbance was measured at 532 nm using a spectrophotometer (Dchanche et al., 2024).

The concentration of MDA is expressed as nmol/mle.

$$[MDA](µmol/L) = \frac{ΔAbs}{ε×L}$$

Where ΔAbs (variation of the different absorbance) = OD test - OD blank; L: path length = 1 cm; ε: molar extinction coefficient = 1.56×105 mmol-1.cm-1. The concentration of MDA can also be converted in mmol/L.

**2.8 Measurement of glutathione level**

The assay is based on the reaction of glutathione (GSH) with 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB also known as Ellman's reagent) that produces the 2-nitro-5-thiobenzoate (TNB) chromophore, which has a maximal absorbance at 412 nm, and oxidized glutathione–TNB adduct (GS–TNB). The reaction between the thiol (SH) end of glutathione and DTNB (Ellman reagent; 5′5-dithiobis [2-nitrobenzoic acid]) results in the formation of a yellow-coloured complex that can be measured with colorimetric technic at 412 nm (Ellman, 1959). The homogenates (200 µL) were introduced in test tubes and distilled water (200 µL) was introduced in a blank tube, respectively. Thereafter, 3 mL of Ellman reagent was added in all the tubes. The mixture was incubated at room temperature for one hour and the absorbance was measured at 412 nm against the blank.

The concentration of GSH is expressed as μmol/ml.

$$[GSH](μmol/mL ) = \frac{ΔAbs×Vt}{ε×L×Vi}$$

Where ΔAbs (variation of the different absorbance) = OD test - OD blank; Vt: the total volume of the medium (tank) (mL); Vi: is the volume of the sample in the spectrometric tank (mL); L: path length = 1 cm; ε: the extinction coefficient = 13600 mol-1.cm-1.

**2.8 Measurement of superoxide dismutase activity**

Xanthine-xanthine oxidase is used to generate O2•− and nitro-blue tetrazolium reduction is used as an indicator of O2•− production. Superoxide dismutase will compete with nitro-blue tetrazolium for O2•−; the percent inhibition of nitro-blue tetrazolium reduction is a measure of the amount of superoxide dismutase (SOD) present. Catalase is included to remove hydrogen peroxide (H2O2) produced by SOD. The principle of this assay is based on the inhibition of adrenalin reduction into adrenochrome by the anion super oxides. The absorbance adrenochrome is maximal after 20 seconds and 80 seconds at 480 nm and is directly proportional to the concentration of SOD in the homogenate. The homogenate (134 µL) is introduced in test tubes and distilled water (134 µL), respectively. A volume of 1666 µL of carbonate-bicarbonate buffer (0.05 M; pH 10.2) was added in all tubes alongside with 200 µL of adrenalin (0.3 mM). For the blank tube, 1666 µL of carbonate-bicarbonate buffer was introduced in the blank tube, and a solution of adrenaline (200 µL) and distilled water (134 µL) were added. The mixture of each tube was homogenised and then the absorbance was read twice after 20 and 80 seconds at 480 nm (Misra and Fridovish, 1972). One unit of the activity SOD is the amount of SOD that inhibits the rate of formazan dye formation by 50%. The SOD activity is expressed as U/g of tissue and obtained from the percentage of inhibition of adrenalin reduction into adrenochrome within one-minute duration.

$$\% of inhibition= 100-\frac{ΔAbs test}{ΔAbs blank} x 100$$

Where ΔAbs (variation of the different absorbance) = OD80s - OD20s; if one unit of SOD (1U/ml) induced 50% of inhibition, therefore n unit will induce X% of inhibition.

 $N=\left(X\%\right) x\frac{1}{50\%}$

**2.9 Measurement of catalase activity**

The method is based on the fact that dichromate in acetic acid reduces to chromic acetate when heated in the presence of H2O2 with the formation of perchloric acid as an unstable intermediate ((Dchanche et al., 2024). The breakdown of hydrogen peroxide (H2O2) into oxygen (O2) and water (H2O) is mediated by the enzyme catalase (CAT). The chromic acetate thus produced is measured colorimetrically at 570 nm. Since dichromate has no absorbance in this region, the presences of the compound in the assay mixture do not interfere with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H202 for different periods of time. The reaction is stopped at specific time intervals by the addition of dichromate/acetic mixture and the remaining H202 is determined by measuring chromic acetate colorimetrically after heating the reaction. Potassium dichromate is actually used in revelatory test of hydrogen peroxide (H2O2), where the association lead to the appearance of an unstable blue green precipitate of perchloric acid. The perchloric acid resulting is degraded under heat to form a green complex that can be measured with colorimetric technic at 570 nm. The working dichromate/acetic acid solution was made with a mixed 5% of potassium dichromate with glacial acetic acid (1:3). The stock solution was diluted with water to make the working dichromate/acetic acid solution (1:5). Afterwards, this working reagent and sample were pipetted into tubes as follows: The specific activity of catalase was calculated according to the law of Beer-lambert, and expressed as U/ml.

$Catalase activity (U/ml)= \frac{OD. Vt}{t.a.m.Vi} x 10$3

OD: absorbance at 570 nm;

a: the slop of the equation y = ax + b, obtained from the standard curve.

t: time of reaction in minute;

Vi: sample volume;

Vt: total volume of the reactive medium

**2.10 Measurements of nitric oxide level.**

Nitric oxide (NO) has an extremely short half-life (< 10 seconds), which makes it difficult to detect and study. However, as NO is metabolized to nitrate and nitrite in the cell, quantitation of these stable anions can be used to measure the amount of NO that was originally present in a sample. Sulfanilic acid is quantitatively converted to a diazonium salt by reaction with nitrite in acid solution. The diazonium salt is then coupled to *N*-(1-naphthyl) ethylenediamine, forming an azo dye that can be spectrophotometrically quantitated based on its absorbance at 548 nm (Dchanche et al., 2024). Griess Reagent is used to convert the nitrite into a purple-colored azo compound, which is quantitated by a spectrophotometer at 546 nm. This method involves the use of the Griess diazotization reaction to spectrophotometrically detect nitrite formed by the spontaneous oxidation of NO under physiological conditions. Equal volumes of *N*-(1-naphthyl) ethylenediamine (solution A) and sulfanilic acid (solution B) were mixed together in the absence of light to form the Griess reagent. The homogenate (100 µL) is introduced in test tubes or sodium nitrite (NaNO2; 1 mM, 100 µL) in blank tubes, respectively. Thereafter, a volume of 400 µL of distilled water was added in all tubes alongside with 500 µL of Griess reagent. The mixture was incubated in the absence of light for 10 minutes, at room temperature and the absorbance was measured at 546 nm. The concentration of nitric oxide for each sample was calculated as follows:

$$Concentration of NO (µM/ml) = \frac{ODtest-ODblank}{a}$$

OD: absorbance at 570 nm; a: the slop of the equation y = ax + b, obtained from the standard curve.

**2.11 Statistical analysis**

Statistical analysis was performed using GraphPad Prism version 8 (GraphPad, Inc., San Diego CA, USA). Quantitative variables were presented as mean ± standard deviation. The comparison of parametric variables data between various groups was compared using one way ANOVA followed by the t-test or using unpaired t test. P < 0.05 was considered statistically significant.

3. results and discussion

**3.1 Oxidant and antioxidant biomarkers and nitric oxide among Toxoplasma gondii infected and non-infected cords**

Results presented in the Table 1 showed the cord blood oxidant and anti-oxidant biomarkers levels as well as NO level in *T. gondii* seropositive and seronegative cords. It was observed that the level of MDA was significantly (p<0.0001) higher in infected cord (0.84±0.08) than in the non-infected cord (0.32±0.02) and similarly the NO was significantly (p<0.0001) higher levels in infected cord (42.2±6.37) compared non-infected cord (20.1±0.70). In contrast, the results indicate that the level of SOD were significantly (p<0.0001) lower in infected cord (29.8±7.05) than non-infected cord (64.6±4.63). The level of CAT (p<0.0001) and GSH (P<0.0001) was also significantly lower in infected cord than non-infected cord.

Table 1: Cord blood levels of MDA, SOD, CAT, GSH and NO in acute and chronic *T. gondii* infection in cord

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Positive Cord****N=77** | **Negative Cord****N = 20** | **p value** |
| MDA (µmol/L) | 0.84±0.08 | 0.34±0.02 | < 0.0001 |
| SOD (U/mg) | 29.8±7.05 | 64.6±4.63 | < 0.0001 |
| CAT (µM/L) | 1.39±0.13 | 3.23±0.33 | < 0.0001 |
| GSH (µg/mL) | 0.03±0.02 | 0.33±0.01 | < 0.0001 |
| NO (µM/L) | 42.2±6.37 | 20.1±0.70 | < 0.0001 |

*Data represented as mean ± SD; P<0.05 was considered to be significant and are highlighted in bold, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: catalase; GSH: Reduced glutathione; NO: Nitric oxide****.***

**3.2 Oxidant and antioxidant biomarkers and nitric oxide among acute and chronic cord Toxoplasma gondii infections**

The Table 2 illustrates the malondialdehyde, catalase, glutathione, superoxide dismutase and nitric oxide levels in acute and chronic *T.* *gondii* infection in cord. Comparing the NO level, there was not significant (p = 0.07) difference in acute and in chronic. MDA level was significantly higher in chronic infection compared to acute infection (p = 0.016). There were significantly higher level of SOD (p = 0.004) as well as the level CAT (p = 0.005), but a significantly lower level of GSH (p = 0.005) in acute infection compared to chronic infection.

**Table 2:** Malondialdehyde, catalase, glutathione, superoxide dismutase and nitric oxide levels in acute and chronic cord blood during *Toxoplasma* *gondii* infection

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Acute infection****N= 45** | **Chronic infection****N = 32**  | **P value** |
| MDA (µmol/L) | 0.82±0.01 | 0.87±0.01 | **0.016** |
| SOD (U/mg) | 31.7±6.90 | 27.1±6.44 | **0.004** |
| CAT (µM/L) | 1.42±0.13 | 1.34±0.10 | **0.005** |
| GSH (µg/mL) | 0.02±0.008 | 0.03±0.01 | **0.005** |
| NO (µM/L) | 41.1±6.32 | 43.7±6.11 | 0.078 |

*Data represented as mean ± SD; comparison of two groups by two tail t-test. \*P<0.05 was considered to be significant and are highlighted in bold, MDA: Malondialdehyde, SOD: Superoxide dismutase; CAT: catalase; GSH: Reduced glutathione; NO: Nitric oxide*

**3.3 Effect of *T. gondii* on correlation between birth weight and umbilical cord blood serum levels of oxidative stress biomarkers.**

The MDA, SOD, CAT and GSH as well as NO levels among *T. gondii* positive and negative cord in relation to birth weight were presented in Table 3. In neonate with *T. gondii* negative umbilical cord blood, results did not any difference between the levels of MDA (p=0.96), SOD (p=0.67), CAT (p=0.32), GSH (p=0.52) and NO (p=0.40) in cord of neonate born with low birth weight, normal birth weight and high birth weight. In contrast, neonate with *T. gondii* positive cord, neonate born with normal birth showed a significant high levels of MDA (p<0.0001), SOD (p<0.0001), CAT (p<0.0001), and GSH (p<0.0001), with a low level of NO (p<0.0001) compared to those birth with low birth weight.

**Table 3:** Oxidant and antioxidant biomarkers and nitric oxide among infected and non-infected cord *Toxoplasma gondii* infections in relation to birth weight

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Positive Cord** |  | **Negative Cord**  |
|  | **LBW****N =32** | **NBW****N = 42** | **Sig.** | **LBW****N = 8** | **NBW****N = 5** | **HBW****N = 7** | **Sig.** |
| MDA (µmol/L) | 0.78±0.02 | 0.91±0.06 | **p<0.0001** | 0.35±0.03 | 0.35±0.02 | 0.34±0.02 | F(2, 17) = 0.04; \*p=0.96 |
| SOD (U/mg) | 23.4±4.03 | 35.1±3.92 | **p<0.0001** | 64.3±4.34 | 63.4±7.58 | 65.8±2.14 | F(2, 17) = 0.39; \*p=0.67 |
| CAT (µM/L) | 1.28±0.02 | 1.48±0.10 | **p<0.0001** | 3.16±0.27 | 3.43±0.54 | 3.18±0.13 | F(2, 17) = 1.20; \*p=0.32 |
| GSH (µg/mL) | 0.02±0.01 | 0.04±0.02 | **p<0.0001** | 0.34±0.04 | 0.30±0.07 | 0.33±0.06 | F(2, 17) = 0.67; \*p=0.52 |
| NO (µM/L) | 47.3±6.18 | 38.0±1.71 | **p<0.0001** | 20.2±0.70 | 19.8±0.64 | 20.3±0.74 | F(2, 17) = 0.94; \*P=0.40 |

*Data represented as mean ± SD. P<0.05 was considered to be significant and are highlighted in bold p value: Unpaired t test p values.pe valuectedtidepressive effect serum cortisone in ratss \*p value: One way ANOVA p value. pe valuectedtidepressive effect serum cortisone in ratssMDA: Malondialdehyde, SOD: Superoxide dismutase; CAT: catalase; GSH: Reduced glutathione; NO: Nitric oxide*

**3.4 Correlation between birth weight and cord blood oxidative biomarkers in *T. gondii* infected and non-infected cords**

In the neonate born with positive umbilical cords, the results showed that the birth weight is significantly and negatively correlated with cord blood levels of MDA (p = 0.001) and NO (p = 0.001), while the levels of CAT (p = 0.001), GSH (p = 0.001) and SOD (p = 0.001) are significantly and positively correlated with the birth weight. I contrast, the concentrations of MDA, CAT, GSH, SOD and NO in *T. gondii* negative cords did not showed significant correlation with the birth weight (p>0.05) (Table 4).

**Table 4:** Correlation between the birth weight and the cord bloodmalondialdehyde, catalase, nitric oxide, reduced gluthation and superoxide dismutase levels among infected and non-infected cords.

|  |  |  |
| --- | --- | --- |
| **Variables** | **Positive Cord** | **Negative Cord** |
| **R** | ***p* value** | **R** | ***p* value** |
| MDA (µmol/L) | -0.89 | **0.001** | -0.01 | 0.95 |
| SOD (U/mg) | 0.96 | **0.001** | 0.01 | 0.99 |
| CAT (µM/L) | 0.90 | **0.001** | -0.03 | 0.90 |
| GSH (µg/mL) | 0.96 | **0.001** | 0.03 | 0.91 |
| NO (µM/L) | -0.83 | **0.001** | -0.03 | 0.89 |

*P<0.05 was considered to be significant and are highlighted in bold.* *MDA: Malondialdehyde, SOD: Superoxide dismutase; CAT: catalase; GSH: Reduced glutathione; NO: Nitric oxide*

**3.5 DISCUSSION**

Oxidative stress is the shifting of pro-oxidant/ anti-oxidant balance usually present in normal cells towards the prooxidant side, which is manifested by elevated levels of free radicals responsible for cell damage. Researches have been demonstrated that free oxygen radicals generated during labour and the imbalance between oxidants and antioxidants in the foetus are responsible of the occurrence of perinatal and neonatal disorders, such as perinatal asphyxia and hypoxic–ischemic encephalopathy in term infants, bronchopulmonary dysplasia, respiratory distress syndrome, necrotising enterocolitis, especially in premature babies, and sudden infant death syndrome (Moore et al., 2019; Toboła-Wróbel et al., 2020; Zych et al., 2025). Although in several cord and pregnancy diseases, oxidative stress has been described (Tamirat et al., 2021), data on umbilical cord *T. gondii* and oxidative stress are infrequent. This study therefore tries to find the association of *T. gondii* infection and oxidative stress in cord blood, and whether increasing levels of oxidative stress biomarkers in the cord blood link with low birth.

Comparing full-term newborns, the results of this study highlight that high levels of MDA and NO alongside with low levels of SOD, CAT and GSH were found in umbilical cords positive of *T. gondii* than non-infected cord. The high MDA and NO observed in the umbilical cord blood with *T. gondii* infection may be an indicator of excessive ROS/RNS production, intensifying oxidative stress. This finding indicates that the transplacental transmission of *T. gondii* causes an oxidative stress in cord blood, therefore the neonate. This finding is similar to results from a similar studies reported an oxidative stress in malaria infected cords (Omer et al., 2021). *T. gondii* in cord may cause macrocromolecular damages by modifying intracellular calcium homeostasis and several metabolic pathways leading to apoptotic cell death, which has been shown in other ROS-related diseases (Rua et al., 2014).

It also observed in this study that there are high level of MDA accompanied by high levels of SOD and CAT, and low level of GSH in chronic infection compared to acute infection. This finding demonstrates that *T. gondii* is most associated with oxidative stress in a chronic stage than in acute. Glutathione is one of the important enzymes in stopping the lipid peroxidation process, in which the starting free radical is regenerated and available to restart another lipid peroxidation process, by reacting with the lipid peroxide intermediates and preventing the perpetuation of the process (Zych et al., 2025). Therefore, the observed high MDA observed in the umbilical cord blood with *T. gondii* chronic infection may be a consequence of the observed low f GSH level.

Antioxidant enzymes, ROS/RNS, factors in the production of ROS/RNS, non-enzymatic antioxidants, and products of oxidative stress were not constantly associated with assumed growth restriction or low birth. Some studies showing positive associations while other studies showing negative or absent associations (Yuba et al., 2024; Blok et al., 2024). However, neonates born with *T. gondii* positive cord and normal birth showed low level of NO compared to those of birth with low birth weight. The higher level of NO observed in the umbilical cord blood of newborns with low birth and infected umbilical cords may be an indicator of excessive RNS production, intensifying oxidative stress. This may also result from the activation of enzymes involved in free radical processes, due to the changes in the concentration of the enzyme ‘cofactor (Andrade et al., 2016). Therefore, the findings depict NO as a potential cause of low birth weight in case of umbilical cord *T. gondii*. The lower level of CAT, SOD and GSH observed in the umbilical cord blood of newborns with low birth and infected umbilical cords may also be the cause of excessive ROS production. This can be a result of the inactivation of enzymes involved in free radical processes, due to the depletion of enzyme activity (Sajjad et al., 2000; Andrés et al., 2023). Copper is one of the cofactors of copper–zinc superoxide dismutase, which eliminates ROS. Low copper content may affect the decrease in enzyme activity and change of synthesis of catalase and Mn-SOD, potentially affecting neurodegenerative disorders in newborns (Arbuckle et al., 2016). *Toxoplasma gondii* infection may the concentration of copper in cord blood or neonate. In addition, in the neonate born with positive umbilical cords, this study showed a negative correlation between MDA and NO levels from the cord blood and birth weight in one hand and in other hand a positive correlation between CAT, GSH from the cord blood and SOD and birth weight. Therefore, the findings describe the increase of MDA and NO, consequently the ROS/RNS as potential factors contributing in low birth during a transplacental transmission of *T. gondii*. A positive and significant correlation was also found between the levels of SOD, GSH, CAT and birth weight. These observations highlight the importance of anti-oxidative biomarkers in foetal growth.

**4. CONCLUSION**

Umbilical cord *T. gondii* infection can cause oxidative stress of cord blood with MDA and NO being the potential biochemical factors, which alongside low CAT, SOD and GSH might lead to low birth weight.

**ETHICAL APPROVAL**

Ethical Clearance (No:2022/0688H/UBa/IRB) was gotten from the Institutional Review Board of the Faculty of Health Sciences of the University of Bamenda

References

Andrade, V.M., Aschner, M.; Marreilha Dos Santos, A.P. (2017) Neurotoxicity of Metal Mixtures. Adv. Neurobiol., 18, 227–265.

Andrés, C.M.C.; Pérez de la Lastra, J.M.; Andrés Juan, C.; Plou, F.J.; Pérez-Lebeña, E. (2023). Superoxide Anion Chemistry-Its Role at the Core of the Innate Immunity. Int. J. Mol. Sci., 24, 1841.

Arbuckle, T.E.; Liang, C.L.; Morisset, A.S.; Fisher, M.; Weiler, H.; Cirtiu, C.M.; et al. (2016). Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. Chemosphere, 163, 270–282.

Beghin, D. Impact de la grossesse sur la pharmacocinétique des médicaments: Quelles répercussions en pratique? Impact of pregnancy on drug pharmacokinetics: What implication in clinical practice? Gynecol. Obstet. Fertil. Senol., 50, 422–425.

Blok, E. L., Burger, R. J., Bergeijk, J. E. V., Bourgonje, A. R., Goor, H. V.,

Dchanche, D.Y., M.S. Anchang, N.K. Emégam, M.L. Ngenteh and B.B.L. Fubi et al. (2024). Ocimum gratissimum L. (Labiateae) aqueous extract prevents behavioural impairment, motor incoordination and brain oxidative stress induced by prenatal stress in female rats. Asian J. Anim. Vet. Adv.,19: 26-36.

Ellman, G. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82: 70–77.Tissue sulfhydryl groups*. Archives of .Biochemistry. Biophysics. 1959; 82(1):70-77.*

Ganzevoort, W., & Gordijn, S. J. (2024). Oxidative stress biomarkers for fetal growth restriction in umbilical cord blood: A scoping review. Placenta, 154, 88-109.

Omer S., Clara Franco‑Jarava, Ali N., Mona O., Mutasim A., Israel M. et al. (2021). Impact of placental malaria on maternal, placental and fetal cord responses and its role in pregnancy outcomes in women from Blue Nile State, Sudan. Malar J (2021) 20:35

Mishra HP, & Fridovich I. (1972). “The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase.” Journal of Biological Chemistry; 247: 3170-3175

Moore, T. A., Ahmad, I.M., Schmid, K.K., Berger, A.M., Ruiz, R.J., Pickler, R.H. et al. (2019). Oxidative Stress Levels Throughout Pregnancy, at Birth, and in the Neonate. Biol. Res. Nurs. 2019, 21, 485–494.

Pinheiro, E.A.; Stika, C.S. (2020). Drugs in pregnancy: Pharmacologic and physiologic changes that affect clinical care. Semin. Perinatol., 44, 151221.

Rua, E. A. O., Marcella L. P., Jean P. L. R., Breno V. N., Silvana S. M., Elisardo C. V. et al. (2014). Effects of tobacco smoking during pregnancy on oxidative stress in the umbilical cord and mononuclear blood cells of neonates. Journal of Biomedical Science (2014) 21:105

Sajjad, Y.; Leonard, M.; Doyle, M. (2000). Antioxidant levels in the cord blood of term fetus. J. Obstet. Gynaecol., 20, 468–471.

Tamirat, K. S., Sisay, M. M., Tesema, G. A., and Tessema, Z. T. (2021). “Determinants of adverse birth outcome in Sub-Saharan Africa: analysis of recent demographic and health surveys,” BMC Public Health, 21 (1), pp. 1–10.

Tadese M., Tessema S. D., and Taye B. T. (2021). “Adverse Perinatal Outcomes Among Grand Multiparous and Low Multiparous Women and Its Associated Factors in North Shewa Zone Public Hospitals: The Role of Parity,” Int. J. Gen. Med., vol. 14, p. 6539, 2021

Tadese M, Dagne K, Wubetu AD, Abeway S, Bekele A, Misganaw Kebede W, et al. (2022) Assessment of the adverse pregnancy outcomes and its associated factors among deliveries at Debre Berhan Comprehensive Specialized Hospital, Northeast Ethiopia. PLoS ONE 17(7): e0271287.

Toboła-Wróbel, K.; Pietryga, M.; Dydowicz, P.; Napierała, M.; Br ˛azert, J.; Florek, E. (2020). Association of Oxidative Stress on Pregnancy. Oxid. Med. Cell. Longev., 2020, 6398520.

Vornic, I.; Buciu, V.; Furau, C.G.; Gaje, P.N.; Ceausu, R.A.; Dumitru, C.-S.; et al. (2024). Oxidative Stress and Placental Pathogenesis: A Contemporary Overview of Potential Biomarkers and Emerging Therapeutics. Int. J. Mol. Sci., 25, 12195.

Yuba, T.; Koyama, Y.; Kinishi, Y.; Uokawa, R.; Ootaki, C.; Shimada, S. e al. (2021). Analysis of Maternal and Fetal Oxidative Stress During Delivery with Epidural Analgesia. Reprod. Sci. 2024, 31, 2753–2762.

Zsuzsanna S‑S. (2022). Erzsebet F., Eniko N‑N., Lorand D., Mircea C., Bela S. (2021). Oxidative stress and peripartum outcomes (Review). Experimental and Therapeutic Medicine, 22: 771.

Zych, B., Górka, A., Myszka, A., Siekierzy ´nska, A., Błaz,˙ W., Błoniarz, D. (2025). The Impact of the Delivery Method on Oxidative Stress in Neonates: A Cross-Sectional Study. J. Clin. Med., 14, 2269.