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Nitric Oxide and Antioxidant Enzymes in Venous and Cord Blood of Late Preterm and Term Omani Mothers

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أكسيد النيتريك والإنزيمات المضادة للأكسدة في الدم الوريدي ودم الحبل السري للأمهات العُمانيات للولدان المُبْتَسَرين المتأخرين والولدان الناضجين

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الملخص: **الهدف**: تقييم دور ضغط الأكسدة في الولادة عند الأمهات العمانيات بعد ورود تقارير متزايدة على أن الولدان حديثي الولادة قبل الأوان في وقت متأخر في خطر أكبر من حديثي الولادة الناضجين من حيث وَفَيَاتُ الفَتْرَة المحيطَة بالولادَة. **الطريقة**: تم جمع عينات من الدم الوريدي أثناء الولادة وعينات أخرى من دم الحبل السري بعد الولادة لأمهات الولدان المُبْتَسَرين المتأخرين والولدان الناضجين في مستشفى جامعة السلطان قابوس، عُمان. تم قياس تركيز أكسيد النيتريك في البلازما ونشاط كاتلاز كريات الدم الحمراء كما تم قياس نشاط بيروكسيديز الجلوتاثيون لكريات الدم الحمراء. **النتائج**: كان تركيز أكسيد النيتريك عند أمهات الولدان المُبْتَسَرين المتأخرين أعلى بشكل ملحوظ 17.1 ± 3.3 (مايكرومول/ لتر) بالمقارنة مع أمهات الولدان الناضجين 11.0 ± 5.5 ($P < 0.0001$)، وقيم أدنى لنشاط بيروكسيديز الجلوتاثيون لكريات الدم الحمراء 94.1 ± 12.9 (وحدة لكل جرام من الهيموغلوبين) مقابل 110.4 ± 12.3 ($P < 0.0001$). وكانت أمهات الولدان المُبْتَسَرين المتأخرين أصغر سنا بكثير من أمهات الولدان الناضجين ($P = 0.027$). وكان الولدان المُبْتَسَرين المتأخرين أقل وزنا بكثير من الولدان الناضجين ($P < 0.0001$)، وكان نشاط بيروكسيديز الجلوتاثيون لكريات الدم الحمراء منخفضا بشكل ملحوظ ($P = 0.001$) في الولدان المُبْتَسَرين المتأخرين بالمقارنة مع الولدان الناضجين. ولم يظهر أي فرق في نشاط كاتلاز كريات الدم الحمراء بين المجموعتين. **الخلاصة**: كانت قيم أكسيد النيتريك في البلازما أعلى عند أمهات الولدان المُبْتَسَرين المتأخرين من نظيرتها لأمهات الولدان الناضجين، كما كان نشاط بيروكسيديز الجلوتاثيون لكريات الدم الحمراء عند المجموعة الأولى منخفضا مقارنة بالمجموعة الثانية. ولوحظ كذلك انخفاض في نشاط بيروكسيديز الجلوتاثيون لكريات الدم الحمراء عند الولدان المُبْتَسَرين المتأخرين بالنسبة للولدان الناضجين والذي يدل على خل للموازنة الموالية للأكسدة والمضادة لها نظرا لزيادة عبء الأكسدة في الولادة قبل الأوان.

مفتاح الكلمات: أكسيد النتريك، كتالاز، بيروكيديز الجلوتاثيون، ولد مُبْتَسَر متأخر، أمهات الأطفال الناضجين، حديثي الولادة؛ الإجهاد التأكسدي، عُمان.

ABSTRACT: ***Objectives:*** This study assessed the role of oxidative stress in parturition in Omani mothers following growing reports that late preterm neonates were at greater risk than term neonates of perinatal death. ***Methods:*** Venous blood samples were collected during labour, and cord (neonatal) blood samples were taken after childbirth in late preterm and term from women at Sultan Qaboos University Hospital, Oman. Plasma nitric oxide (NO) concentrations, erythrocyte catalase (CAT). Erythrocyte glutathione peroxidase (GPx) activities were measured using spectrophotometric methods. ***Results*:** When compared with term mothers, late preterm mothers had markedly higher NO concentrations (μmol/L) 17.1 ± 3.3 versus 11.0 ± 5.5 (P <0.0001), and lower GPx values (U/g Hb) 94.1 ± 12.9 versus 110.4 ± 12.3 (P <0.0001). Late preterm mothers were significantly younger (P = 0.027) than term mothers and had neonates that weighed significantly less (P <0.0001) than term neonates. GPx activity was significantly reduced (P = 0.001) in late preterm neonates as compared to term neonates. CAT showed no change in activity in any comparison. ***Conclusion:*** Distinctly higher values of NO and lower GPx activity were found in late preterm mothers relative to term mothers; also, lower GPx in late preterm neonates relative to term neonates suggested a pro-oxidant-antioxidant imbalance due to the greater oxidative burden in late preterm parturition.

Keywords: Nitric oxide; Catalase; Glutathione peroxidase; Late preterm birth; Term birth; Neonates; Oxidative stress; Oman.

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Advances in Knowledge

- *Oxidative stress is more a feature of parturition at late preterm than at term.*
- *Late preterm mothers are at greater risk of oxidative stress than term mothers.*
- *Late preterm neonates are more susceptible to oxidative stress than term neonates.*

Application to Patient Care

- *Identifying modifiable factors in the obstetric setting may be an inexpensive and noninvasive therapy for preventing preterm parturition.*
- *Antioxidants (including the carotenoids, and vitamins C and E) and antioxidant cofactors (such as copper, selenium, and zinc) are capable of disposing, scavenging, or suppressing the generation of reactive oxygen species implicated in oxidative stress.*
- *Physicians should identify antioxidant-laden diets and educate pregnant Omani women about their benefits, and also advise them to take daily multivitamins and microminerals throughout pregnancy and beyond.*
- *Regular assays of plasma nitric oxide (NO) and erythrocyte glutathione peroxidase (GPx) should be considered in suspected late preterm pregnancy.*

The gestational age window termed "late preterm birth" occurs between 34 weeks 1 day and 36 weeks 6 days.[1–3] The greatest number of obstetric interventions and adverse neonatal outcomes occur at late preterm births.[4] Compared with term neonates, late preterm neonates are at higher risk for hospital readmissions, post-neonatal mortality, sudden infant death syndrome, white matter injury, and neuro-developmental problems well into the school age years.[5–8]

Nitric oxide (NO) is the product of a five-electron oxidation of L-arginine mediated by one of three NO synthases: endothelial, neuronal, and inducible NO synthases (eNOS, nNOS and iNOS).[9] Neuronal NOS and eNOS are responsible for the continuous basal release of NO. Endothelial NOS is expressed in theca cells, granulosa cells, and the surface of oocyte during follicular development.[10,11] Inducible NOS expressed in monocytes and macrophages in response to pro-inflammatory cytokines, catalyses the synthesis of a large amount of NO in pathological conditions.[12,13] NO itself comprises a nitrogen atom bound to an oxygen atom, forming a reactive nitrogen species depicted as $^{\cdot}NO$, a short-lived, readily diffusible molecule that reacts with superoxide (O_2^{-}) to form the highly reactive and cytotoxic pro-oxidant, peroxinitrite anion ($ONOO^{-}$).[13–15] Both glutathione peroxidase (GPx) and catalase (CAT) function as enzymatic antioxidants by neutralising pro-oxidants and thus prevent damage to the cellular structure. The selenoprotein, GPx, is cytosolically located in most tissues. It catalyses the breakdown of hydrogen peroxide (H_2O_2) and organic peroxide (ROO) supported by reduced glutathione (GSH) to form glutathione disulphide (GSSG), water, and organic alcohol (ROH). In erythrocytes from adult humans, all GPx activity appears to be selenium-dependent.[16] Catalase, a haemoprotein, specifically converts H_2O_2 to yield O_2, and H_2O, and does not decompose ROO.

In this context, information about oxidative stress in parturition at late preterm and term is lacking. The objective of this study was to assess the strength of oxidative stress at parturition by measuring and comparing concentrations of plasma NO, and activities of erythrocyte GPx and CAT in venous and cord blood of Omani mothers who delivered at late preterm and at term.

Methods

Ethical approval was granted for the study by the research review board at the authors' institution. Enrollment was voluntary, and all participants signed a consent form previously approved by the institution's ethics committee for the protection of human subjects in research. A total of 37 mothers who delivered at late preterm were registered, as well as 37 mothers who delivered at term. They were non-smokers, normotensive, and had singleton gestations ranging from 34 to 42 weeks. Gestational age at entry was determined by an obstetric estimate of the last menstrual period, uterine size, and ultrasound examination.[17] Late preterm mothers delivered between 34 weeks and <37 weeks and their ages ranged from 14–44 years (mean 28 years). Term mothers delivered between 37 and 42 weeks and were aged 22–45 years (mean 33 years). No medical complications were encountered during pregnancy and all deliveries occurred vaginally and were free of artificial support. The cord blood was deemed neonatal. Late preterm neonates (20

Table 1: Comparisons of parameters (mean ± SD) of birth weights, nitric oxide, glutathione peroxidase, and catalase of term neonates versus late preterm neonates

Parameter	Late Preterm Neonates	Term Neonates	*P* value
	(n = 37)	(n = 37)	
Birth weight (g)	2,463 ± 415	3,292 ± 491	<0.0001
NO (µmol/L)	5.32 ± 3.67	5.07 ± 2.96	0.910
GPx (U/g Hb)	48.8 ± 22.7	77.6 ± 26.9	0.001
CAT (nmol/min/mL)	96.5 ± 44.3	91.3 ± 45.8	0.993

Note: Statistical significance was considered at P <0.05.

Legend: NO = nitric oxide; GPx = glutathione peroxidase; CAT= catalase.

males, 17 females) born to late preterm mothers, had a mean ± SD birth weight of 2,463 ± 415 g. Term neonates (17 males, 20 females) born to term mothers, had a mean ± SD birth weight of 3,292 ± 491 g.

Maternal venous blood samples were taken during labour from the median cubital vein into evacuated lithium heparinised tubes. Samples of umbilical cord blood were collected immediately after delivery by aseptic puncture of the umbilical veins connected to the maternal placenta into evacuated lithium heparinised tubes. The specimens were centrifuged at 4° C; cord and venous plasma were separated for assay of NO. Haemolysates were prepared from the packed cells for the assays of GPx and CAT. Measurement of NO concentration was performed in a 96-well microtitre plate using reagents from Cayman (Cayman Chemical Company, Michigan, USA) and was a two-step process based on the method of Green *et al.*[18] The first step is the conversion of nitrate to nitrite utilising nitrate reductase; the second is the addition of the Greiss reagents which, on reaction with nitrite, develop a deep purple azo chromophore. Absorbance is read after 10 minutes at 540 nm using a plate reader, the Thermo Labsystem Multiskan Spectrum (Thermo Electron Corporation, Zantaa, Finland). Standards, controls and samples were measured in triplicate and expressed in µM

Haemolysates were prepared from the remaining packed cells as previously described for the GPx assay, using Ransel kits.[19] Ransel controls (Randox Laboratories, Crumlin, UK) were used to monitor the GPx activity.

Catalase activity in the haemolysates and controls was assayed in a 96-well microtitre plate, using catalase assay kits (Cayman Chemical Company, Michigan, USA), which utilise the peroxidative function of CAT for determination of enzyme activity. The method is based on the reaction of CAT with methanol in the presence of an optimal concentration of H_2O_2 to form formaldehyde, measured with purpald (4-amino-3-hadrazino-5- mercapto-1,2,4-triazole) as the chromogen.[20,21] Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colourless to a purple colour.[19,20] The absorbance was read at 540 nm using a plate reader, the Thermo Labsystem Multiskan Spectrum (Thermo Fisher Scientific, Massachusetts, USA).

The imprecision study was done using the internal quality control sera supplied in the commercial kits. Analyses of twelve aliquots of the controls revealed an intra-assay coefficient of variations (%) of 2.9, 3.6 and 4.1 for NO, GPx and CAT, respectively; the inter-assay CV values (%) performed over six days were 3.6, 5.0 and 5.4 for NO, GPx and CAT, respectively.

Each data set was confirmed to have Gaussian distribution by the one-sample Kolmogorov-Smirnoff test and, additionally, by histogram plots. Then all data were presented as mean ± SD. The mean differences between more than two groups were determined by one-way analysis of variance (ANOVA) using Scheffe's post-hoc test for a multiple comparison. Probability (*P*) values were two-tailed and *P* <0.05 was considered to be significant.

Results

The data comparisons (mean ± SD) of the parameters of late preterm versus term neonates are presented in Table 1. Late preterm neonates weighed significantly (*P* = <0.0001) less and had lower GPx activity (*P* = 0.001) as compared to term neonates. NO concentrations and CAT activity were similar in both groups of neonates. Table 2 represents maternal data (mean ± SD) of age, NO, GPx and CAT. Late preterm mothers were significantly younger (*P* = 0.027), had strikingly higher NO concentrations (*P* <0.0001), and a markedly lower level of GPx activity (*P* = <0.0001) relative to term mothers. Catalase activities of both mothers were similar.

Table 2: Comparisons of parameters (mean ± standard deviation) of age, nitric oxide, glutathione peroxidase, and catalase of term mothers versus late preterm mothers

Parameter	Late Preterm Mothers (n = 37)	Term Mothers (n = 37)	*P* value
Age (years)	27.5 ± 6.5	32.5 ± 6.0	0.027
NO (µmol/L)	17.1 ± 3.3	11.0 ± 5.5	<0.0001
GPx (U/g Hb)	94.1 ± 12.9	110.4 ± 12.3	<0.0001
CAT (nmol/min/mL)	93.9 ± 32.6	96.3 ± 32.3	0.998

Note: Statistical significance was considered at P <0.05.
Legend: NO = nitric oxide; GPx = glutathione peroxidase; CAT= catalase.

Data on the effect of gender on neonatal parameters are omitted because the differences did not attain statistical significance.

Discussion

Earlier studies carried out on animal and human tissues generally reported decreasing NO synthesis concomitant with declining NOS activity as gestation approached term.[22–28]Higher NO values found in late preterm mothers compared with term mothers Table 2 complement those reports.[22–28] Some earlier studies reported a greater expression of the isoforms of NOS (eNOS, iNOS) in the myometrium of the preterm 'not in labour' group compared with all other groups: non-pregnant women, pregnant women in the early third trimester, and pregnant women at term (both before and after labour).[27,28] Since iNOS promotes inflammation, and inflammation in turn promotes pro-oxidant formation, this may explain why late preterm mothers and not term mothers had more elevated plasma NO concentrations and reduced erythrocyte antioxidant (GPx) activity Table 2.[12] High pro-oxidant levels, coupled with reduced antioxidants, signify a disturbance of the pro-oxidant-antioxidant balance otherwise known as oxidative stress.[29,30] The placenta synthesises, on a weight-for-weight basis, more NO than the foetal membrane or the myometrium.[26] From early pregnancy, the mitochondria replete placenta influences maternal homeostasis and consumes about 1% of the basal metabolic rate of the pregnant woman.[31] The placenta in addition, is highly vascular and, on exposure to high maternal oxygen tension, generates superoxide ($^1O_2^{\cdot-}$); about 5% of all electrons in the mitochondrial respiratory chain seep out of the mitochondria.[31] Superoxide dismutase is the vanguard cytoprotective enzyme in that it disproportionates $^1O_2^{\cdot-}$ to O_2 and H_2O_2 the latter being a substrate for GPx and CAT. Glutathione peroxidase and CAT are both predominantly intracellular cytoprotective enzymes, but in addition to scavenging H_2O_2, GPx and not CAT, also scavenges lipid (organic) hydroperoxides (ROOH). Hence, it follows that GPx, because of its greater antioxidant burden as compared to CAT was the cytoprotective enzyme most utilised and consistently depleted in this study [Tables 1 and 2]. According to a literature search, there are no previous studies related to the effect of GPx and CAT on late preterm and term labour or parturition to which it is possible to make comparisons.

Conclusion

The findings of the present study suggest that oxidative stress was more a feature of childbirth at late preterm than at term. It is anticipated that this information would be of use to the medical community in general, and particularly, to obstetricians, midwives, and neonatologists who deal with neonatal morbidity, high neonatal hospital readmissions, and post-neonatal mortality, including sudden infant death syndrome among others.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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