



## Original Research Article

## Evaluation of urinary oxidative stress markers, inflammatory sialoproteins and neopterin in pregnant women with intrauterine growth restriction

Nachimuthu Maithilikarpagaselvi<sup>1,\*</sup>, Agrawal Shyamsundar Muskan<sup>1</sup>, Palai Pallavee<sup>2</sup>,  
Murugaigaiyan Sathish Babu<sup>1</sup>, Ramasamy sathiya<sup>1</sup>, Ramalingam Sripradha<sup>1</sup>

<sup>1</sup>Dept. of Biochemistry, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth, Puducherry, India

<sup>2</sup>Dept. of Obstetrics & Gynecology, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth, Puducherry, India



## ARTICLE INFO

## Article history:

Received 31-07-2019

Accepted 21-01-2020

Available online 21-02-2020

## Keywords:

Intrauterine growth restriction  
neopterin  
oxidative stress index

## ABSTRACT

**Introduction:** Intrauterine growth restriction (IUGR) represents major cause for perinatal morbidity and mortality. Oxidative stress and altered inflammatory response may contribute to the pathogenesis of IUGR. The present study was aimed to investigate the urinary and blood levels of inflammatory and oxidative stress markers in women with IUGR.

**Materials and Methods:** 23 pregnant women with intrauterine growth restriction and 30 healthy pregnant women were included in the present study. Detailed demographic data were collected. Total protein, albumin, total anti-oxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), malondialdehyde (MDA), neopterin and protein bound sialic acid (PBSA) were estimated in the blood. TOS, TAS and neopterin were measured in urine samples of women with IUGR.

**Results:** Serum MDA ( $16.2 \pm 1.8$ ) and Sialic acid ( $2.5 \pm 0.8$ ) levels were significantly elevated in women with IUGR. Urinary TOS ( $20.4 \pm 4.4$ ) and OSI ( $5.4 \pm 1.2$ ) were significantly increased in pregnant women with IUGR. The cut off value in ROC analysis for urinary oxidative stress index was 4.6 for the diagnosis of IUGR.

**Conclusion:** Oxidative stress in maternal blood is positively associated with the development of IUGR in pregnant women. Estimation of these parameters in serum and urine could serve as screening tool for the early prediction and diagnosis of IUGR which will further aid in the better management of neonatal health.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by/4.0/>)

### 1. Introduction

Intrauterine growth restriction (IUGR) refers to a fetus with an estimated fetal weight below the 10<sup>th</sup> percentile for gestational age on ultrasound.<sup>1</sup> IUGR represents the major risk factor for perinatal mortality and morbidity. IUGR babies are more prone to develop sepsis, altered Apgar score and perinatal asphyxia.<sup>2</sup> Early diagnosis of IUGR during pregnancy is essential in order to reduce the complications associated with IUGR and for the better management of neonatal health. The conservation of delicate balances of pro-inflammatory and anti-inflammatory environment

is essential for the maintenance of normal pregnancy. Disturbance in this immunological response in the pregnancy leads to stimulation of the maternal immune system ultimately culminate in complications such as placental damage, abortion, retardation of fetal growth and preterm labour.<sup>3</sup> Activated maternal inflammatory response also produces the free radicals leading to oxidative stress resulting in elevation of various oxidative markers.<sup>4</sup> Free radical generation leads to oxidative stress which ultimately results in reduced antioxidant status and increased oxidant status. Oxidative stress in pregnant women with IUGR showed increased oxidant status and decreased antioxidant enzymes.<sup>5</sup>

\* Corresponding author.

E-mail address: [maithilibiochem@gmail.com](mailto:maithilibiochem@gmail.com) (N. Maithilikarpagaselvi).

It was shown that various inflammatory markers have been elevated in women with IUGR. Studies have shown that elevation of various markers of inflammatory response such as interleukin-6, (IL-6), high sensitive C-reactive protein (hsCRP) and tumor necrosis factor alpha (TNF- $\alpha$ ) and neopterin have been associated with IUGR in pregnant women.<sup>6,7</sup> Among the markers, Neopterin is a biomarker of cellular immunity, a catabolic product of guanosine triphosphate metabolism. Neopterin was found to be elevated in auto-immune disorders, viral infection, inflammatory disease and nephritic syndrome and also used as diagnostic markers for the diagnosis of the same.<sup>8,9</sup> Erkenekl et al have demonstrated that serum neopterin levels were significantly higher in pregnant women with fetal growth restriction.<sup>6</sup> Alterations in the immune status and redox imbalance also enhance the release of sialic acid (N-acetylated derivative of neuraminic acid), inflammatory marker from various glycoproteins which is also associated with various complications in pregnancy.<sup>10,11</sup> Evaluation of Neopterin levels, hsCRP, sialic acid and oxidative stress marker could be used as biomarker for diagnosis and prediction of adverse outcome in pregnant women with IUGR. This will further aid in the better management of the neonatal health. To the best of our knowledge, there was only one report on elevation of neopterin levels in pregnant women with IUGR.<sup>6</sup> Hence the present was designed to investigate the relationship between inflammatory marker of natural immunity and oxidant status in pregnant women with IUGR.

## 2. Materials and Methods

The study was conducted between June 2017 and December 2018 in the Department of Biochemistry in collaboration with Department of Obstetrics and gynaecology, Mahatma Gandhi Medical College and Research Institute (MGM-CRI), Puducherry, India. The subjects were recruited from the antenatal OPD of Obstetrics & Gynaecology, MGMCR & RI. The study was approved by Institutional Human ethical committee (IHEC), MGMCR. The study was initiated after obtaining written informed consent from the study subjects. The case control study comprised of 23 pregnant women with IUGR and 30 women without IUGR in the age group of 22 to 32. IUGR were diagnosed by ultrasonography in the third trimester (27-40 weeks), if the estimated fetal weight (EFW) falls below the 10<sup>th</sup> percentile for gestational age is considered as intrauterine growth restriction (IUGR). Gestational age was calculated from the last menstrual period and confirmed by the ultrasound measurement. The Gestational age, maternal age, Gravida and Parity matched healthy pregnant women with and without IUGR were included in this study. Pregnant women with multiple gestations, pre-eclampsia, Diabetes, thyroid disease, Hypertension, renal disease and fetuses with structural or chromosomal anomalies were excluded from

this study.

Detailed history, gestational age and demographic data were obtained from the study subjects

Two millilitre of venous blood samples was collected in EDTA vial after an overnight fasting of 12 hours. The blood samples were centrifuged at 3000g for 10 min and the plasma was separated. Simultaneously urine samples of the study subjects also collected in preservative. The biochemical parameters such as total protein, albumin, MDA, TAS, TOS and protein bound sialic acid were estimated immediately in plasma and urine samples. The remaining plasma and urine samples were stored at - 40° C for two months for neopterin estimation. The plasma levels of total protein and albumin were estimated using standard reagents kits adapted to fully automated clinical chemistry analyser. Serum and urinary neopterin levels were estimated by ELISA using a commercial kit (Bioassay Technology Laboratory, Shanghai, China). The protein bound sialic acid was measured by Aminoff's method.<sup>12</sup> Total antioxidant status in plasma and urine samples were measured through ferric reducing antioxidant power (FRAP) assay.<sup>13</sup> This assay is based on reduction of ferric tripyridyltriazine (Fe<sup>3+</sup>, TPTZ) complex to ferrous form which produces blue colour that was measured at 593 nm. Results were obtained from a standard graph of FeSO<sub>4</sub> (0-1000  $\mu$ mol/L) and expressed as  $\mu$ mol/l. Total oxidant status (TOS) was measured according to the method of Ereli et al.<sup>14</sup> Oxidative stress index (OSI) was calculated as total oxidant status (TOS) divided by total antioxidant status (TAS) multiplied by 100.

### 2.1. Statistical analysis

All results are presented as mean  $\pm$  S.D and compared using the non-parametric Mann-Whitney test for two groups. The data were analyzed using SPSS. 19 software. Data variations between the groups were analyzed by independent 't' test. A P value of < 0.05 was considered as statistically significant.

## 3. Results

The baseline demographic and clinical characteristics of pregnant women with and without IUGR are shown in Table 1. There was no significant difference in the maternal age, gestation age, gravida, parity and blood pressure of pregnant women with IUGR and without IUGR.

The biochemical parameters of women with IUGR and without IUGR were shown in table 2. There was no significant difference between total protein and albumin among cases and control. There was a significant increase in the plasma levels of Malondialdehyde (MDA) and protein bound sialic acid (PBSA) in women with IUGR. No significant difference was found between serum neopterin, TOS and TAS in cases when compared to control. In consistent with these findings we observed increased serum

levels of neopterin, TOS and decreased TAS in women with IUGR, though these findings were not statistically significant.

The results of urinary total anti-oxidant, oxidant status and neopterin levels are shown in Table 3. Urinary total anti-oxidant status was lower in women with IUGR ( $386 \pm 64$ ), but not statistically significant. Urinary total oxidant status in women with IUGR ( $20.4 \pm 4.4$ ,  $p < 0.05$ ) was significantly increased when compared with the control subjects ( $16.7 \pm 4.6$ ). On comparison with control group ( $4.3 \pm 2.2$ ) significant increase was found in urinary oxidative stress index was in women with IUGR ( $5.4 \pm 1.2$ ,  $p < 0.05$ ). The urinary neopterin levels were higher in women with IUGR ( $15.7 \pm 2.2$ ,  $p > 0.05$ ) when compared to control women ( $14.3 \pm 3$ ). But these differences were not statistically significant between the two groups.

Receiver operating characteristic curve for urinary Neopterin and OSI are shown in Figure 1. We found that both Neopterin and OSI had good diagnostic accuracy in the diagnosis of IUGR. Area under the curve of the Receiver operative curve (ROC) of Neopterin and OSI was found to be 15.5 (78% sensitivity and 73% specificity) and 4.6 (70 % sensitivity and 60 % specificity) respectively.

**Table 1:** The demographic and clinical features of pregnant women with IUGR and without IUGR

Parameters	Control (n=30)	Case (n=23)	P value
Age (Years)	$25.6 \pm 1.8$	$26.0 \pm 1.6$	0.458
Gestational age (weeks)	$33.8 \pm 2.1$	$32.4 \pm 2.4$	0.308
Parity	0.7 (0,1)	0.8 (0,1)	0.752
Gravida	1.7 (1,2)	1.6 (1,2)	0.688
SBP	$107.2 \pm 5.0$	$108.7 \pm 3.7$	0.190
DBP	$82.2 \pm 9.5$	$81.7 \pm 9.3$	0.827

SBP -Systolic blood pressure, DBP -Diastolic blood pressure

#### 4. Discussion

Pregnancy associated complications such as Intrauterine growth restriction, preterm delivery, pre-eclampsia, etc. have a great impact on maternal and neonatal health. Early diagnosis of IUGR and pregnancy associated complications during the antepartum would lead to better management of maternal and neonatal health. Hence the present study investigated the serum and urinary biomarkers in pregnant women with IUGR which will aid the early diagnosis of the complications.

It has been reported that there is an imbalance between oxidant status and the antioxidant system in pregnant women with IUGR.<sup>15</sup> In the present study we found, significant increase in the urinary total oxidant status (TOS) and oxidative stress index (OSI) in women with IUGR when compared with the control subjects. Concordance with this result, we also observed increased serum total

**Table 2:** Biochemical Parameters of pregnant women with IUGR and without IUGR

Parameters	Control (n=30)	Case (n=23)	P value
Total protein (g/dl)	$7.6 \pm 0.4$	$7.4 \pm 0.4$	0.643
Albumin (g/dl)	$4.3 \pm 0.5$	$4.2 \pm 0.3$	0.842
FRAP ( $\mu\text{mol/L}$ )	$485 \pm 108$	$463 \pm 62$	0.345
TOS ( $\mu\text{mol H}_2\text{O}_2$ Equiv./L)	$14.0 \pm 4.9$	$15.6 \pm 3.0$	0.131
OSI	$3.1 \pm 1.5$	$3.4 \pm 0.8$	0.156
MDA ( $\mu\text{mol/L}$ )	$5.2 \pm 1.4$	$16.2 \pm 1.8^{***}$	0.000
Neopterin (nmol/L)	$8.2 \pm 2.8$	$9.4 \pm 2.3$	0.166
Protein bound sialic acid ( $\mu\text{g/mg}$ of protein)	$1.2 \pm 0.5$	$2.5 \pm 0.8^{***}$	0.000

Data are expressed as mean  $\pm$  S.D, \*comparison of control Vs IUGR group

\*\*\* $P < 0.01$ , \*\*\*\* $P < 0.001$

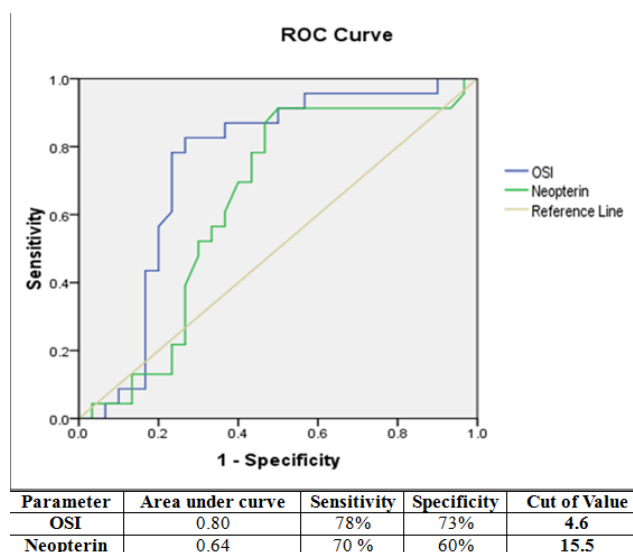
FRAP -Ferric reducing power of plasma, TOS -Total oxidant status, MDA -Malondialdehyde and OSI- Oxidative stress index.

**Table 3:** Urinary oxidant-anti-oxidant status and inflammatory markers of pregnant women with and without IUGR

Parameters	Control (n=30)	Case (n=23)	P value
TAS ( $\mu\text{mol/L}$ )	$426.7 \pm 105$	$386 \pm 64$	0.074
TOS ( $\mu\text{mol H}_2\text{O}_2$ Equiv./L)	$16.7 \pm 4.6$	$20.4 \pm 4.4^{**}$	0.003
OSI	$4.3 \pm 2.2$	$5.4 \pm 1.2^{**}$	0.007
Neopterin (nmol/L)	$14.3 \pm 3.0$	$15.7 \pm 2.2$	0.133

Data are expressed as mean  $\pm$  S.D, \*comparison of control Vs IUGR group

\*\* $P < 0.01$



**Fig. 1:** Receiver operating characteristic curve (ROC) for determining cut off value of urinary OSI and neopterin

oxidant status (TOS) and decreased urinary total anti-oxidant status (TAS) in women with IUGR, though these findings were not statistically significant. The immune system and inflammation play an important role in fetus tolerance and maintenance of normal pregnancy.<sup>16</sup> Stimulation of cellular and humoral immune response is involved in the development of various pathological conditions such as pre-eclampsia, spontaneous abortion, intrauterine growth restriction and pre-term delivery.<sup>17</sup> Maternal inflammatory response and oxidative stress are positively associated with the development of IUGR in pregnant women.<sup>[18,19]</sup> Altered immune response triggers the production of pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and thereby causes low grade inflammation.<sup>20</sup> Activated maternal inflammatory response further produces free radicals, causes elevation of various oxidative markers such as Malondialdehyde (MDA), lipid peroxides, etc and thereby leading to oxidative stress. Due to these processes, human placenta is susceptible to oxidative stress and oxidative damage in the early gestation which may contribute to the development of IUGR in pregnancy.

In our study urinary neopterin levels were higher in women with IUGR when compared to control women though it was not statistically significant between the two groups. Neopterin, is an early biomarker of natural immunity and produced by activated macrophages in response to inflammation.<sup>21</sup> Erkenekli et have demonstrated that serum neopterin levels were significantly elevated in pregnant women with fetal growth restriction and was associated with marked activation of the natural immune system. Increased neopterin levels also enhance the production of free radicals from the macrophages which indicates the immunological activation of oxidative stress. Consistent with these findings, we also found increase in another serum inflammatory marker, protein bound sialic acid in women with IUGR on comparison with pregnant women without IUGR. Alterations in the immune status and redox imbalance also enhance the release of sialic acid, from various glycoproteins.<sup>10,11</sup> From the above mentioned findings, the present study indicated the existence of altered redox imbalance and inflammatory response in pregnant women with IUGR.

## 5. Conclusion

Maternal inflammatory response and oxidative stress is positively associated with the development of IUGR in pregnant women. Detection of these oxidative stress and inflammatory markers in serum and urine could serve as screening tool for the early prediction and diagnosis of IUGR. Assessment of these parameters could be used as biomarkers to predict the extent of inflammation and redox imbalance in pregnant women with IUGR which will further

aid in the better management of the neonatal health.

## 6. Source of funding

None.

## 7. Conflict of interest

None.

## References

- Novac MV, Niculescu M, Manolea MM, Dijmărescu AL, Iliescu DG, et al. Placental findings in pregnancies complicated with IUGR - histopathological and immunohistochemical analysis. *Romanian J Morphol Embryol Rev Roum Morphol Embryol*. 2018;59:715–720.
- Garite TJ, Clark R, Thorp JA. Intrauterine growth restriction increases morbidity and mortality among premature neonates. *Am J Obstet Gynecol*. 2004;191:481–487.
- Peltier MR. Immunology of term and preterm labor. *Reprod Biol Endocrinol*. 2003;1.
- Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. *Int J Dev Biol*. 2014;58:219–229.
- Karowicz-Bilinska A, Kedziora-Kornatowska K, Bartosz G. Indices of oxidative stress in pregnancy with fetal growth restriction. *Free Radic Res*. 2007;41:870–873.
- Erkenekli K, Keskin U, Uysal B, Kurt YG, Sadir S, et al. Levels of neopterin and C-reactive protein in pregnant women with fetal growth restriction. *J Obstet Gynaecol J Inst Obstet Gynaecol*. 2015;35:225–228.
- Raghupathy R, Al-Azemi M, Azizieh F. Intrauterine Growth Restriction: Cytokine Profiles of Trophoblast Antigen-Stimulated Maternal Lymphocytes. *J Immunol Res*. 2011;.
- Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. *Curr Drug Metab*. 2002;3:175–187.
- Reibnegger G, Auhuber I, Fuchs D, Hausen A, Judmaier G, Prior C. Urinary neopterin levels in acute viral hepatitis. *Hepatol Baltim Md*. 1988;8:771–774.
- Taoka Y. Biochemistry and clinical significance of sialic acids. *Rinsho Byori*. 1983;54:2–25.
- Tewarson SL, Mittal VP, Singh M, Gupta GP. Serum sialic acid—an important cancer marker. *Indian J Cancer*. 1993;30:125–131.
- Aminoff D. Methods for the quantitative estimation of N-acetylneuraminic acid and their application to hydrolysates of sialomucoids. *Biochem J*. 1961;81:384–392.
- Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Anal Biochem*. 1996;239:70–76.
- Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38:1103–1111.
- Kressig P, Beinder E, Schweer H, Zimmermann R, Mandach UV. Post-delivery oxidative stress in women with preeclampsia or IUGR. *J Perinat Med*. 2008;36:310–315.
- Mor G, Cardenas I. The Immune System in Pregnancy: A Unique Complexity. *Am J Reprod Immunol N Y N*. 1989;63:425–433.
- Haedersdal S, Salvig JD, Aabye M, Thorball CW, Ruhwald M, et al. Inflammatory markers in the second trimester prior to clinical onset of preeclampsia, intrauterine growth restriction, and spontaneous preterm birth. *Inflammation*. 2013;36:907–913.
- Mert I, Oruc AS, Yuksel S, Cakar ES, Buyukkagnici U, et al. Role of oxidative stress in preeclampsia and intrauterine growth restriction. *J Obstet Gynaecol Res*. 2012;38:658–664.
- Tjoa ML, Vugt JV, Go A, Blankenstein MA, Oudejans C, et al. Elevated C-reactive protein levels during first trimester of pregnancy are indicative of preeclampsia and intrauterine growth restriction. *J Reprod Immunol*. 2003;59:29–37.
- Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, et al. Inflammation and Pregnancy. *Reprod Sci*. 2009;16:206–215.

21. Fuchs D, Weiss G, Wachter H. Neopterin, biochemistry and clinical use as a marker for cellular immune reactions. *Int Arch Allergy Immunol.* 1993;101:1–6.

### Author biography

**Nachimuthu Maithilikarpagaselvi** Assistant Professor

**Agrawal Shyamsundar Muskan** Student

**Palai Pallavee** Professor

**Murugaigaiyan Sathish Babu** Associate Professor

**Ramasamy sathiya** Tutor

**Ramalingam Sripradha** Tutor

**Cite this article:** Maithilikarpagaselvi N, Muskan AS, Pallavee P, Babu MS, sathiya R, Sripradha R. Evaluation of urinary oxidative stress markers, inflammatory sialoproteins and neopterin in pregnant women with intrauterine growth restriction. *Indian J Obstet Gynecol Res* 2020;7(1):54-58.