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RESEARCH ARTICLE

The Urinary Oxidative Stress Biomarkers in Preterm and Post-term Delivery

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Abstract

Objectives: this study aimed to investigate the correlation between urinary oxidative stress biomarkers with preterm and post-term pregnancy duration.
Methods: a case-control study design was employed to enroll 90 women, with 30 women were term (control group), 30 women were preterm (first case group) and 30 women were post-term (second case group). Urine samples were collected before admission to operation theatre. Age and BMI were taken for each woman. Urinary oxidative stress biomarkers (Malondialdehyde, MDA, Hexanoyl-Lysine Adduct, HEL, and dityrosine, DT) were estimated. Enzymatic antioxidant superoxide dismutase (SOD) was also measured in urine as well.
Results: the results showed that MDA, HEL and DT were significantly correlated with preterm but not post-term delivery. SOD level was significantly correlated with both preterm and post-term pregnancy.
Conclusions: Oxidative stress biomarkers may be an important contributor of premature birth. High levels of SOD may be implicated in the aetiology of post-term pregnancy. The causal relationship between oxidative stress biomarkers and gestational age may be further investigated by longitudinal studies.

INTRODUCTION

Oxidative Stress (OS) occurs when the production of free radicals surpasses the rate of their buffering by the cellular defence mechanisms.(1) Several studies have demonstrated that the access of OS may be associated with early pregnancy complications such as preterm labor, preeclampsia and premature rupture of membrane.(2) These free radicals cause cytotoxic damage to cellular proteins, lipids and DNA resulting in early pregnancy termination or complication. The cellular defense mechanisms include enzymatic and nonenzymatic antioxidant buffering systems which counteract their adverse effects.(3) While Ferguson et al (2015) suggests that the access of urinary OS biomarkers may be associated with preterm labor and pregnancy duration.(4) A study done by Kaya S et al (2013) showed that the decreased OS status might be associated with prolonged (post-term) pregnancy.(5)

Several OS biomarkers have been described as parameters to estimate OS level in urine. Malondialdehyde (MDA) and Hexanoyl-Lysine Adduct (HEL) have been widely used as indicators of oxidative damage of lipids caused by free radicals in both blood and urine.(6) Similarly, measuring Dityrosine (DT) is helpful as a specific biomarker for protein oxidation by reactive oxygen species.(6) On the other hand, superoxide dismutase (SOD) is one of the most important anti- oxidative enzymes that help to protect against cell injury caused by free radicals.(6)

However, there is little research which was done to investigate the association between OS biomarkers in urine and duration of pregnancy among Iraqi women. Therefore, the current study aimed to examine the association between urinary OS biomarkers and pregnancy duration among Iraqi women in an attempt to provide evidence about whether OS status can predict the pregnancy duration. The current study utilized MDA, HEL, DT and SOD as reliable indicators to measure OS level in urine.

Materials and Methods

Study Design and participants

A case-control study design was employed to guide the research hypotheses. Purposive sampling was performed to approach study sample. Thirty term delivering women were recruited as the study control, thirty preterm women were enrolled as the first case group, and thirty post-term women were selected as the second case group. All women enrolled in this study were selected from January to September 2014 from hospitals in Baghdad.. The sample size was estimated to achieve a statistical power of 0.80 (at $p<0.05$, 95% confidence interval, assuming R is equal to 3). We excluded women who had any of these conditions: hypertension, thyroid disease, diabetes mellitus, smoking, evidence of active infection, fever, chronic inflammatory diseases (including rheumatoid arthritis, joint pain, osteoarthritis, abdominal complain, inflammatory bowel disease); currently taking any medication, Cytomegalovirus (CMV) and toxoplasmosis infection. These conditions were excluded by gynaecologist. Permission from these women was sought before enrolling in this study.

Sampling and Methods

Before admission to operation theatre, urine samples were collected. Women were asked to collect 10 to 15 ml of urine. Women should not have voided for at least two hours before sample collection to increase the chance of detecting markers in urine. The weight and height of women were also recorded to measure the body mass index (BMI). Urine samples were used to measure oxidative stress biomarkers (MDA, HEL and DT) and enzymatic antioxidant activity (SOD). These were measured using laboratory kits for MDA (OxiSelect MDA Adduct, Canada), HEL (Hexanoyl-Lys adduct (HEL) ELISA Kit, Japan), DT (Dityrosine (DT) ELISA, Japan) and SOD (OxiSelect Superoxide Dismutase Activity Assay, Canada).

Statistical Analysis

Data were entered into SPSS statistical software (v. 22). Descriptive data analysis was first done to describe the participants demographic and biochemical criteria. The mean and Standard Error (SE) was used to describe the continuous variables. P value less than 0.05 was considered significant. Pearson correlation test was performed to examine the association between urinary OS biomarkers and gestational age. Analysis of Variance (ANOVA) was performed to assess the difference between study groups in urinary OS level. If the difference by ANOVA analysis is significant, independent sample t-test was performed to assess the difference in urinary OS between preterm and post-term women in comparison to term women as study control.

Results

Ninety (30 preterm, 30 term and 30 post-term) women were enrolled in this study. The mean age of study participants is 29.73 (SE: 0.54) years. The summary of biochemical criteria for study participants are summarized in Table-1. The results showed that study groups were not significantly different in age and BMI ($P > 0.05$). Data were presented as mean and standard error.

Table-1:The comparison between study groups in anthropometric and biochemical markers

Variables	Preterm(N=30) Mean ± S.E	Post-term (N=30) Mean ± S.E	Term (N=30) Mean ±S.E	P-value
Age (years)	28.23 ± 0.43	31.71 ± 0.63	29.31 ± 0.73	0.115
BMI (kg/m^2)	31.32 ± 0.33	28.71 ± 0.91	29.46 ± 0.43	0.111
MDA(pmol/l)	17.43± 0.71	9.97 ± 0.69	11.67 ± 0.87	<0.001*
DT (µmol/l)	8.87 ± 0.42	4.12 ± 0.40	4.62 ± 0.51	<0.001*
HEL (nmol/l)	97.97 ± 1.72	92.80 ± 1.22	93.83 ± 0.91	0.017*
SOD (nmol/l)	1.97 ± 0.19	3.17 ± 0.29	2.43 ± 0.11	<0.001*

* Significant at $p <0.05$. BMI: Body Mass Index; S.E.; standard error. MDA: Malondialdehyde; HEL: Hexanoyl-Lysine Adduct; DT: Dityrosine; SOD: Superoxide Dismutase

According to the results presented in Table-1, all OS biomarkers were significantly different between study groups. It showed that urinary MDA level was significantly different between preterm, post-term and term women (F= 26.44, df: 2, p< 0.001). Similarly, urinary DT levels were significantly different between study groups (F= 34.14, df: 2, p<0.001). Conversely, study groups were significantly different in HEL levels in urine (F= 4.25, df: 2, p= 0.017). Finally, the results from table 1 showed that the preterm, post-term and term women groups were significantly different in urinary SOD levels (F= 8.54, df: 2, p<0.001). For MDA, HEL and DT, the highest levels were recorded among preterm women while post-term women had the lowest levels. On the other hand, post-term women had the highest level of SOD while preterm women had the lowest level of SOD. Furthermore, independent samples t-test was performed to assess the statistical difference in urinary OS levels between preterm, post-term women groups in comparison to term women as study control. Table-2 depicts the difference in urinary OS biomarkers between preterm and term women groups.

Table-2: The difference between preterm and term women in urinary oxidative stress biomarkers

Oxidative stress biomarkers in urine	T value	P value
MDA (pmol/l)	-5.13	<0.001*
DT (µmol/l)	-6.43	<0.001*
HEL (nmol/l)	-2.45	0.017*
SOD (nmol/l)	3.51	0.001*

* Significant at P<0.05; MDA: Malondialdehyde; HEL: Hexanoyl-Lysine Adduct; DT: Dityrosine; SOD: Superoxide Dismutase

The results presented in Table-2 showed that all OS biomarkers were significantly different between preterm and term women groups. According to the t value, the direction of relationship between OS biomarkers (MDA, HEL and DT) was negatively associated with gestational age. On the other hand, the enzymatic antioxidant (SOD) was positively associated with gestational age. That's to say, the SOD level in urine seemed to increase with increasing gestational age. On the other hand, MDA, HEL and DT seemed to decrease with increasing gestational age. In addition, the difference between post-term and term women in urinary OS was examined utilizing t-test as well. Table-3 summarized the results.

Table-3: The difference between post-term and term women in urinary oxidative stress biomarkers

Oxidative stress biomarkers in urine	T value	P value
MDA (pmol/l)	-1.53	0.130
DT (µmol/l)	-0.777	0.440
HEL (nmol/l)	-0.681	0.499
SOD (nmol/l)	2.42	0.021*

* Significant at P<0.05; MDA: Malondialdehyde; HEL: Hexanoyl-Lysine Adduct; DT: Dityrosine; SOD: Superoxide Dismutase

The results showed that only SOD level in urine was significantly different between post-term and term women groups. According to t value, the direction of the relationship was negative between gestational age and MDA, HEL and DT. On the other hand, the direction of the relationship was positive for SOD in association with gestational age.

In order to examine whether the urinary OS biomarkers are associated with the duration of pregnancy, Pearson correlation analysis was conducted. The preterm and post-term study groups were compared to the term group as the control, (Table 4) summarizes the results of the correlation between OS level and pregnancy duration.

Table 4: The correlation between urinary OS biomarkers and gestational age

Biomarkers	Preterm group		Post-term group		Y value
	R	P	R	P	
MDA (pmol/l)	-0.585	<0.001**	-0.022	0.181	24.22+ -3.73*X
HEL (nmol/l)	-0.090	0.049*	-0.004	0.611	99.75+-1.35*X
DT (µmol/l)	-0.529	<0.001**	-0.080	0.070	12.99+ -2.37*X
SOD (nmol/l)	0.149	0.023*	0.188	0.015*	0.62 +0.79*X

* Significant at p<0.05, ** significant at p<0.001, MDA: Malondialdehyde; HEL: Hexanoyl-Lysine Adduct; DT: Dityrosine; SOD: Superoxide Dismutase

The result presented in table 4 showed that MDA level in urine is significantly associated with gestational age in preterm women group only. The trend of the relationship between urinary MDA levels in both preterm and post-term groups and gestational age was negative. In other words, the increase in MDA level in urine was associated with decrease the possibility of term pregnancy. Figure 1 (in the appendix) depicts the correlation between urinary MDA level and gestational age. Similarly, the result showed that HEL level in urine is significantly correlated with gestational age in preterm women group only. The direction of the relationship between HEL level and gestational age was also negative. That's to say; HEL exhibited a similar pattern of correlation to gestational age as MDA. Figure 2 (in the appendix) shows the correlation between HEL level and gestational age. It seems that DT had a similar pattern of relationship to other OS biomarkers, with significant correlation with preterm women group only (see figure 3 in the appendix). On the other hand, SOD showed a significant correlation with both preterm and post-term women groups. The direction of the relationship between SOD level and pregnancy duration was positive. SOD levels constantly increased with increasing gestational age (figure 4 in the appendix).

Appendix

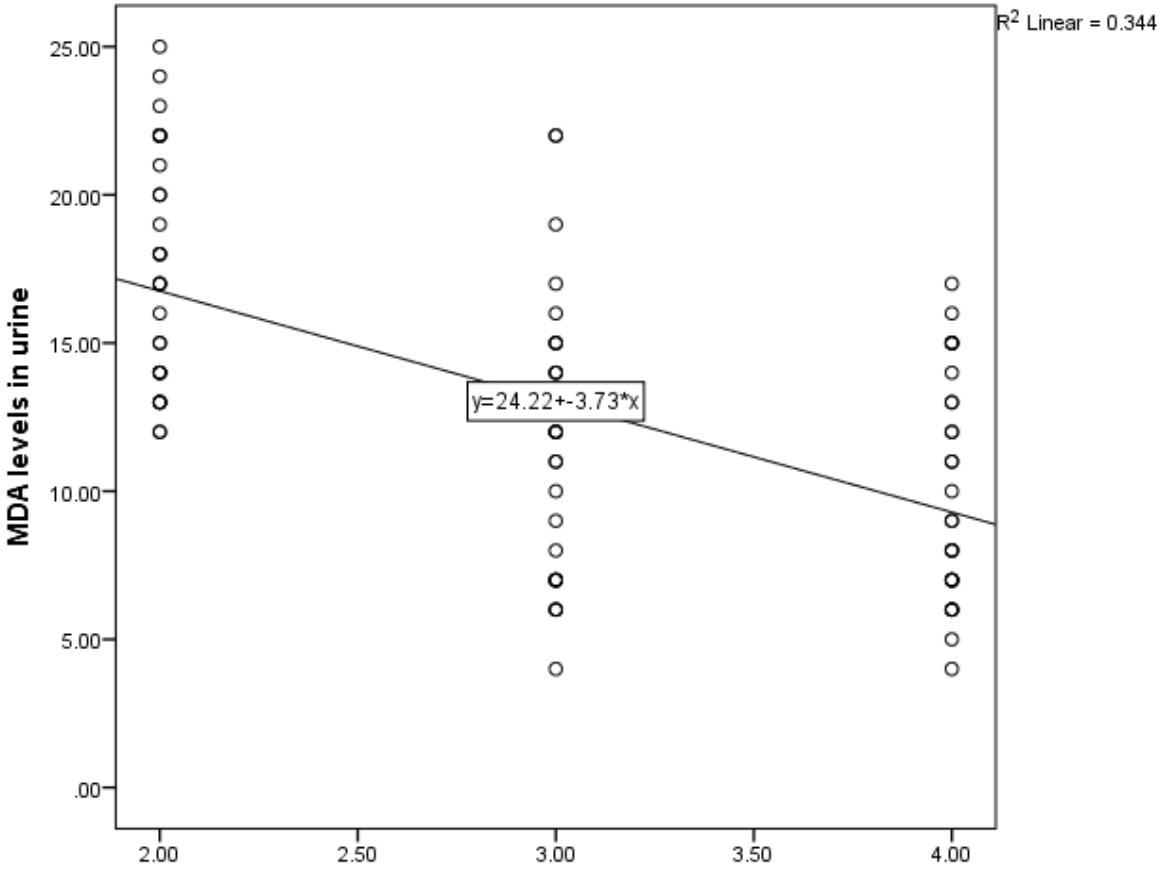

Figure 1: the correlation between MDA level in urine and gestational age.

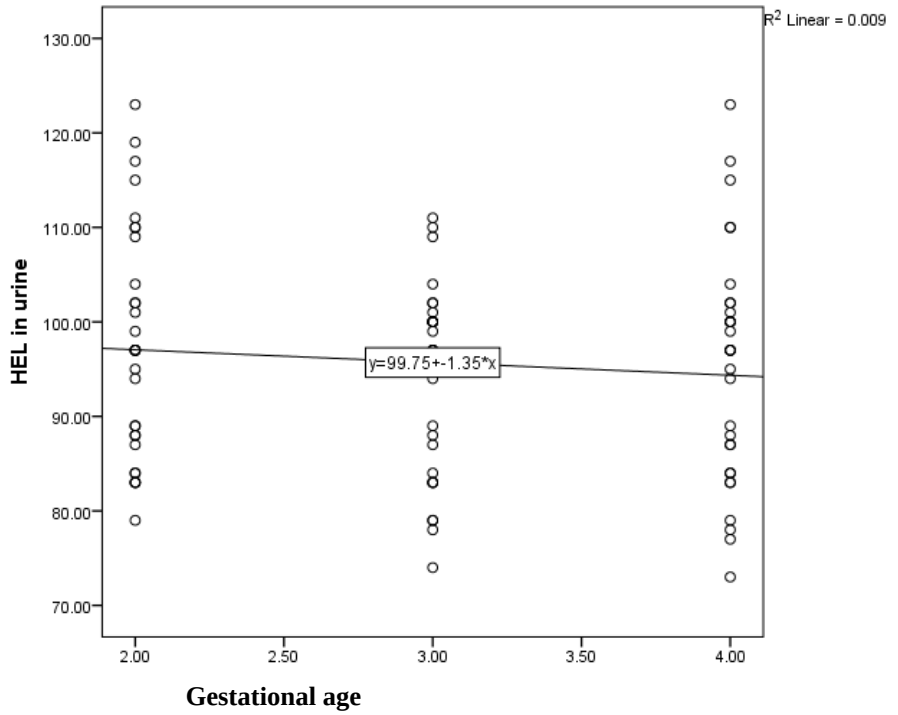

Figure 2: The correlation between HEL level in urine and gestational age

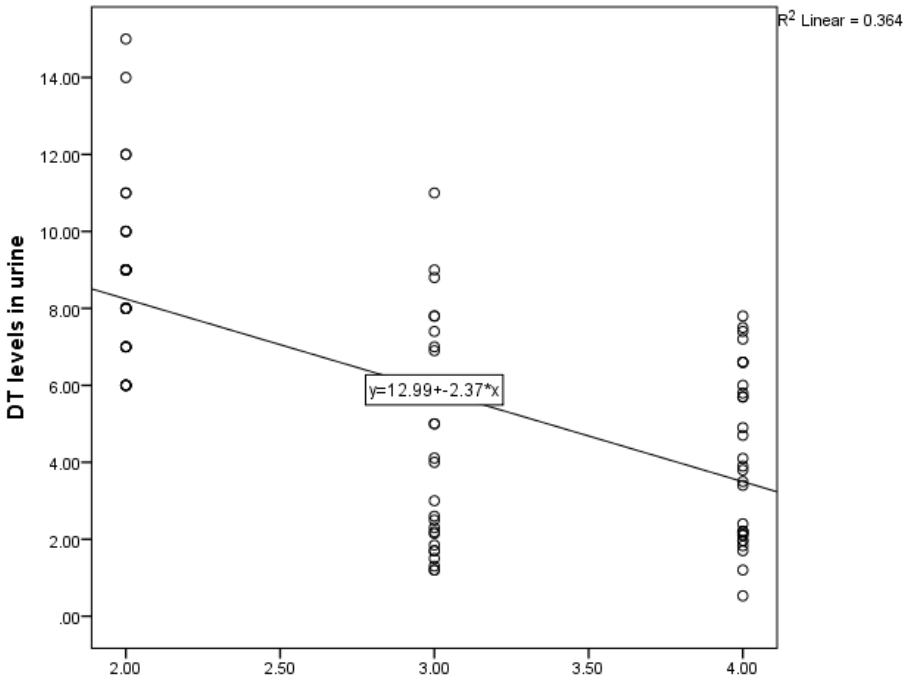

Figure 3: The correlation between DT level in urine and gestational age

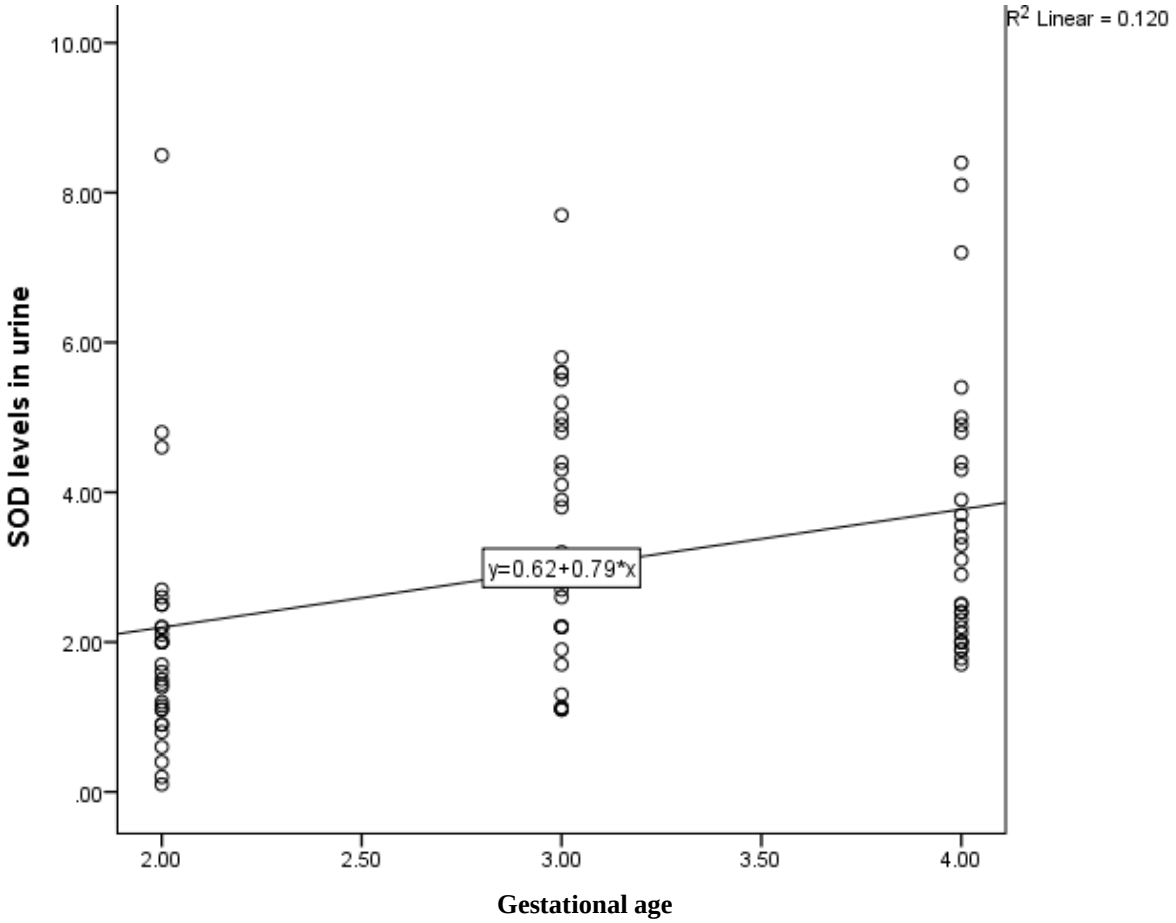

Figure 4: The correlation between SOD level in urine and gestational age

Discussion

With exception of SOD, the results of the present study showed that urinary OS biomarkers are significantly different between preterm and term women groups only. The results also showed that urinary MDA, HEL and DT are negatively associated with duration of pregnancy. On the other hand, SOD was positively associated with pregnancy duration. The findings of the present study are consistent with research done in different settings. Ferguson et al (2015) reported that OS level is associated with preterm labor among women in the United States.[4] Similarly, Dani et al (2004) described the relationship between high OS level and increased risk of preterm delivery.[7] Evidence examined the total oxidant status in amniotic fluid of late preterm infants and confirmed the association between higher OS level and premature birth.[8] Despite the fact that the mechanism of the effect of OS on pregnancy and subsequent preterm delivery is still unclear, Menon R. (2014) hypothesized that OS induces damage to intrauterine tissues especially fetal membrane of the placenta.[9] This damage affects all fetal cellular elements resulting in fetal cell aging. Aging cells generate biomolecular signals which are uterotonic, signaling the labor process. This may also be supported by the results of the present study which reported the highest OS levels among women with preterm labor. Also, the present study reported that women with preterm delivery had the lowest levels of the enzymatic antioxidant activity (SOD). This is consistent with previous study by Cinkaya et al (2010) which reported low levels of total antioxidant status among women with preterm labor.[10] Therefore, antioxidant therapy to re-enforce the total antioxidant status has been proposed as a method to prevent preterm labor.

In addition, the present study showed that the urinary OS levels were not associated with post-term delivery. Despite the fact that OS levels were not significantly different in women with post-term delivery, the results of the present study showed that post-term women had non-significantly lower OS levels as compared to term women. In the medical literature, data about the relationship between OS level and post-term delivery is very scarce. However, one evidence reported that OS levels are not changed in post-term pregnancy.[5] As for the enzymatic antioxidant status, our study reported that SOD levels have significantly increased among women with

post-term delivery. This is consistent with much research that reported higher total antioxidant status among women with post date delivery. Mueller et al (2005) reported enormously high antioxidant activity during post-term pregnancy.[11] Similarly, Leal et al (2011) described a constant increase in SOD levels while pregnancy progresses. The high level of antioxidant status may protect against cellular damage caused by OS and slow down the fetal tissue aging process that may explain the prolonged pregnancy.[12]

Conclusions and Recommendations

High levels of urinary OS biomarkers may be an important contributor to preterm labor. The low level of enzymatic antioxidant activity may be another reason behind premature delivery. The High level of SOD may influence the duration of pregnancy resulting in post-date delivery. A longitudinal study design with repeated measures of OS during pregnancy is recommended to ascertain the relationship between OS and pregnancy duration.

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