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# A comparative study of Serum Malondialdehyde and Hexanoyl-Lysine Adduct in Preterm and Post-term Deliveries

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## Abstract

**Background** High oxidative stress reflects the state when the production of reactive oxygen species exceeds their removal. Malondialdehyde (MDA )and Hexanoyl-Lysine Adduct (HEL) are indicators of oxidative damage of lipids caused by free radicals in blood. The high level of these biomarkers has been implicated in early pregnancy complications.

**Objectives** This study aimed to investigate the correlation between serum oxidative stress biomarkers in preterm and post-term deliveries.

**Methods** A case-control study was designed to recruit 90 pregnant women with 30 women delivering at term (control group), 30 women at preterm (first case group) and 30 women at post-term (second case group). All women underwent elective cesarean section. Blood samples were collected before admission to operation theatre. Women's age, body mass index, lipid profile, renal function test and random glucose were measured. Serum oxidative stress biomarkers (Malondialdehyde and Hexanoyl-Lysine Adduct) were measured as indicators of lipid peroxidation.

**Results** The results showed that study groups were significantly different in serum MDA and HEL ($P \leq 0.01$). Both serum MDA and HEL levels were significantly higher in preterm women group and their level steadily decreased as pregnancy progressed. Serum MDA was not significantly different between term and post-term women groups (P>0.05).

**Conclusions** Oxidative stress biomarkers may be important contributors of premature birth. Low level of serum HEL may play a role in delayed onset of labor. The causal relationship between oxidative stress biomarkers and pregnancy outcome may be further investigated by longitudinal studies.

**Key words** Oxidative Stress, Preterm women, Post-term women, Malondialdehyde, Hexanoyl-Lysine Adduct.

**List of Abbreviation:** MDA = Malondialdehyde, HEL = Hexanoyl-Lysine Adduct, OS = Oxidative stress, ROS = Reactive oxygen species, BMI = body mass index, TG = triglyceride, VLDL-C = Very Low Density Lipoprotein Cholesterol (VLDL-C), HDL-C = High Density Lipoprotein Cholesterol (HDL-C), LDL-C = low Density Lipoprotein cholesterol.

## Introduction

High Oxidative Stress (OS) takes place when the production of reactive oxygen species (ROS) exceeds the physiological level in blood and soft tissue [(1)]. These ROS may cause tissue injury resulting in cytotoxic damage to cellular proteins, lipids and DNA which has been implicated in early pregnancy complications such as preterm labor, preeclampsia and ante partum hemorrhage [(2)]. Body defense response involves enzymatic and non-enzymatic antioxidant buffering pathways which reduce the effect of these free radicals [(3)]. Previous studies have shown that increasing level of OS biomarkers in blood may lead to preterm labor [(4)]. Other studies showed that the administration of antioxidant vitamins during pregnancy was associated with decreased incidence of spontaneous preterm labor with a positive dose-response across all groups [5]. However, the oxidative pathways linking low antioxidants level in serum to preterm labor and premature rupture of membrane have not been fully explained, but such a relationship have been hypothesized by several investigators [6,7].

Several OS biomarkers have been described as indicators to measure OS level in serum. Malondialdehyde (MDA) and Hexanoyl-Lysine Adduct (HEL) have been widely used as estimates of oxidative damage of lipids caused by free radicals in blood [8]. MDA has been confirmed as one of the advanced lipid peroxidation products. Another study was also conducted among Chinese women showed that pregnancy induced hypertension may be a predisposing factor for increasing the oxidative stress biomarker, MDA, which may eventually lead to premature labor [9]. Similarly, HEL is an important biomarker for initial stage of lipid peroxidation [8]. HEL is formed by oxidative modification of oxidized omega-6 fatty acids such as linoleic acid or arachidonic acid [8].

However, the present research has been done to examine the association between OS level and pregnancy outcome among Iraqi women. It intended to assess the association between OS level in serum and the type of delivery (Preterm, term or post-term) among Iraqi women. The purpose of this paper is to provide evidence reflecting the influence of OS level and whether it can predict pregnancy duration.

## Methods

### *Study Design and participants*

A case-control study design was used in this study. Ninety pregnant women were recruited (30 term women were recruited as the study control, 30 preterm women as the first case group, and 30 post-term women as the second case group). Women were approached from hospitals in Baghdad (Al-Hakeem and Al-Imamain Al-Kadhimain Medical City) during the period from 12th of January to 19th of September 2014. 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). The exclusion involved women with the following criteria: 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 and toxoplasmosis infection. These conditions were excluded by gynecologist. The ethical approval was obtained for this study from Al Nahrain University and permission was sought from these women before enrolling in this study.

### *Sampling and Methods*

Prior to admission of enrolled women to operation theatre, blood samples were collected. These women were not in fasting state. Ten millimeters of venous blood had been withdrawn and left to clot in a tube, centrifuged for 10 minutes at 3000 rpm to collect serum. These samples were used to measure serum lipids, random glucose, urea and creatinine to affirm the biochemical similarity between study groups and to exclude women who had abnormal biochemical measures. The weight and height of women were also measured to estimate the body mass index (BMI). Serum was used to determine glucose, lipid profile including total cholesterol, triglyceride (TG), very low density lipoprotein cholesterol (VLDL-C), and high density lipoprotein cholesterol (HDL-C) [measured by the precipitation of chylomicrons] done using colorimetric enzymatic method. Low density lipoprotein cholesterol (LDL-C) was calculated by the formula of Friedewald *et al.* 1972 [10]. Blood samples were also used to measure MDA and HEL levels by OxiSelect MDA Adduct ELISA Kit (CELL BIOLABS, Inc, Canada) and Hexanoyl-Lys adduct (HEL) ELISA Kit (Japan Institute for the Control of Aging JICA).

### *Statistical Analysis*

Data were encoded and entered into SPSS statistical software (v. 22). Descriptive analysis of biochemical and anthropometric characteristics of study sample was first presented. The mean and standard error (SE) were presented to describe study variables. The difference between study groups in anthropometric and biochemical criteria was assessed utilizing Analysis of Variance (ANOVA) test.

Pearson correlation test was performed to examine the correlation between OS level and types of pregnancy. ANOVA analysis was also performed to assess the difference between study groups in OS levels. If significant, independent samples t-test was performed to assess the statistical difference between preterm and post-term women groups (as case groups) and term women group (as study control). Alpha index (p value) of less than 0.05 was considered significant.

## Results

The present study enrolled 90 women (30 pre-term, 30 post-term and 30 term). The mean age of study participants was 29.73 ±SE: 0.54 years. The summary of biochemical criteria for study participants is summarized in Table 1. The results from ANOVA analysis showed that study groups were not significantly different in age, BMI, lipids, random glucose, urea and creatinine (P > 0.05). Data were presented as mean and standard error.

**Table 1. Anthropometric and biochemical criteria of the studied groups**

| 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 |
| Total Cholesterol (mg/dl) | 184.03 ± 1.32 | 178.07 ± 0.79 | 179.80 ± 0.72 | 0.091 |
| HDL-C (mg/dl) | 67 ± 0.96 | 65.60 ± 0.81 | 65.93± 1.92 | 0.738 |
| LDL-C (mg/dl) | 89.73 ± 2.31 | 86.63 ± 1.11 | 87.13 ± 1.73 | 0.421 |
| Triglyceride (mg/dl) | 136.70 ± 3.77 | 129.16 ± 2.12 | 133.77 ± 6.51 | 0.495 |
| LDL-C/HDL-C ratio | 1.39 ± 0.03 | 1.38 ± 0.04 | 1.31 ± 0.03 | 0.124 |
| TC/HDL-C ratio | 2.76 ± 0.04 | 2.73 ± 0.03 | 2.79 ± 0.08 | 0.529 |
| Random Serum glucose (mg/dl) | 143.60 ± 2.12 | 146.83 ± 2.01 | 151.3 ± 1.92 | 0.101 |
| Urea (mg/dl) | 17.22 ± 0.93 | 19.10 ± 0.49 | 17.52 ± 1.11 | 0.274 |
| Creatinine (mg/dl) | 0.59± 0.007 | 0.67 ± 0.003 | 0.64 ± 0.003 | 0.071 |

BMI = Body Mass Index; HDL-C = High Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol, S.E. = standard error.

***Oxidative stress biomarkers (MDA and HEL) and pregnancy types***

ANOVA analysis was utilized to assess the difference between study groups in serum MDA and HEL. Data were expressed as mean ± (SE). Table 2 depicts the analysis results.

**Table 2. Comparison between study groups in oxidative stress biomarkers in blood**

| Oxidative stress biomarkers | Preterm (N=30) Mean ± S.E | Post-term (N=30) Mean ± S.E | Term (N=30) Mean ± S.E | *P* value |
|---|---|---|---|---|
| **MDA (pmol/l)** | 62.37 ± 2.99 | 49.90 ± 3.60 | 51.70 ± 2.52 | 0.010* |
| **HEL (nmol/l)** | 373.13 ± 4.95 | 184.37 ± 3.88 | 297.10 ± 5.76 | <0.001* |

* = *P* < 0.05, MDA = Malondialdehyde, HEL = Hexanoyl-Lysine Adduct

As for the results presented in table 3, pre-term, post-term and term women groups were significantly different in both MDA (F= 8.11, df: 2, *P* = 0.01) and HEL (F= 24.11, df: 2, *P* < 0.001). Both OS biomarkers levels were the highest among preterm women, and the lowest among post-term women. After affirming the difference between study groups in OS levels, independent samples t-test was performed to examine the difference in serum MDA and HEL levels between preterm and post-term women groups in comparison to term women.

**Table 3. The difference in oxidative stress biomarkers in blood between preterm, post-term in comparison to term women**

| Oxidative stress biomarkers in blood | Preterm | | Post-term | |
|---|---|---|---|---|
| | T value | P value | T value | P value |
| **MDA (pmol/l)** | -2.73 | 0.008* | -0.41 | 0.684 |
| **HEL (nmol/l)** | -10.01 | <0.001* | -16.24 | <0.001* |

* = p<0.05, MDA = Malondialdehyde, HEL = Hexanoyl-Lysine Adduct.

The results showed that the preterm and term women groups were significantly different in serum OS biomarkers (t: -2.73, *P* = 0.008 and t: -10.01, *P* < 0.001 for MDA and HEL respectively). Moreover, the direction of the relationship was also examined by interpreting the t value for the difference between preterm and term women groups. The relationship between pregnancy types and both MDA and HEL seems to be negative. In other words, preterm pregnancy was associated with higher levels of both MDA and HEL, which seemed to decrease with increasing gestational age.

Similarly, the difference between post-term and term women groups was also examined. The results showed a significant difference in HEL between post- term and term women (t: -16.24, *P* < 0.001). By examining the t value, the direction of the relationship showed that both MDA and HEL were negatively associated with the gestational age.

***Association between OS biomarkers and pregnancy types***

Pearson correlation analysis was performed to assess the association between serum MDA and HEL levels and pregnancy types. Both preterm and post-term groups were compared to term women group as the study control.

According to the results, MDA level in blood was significantly correlated with gestational age in preterm women group only. While serum HEL was significantly associated with gestational age in both preterm and post-term women groups. The results also confirmed the negative direction of the relationship between serum OS and pregnancy type. The relationship between OS biomarkers and pregnancy is depicted in fig. 1 and 2.

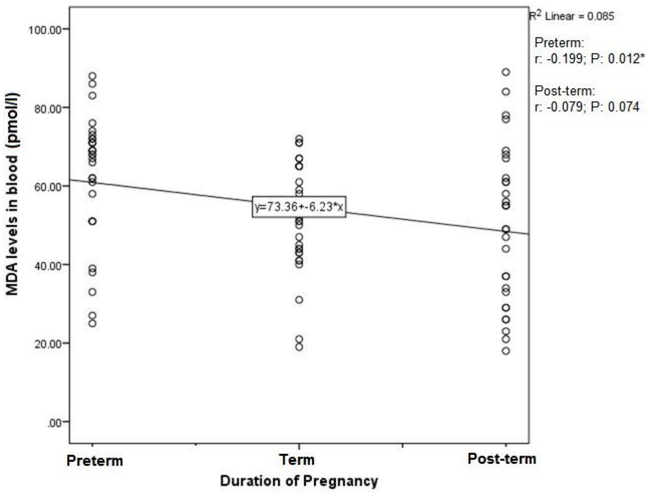


**Fig. 1. The correlation between MDA level in blood and pregnancy duration**

## Discussion

The results of the present study showed that OS biomarkers were negatively correlated with duration of pregnancy among preterm women group, but the only significant correlation was that of HEL in the post-term women. The findings of our results are consistent with previous studies that ascertained similar relationship in different settings. Kasat et al. (2013) reported a higher level of serum MDA among preterm women as compared to term women group [11]. This is further evidenced by Cinkaya et al. (2010) who reported lower total antioxidant status among preterm delivering women as compared to term women group [12]. The high MDA level was also implicated in neonatal complications.

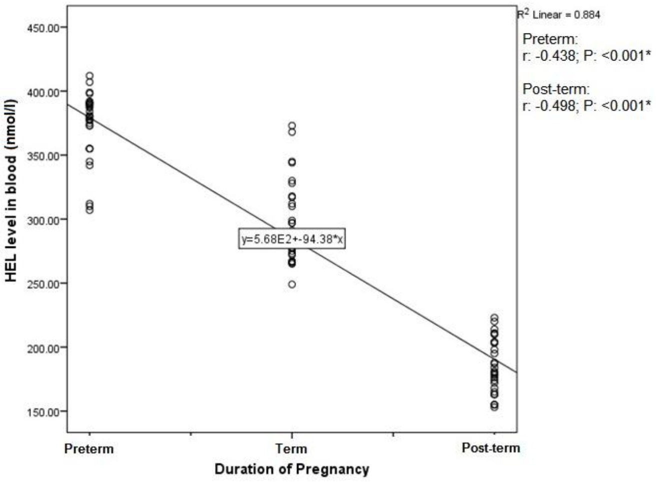


**Fig. 2. The correlation between HEL level in blood and pregnancy duration**

One study conducted in Tikrit University showed that serum level of MDA is correlated with low birth weight and having neonate complications such as respiratory distress syndrome [13]. Although the mechanism through which high OS levels may affect pregnancy outcome is still unclear, Menson (2014) suggested that high OS level has damaging effect on the intrauterine tissues particularly the fetal membrane of the placenta which may result in fetal cell aging [14]. Aging cells form uterotonic biomolecular signals enhancing and promoting the labor process [14]. This is also confirmed by the results of the current study which showed that preterm women had significantly higher levels of serum MDA and HEL in comparison to term and post-term women.

On the other hand, the present study reported that only serum HEL was inversely associated with post-term pregnancy. This was also supported by evidence that showed the decreasing level of OS markers in post-term pregnancy may play a role in delaying the onset of labor [15]. This may also confirm Menson's theory about the effect of OS on fetal tissue aging which triggers early premature labor [14].

At last, it could be said that high serum MDA and HEL levels may be an important contributor to the pathophysiology of premature labor. The OS levels were the highest among preterm women and lowest among post-term women. Further investigation of the causal relationship between OS and pregnancy duration is recommended. The effect of anti-oxidants as a mean to reduce the risk of premature labor may also be evaluated.

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## Author Contribution

Zeena Conception and design, data collection, analysis, interpretation, writing and revision of the manuscript were performed; Dr. Risala help in sampling and Dr. Rayah and Dr. May supervise this paper as part from a thesis.

## Conflict of Interest

**None**

## Funding

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## References

1. Burton GJ, Jauniaux E. Oxidative stress, Best Practice and Research. Clin Obstet Gynaecol. 2011; 25: 287-99.

2. Buhimschi IA, Buhimschi CS, Pupkin M, et al. Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human fetus. Am J Obstet Gynecol. 2003; 189: 181-8.
3. Knuppel RA, Hassan MI, McDermott JJ, et al. Oxidative Stress and Antioxidants: Preterm Birth and Preterm Infants, Preterm Birth - Mother and Child. Am J Biochem. 2012; 3: 136-9.
4. Clinton SK. Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev. 1998; 56: 35-51.
5. Kramer MS, Kahn SR, Platt RW, et al. Antioxidant vitamins, long-chain fatty acids, and spontaneous preterm birth. Epidemiology. 2009; 20: 707-13.
6. Luo ZC, Fraser WD, Julien P, et al. Tracing the origins of "fetal origins" of adult diseases: programming by oxidative stress? Med Hypotheses. 2006; 66: 38-44
7. Masters ET, Jedrychowski W, Schleicher RL, et al. Relation between prenatal lipid-soluble micronutrient status, environmental pollutant exposure, and birth outcomes. Am J Clin Nutr. 2007; 86: 1139-45.
8. Kato Y, James EF, Thompson JF, et al. Physiological oxidative stress and understanding its effect in human disease. Biochem Biophys Res Commun. 2009; 390: 547-51.
9. Zhou JF, Wang XY, Shangguan XJ, et al. Increase oxidative stress in women with pregnancy-induced hypertension. Biomed Environ Sci. 2005; 18: 419-26.
10. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem.1972; 18: 499-502.
11. Kasat S, Subhashchandrappa M, Mallikarjun CR, et al. Malondialdehyde, Total antioxidant capacity and vitamin E levels in preterm and term infants. Soushrutam. 2013; 1: 123-27.
12. Cinkaya A, Keskin HL, Buyukkagnici U, et al. Maternal plasma total antioxidant status in preterm labor. J Obstet Gynaecol Res. 2010; 36: 1185-8.
13. Al-Anee A, Shihab S, Edan HH, et al. Evaluation of serum malondialdehyde in relation to other clinical considerations in premature neonates. Tikrit Med J. 2009; 15: 42-51.
14. Menson R. Oxidative stress damage as a detrimental factor in preterm birth pathology. Front Immunol. 2014; 134: 281-4.
15. Kaya S, Keskin HL, Kaya B, et al. Reduced total antioxidant status in postterm pregnancies. Hippokratia, 2013; 17: 55-9.



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