Evaluation of serum malondialdehyde in relation to other clinical considerations in premature neonates.


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Abstract
In a pioneer study conducted in Iraq, the serum levels of malondialdehyde were tested to evaluate their role in premature neonates. The study was conducted in Tikrit Teaching Hospital during the period from the 1st of February 2006 till the 1st of July 2007. The study had been done on 300 neonates; they were divided into 4 groups, and these includes 100 apparently healthy normal birth weight mature neonates (NBW), 100 apparently healthy low birth weight premature neonates (LBW), 50 low birth weight premature neonates with birth asphyxia (LBW+BA), and 50 low birth weight premature neonates with respiratory distress syndrome (LBW+RDS). The malondialdehyde (MDA) level was measured in sera of the studied neonates and the control groups. The results revealed that: there is a significant statistical difference between the NBW and the LBW neonates regarding the birth weight and the gestational age while there is no significant difference concerning the age of the newborns. Also there is a significant statistical difference between the LBW neonates and the LBW+BA and LBW+RDS neonates regarding the gestational age, age, and birth weight. It was revealed that the mean serum level of MDA is higher in LBW in comparison with the NBW, which is statistically significant at a P value of less than 0.001. The mean serum level of MDA in LBW+BA is significantly (P < 0.001) higher than the mean values in LBW and NBW neonates. As well as the LBW+RDS has a mean serum level of MDA, which is significantly (P < 0.001) higher than the mean level in LBW and NBW neonates. It is also evident that the mean serum level of MDA in LBW+BA is significantly (P < 0.01) higher than the mean value in LBW+RDS. The correlation between serum MDA levels and birth weight, have a strong positive correlation (r = +0.31, significant at the 0.01 level) in NBW neonates. There is a strong positive correlation (r = +0.32, significant at the 0.01 level) between serum MDA levels and the birth weight in LBW+BA. In conclusion; The mean serum MDA level in LBW was significantly higher than that in NBW neonates.

Introduction
Live born infants delivered before 37 completed weeks from the 1st day of the last menstrual period are termed premature by the World Health Organization (WHO). (1)

Infants weighting less than 2500 g at birth is considered as “low birth weight” (2) Many of the infants are small because they are born before their time, others have an abnormally low birth weight for their gestational age. (3)

Normal birth weight (NBW) newborn is a newborn with birth weight ranging from 2.5 to 4.6 kg; boys are slightly heavier than girls. (4)

Birth asphyxia (BA) (or hypoxia ischemia encephalopathy) is a multisystem disease results from neonatal asphyxia or ischemia or both that results from different reasons weather it prenatal, natal and postnatal . birth asphyxia affect each organ in the newborns like the brain, heart, lungs , blood , bones , liver and kidneys. (3)

Respiratory distress syndrome (RDS) is a disease that results from immaturity of lungs due to the deficiency in the lung surfactant. It is usually affect preterm newborn although it is affect rarely the mature ones. The deficiency in surfactant leads to decrease in the lung expansion which leads to poor oxygenation of blood lads to ventilation-perfusion mismatch which results in tissue hypoxia. (4)
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Malondialdehyde is an aldehyde \(^{(5)}\) considered to be the terminal compound and the most important marker for monitoring lipid peroxidation and oxidative damage induced by reactive oxygen species (ROS)\(^{(6,7)}\) which is strongly associated with the development of serious diseases in low birth weight neonates. \(^{(6)}\) It is also considered as a thiobarbituric acid reactive substance. \(^{(8)}\)

Oxidative stress is usually assessed by using 2 markers of lipid oxidation (MDA and lipid hydroperoxides) and 2 markers of protein oxidation (carbonylated total proteins and carbonylated hemoglobin). \(^{(7)}\)

Methods of MDA measurements: \(^{(8)}\)

a) Method A: A spectrophotometric measurement of thiobarbituric acid - reactive substances (TBARS) in the TCA-supernatant of plasma.
b) Method B: A fluorescence measurement of plasma lipid peroxides.
c) Method C: HPLC/532 nm.
d) Method D: HPLC/fluorescence.

Thiobarbituric acid is usually reacting with other compounds that are present in the serum to form colored species that can interfere with the assay of MDA, but little is known about these interfering species. Also the presence of barbituric acid impurities in the thiobarbituric acid reagent is found to produce a product that absorb light at 490 and 513 nm, indicating that thiobarbituric acid should be purified before use. \(^{(9)}\)

Malondialdehyde has the following abbreviated structure: \(\text{C}_3\text{H}_4\text{O}_2.\) \(^{(5)}\)

![Malondialdehyde Structure](image)

Figure (1-3): The structure of Malondialdehyde. \(^{(5)}\)

The aim of the study is to determine the serum level of malondialdehyde in newborn infants.

**Materials & Methods**

The study was conducted in Tikrit Teaching Hospital during the period from the 1st of February 2006 till the 1st of July 2007.

Around 2000 newborns of both sexes, with different presentations in Tikrit Teaching Hospital were checked for their age, gestational age, and their birth weights. Only 300 of them were included in this study. Neonates included in this study were of comparable ages. Neonates included in this study were collected from: the Neonatal intensive care unit, Delivery room, Operating theater and Out-patient clinic.

The birth weight of each newborn was measured twice at the same time by using 2 digital baby scales (with subtracting 2 g from the weight of each neonate: the weight of the umbilical clamp) present at the neonatal care unit soon after delivery of the newborn.

Hundred out of the total low birth weights were found to be diseased newborns; 50 of them with birth asphyxia and 50 with respiratory distress syndrome, their weights were less than 2500 g who were considered as a diseased group in this study.

As a part of each newborn medical history, assessment of gestational age by using Dubowitz scoring system, \(^{(10)}\) sex of the newborn, age of the newborn, whether the mother is hypertensive and/or diabetic was also considered, and full physical examination of each newborn to find whether the newborn was healthy or diseased.

Three milliliters of umbilical venous blood sample were obtained by using an umbilical catheter from each neonate included in this study soon after the physical examination and before any feeding started. Each blood sample was
collected in a plain tube for the estimation of serum malondialdehyde.

Controls:
Two groups were considered to be as control, the first one composed of 100 apparently healthy NBW, and the second group was also composed of 100 apparently healthy LBW neonates.

The same methods and instruments were used in all stages for the apparently healthy control newborns as for the diseased group. A complete history, clinical examination, birth weight, gestational age, and their sex were also considered.

Determination of Serum Malondialdehyde:
A- Principle:
Malondialdehyde in serum was separated and determined as conjugate with TBA. Serum proteins were precipitated by TCA and then removed by centrifugation. The MDA-TBA complex was measured at 534 nm. (11)

B- Reagents:
Reagent-1: (Trichloroacetic acid 17.5%): Seventeen and a half grams of TCA were dissolved in 100 ml of distilled water, the reagent was kept into a sealed glass bottle to prevent evaporation.

Reagent-2: (Trichloroacetic acid 70%): Seventy grams of TCA were dissolved in 100 ml of distilled water, the reagent was kept into a sealed glass bottle to prevent evaporation.

Reagent-3: (Thiobarbituric acid 0.6%): Six hundreds milligrams of TBA was dissolved in 100 ml of distilled water, the reagent was kept into a sealed glass bottle to prevent evaporation.

C- Procedure:
The reaction was performed in 18 x 150 mm Pyrex test tube labeled as test and blank, into which the following reagents were pipette as follow:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1 ml</td>
<td>—</td>
</tr>
<tr>
<td>D.W</td>
<td>—</td>
<td>1 ml</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

The tubes were mixed well and incubated in boiling water bath for 15 min., allowed to cool, then the tubes were let to stand at room temperature for 20 min. Then the tubes centrifuged at 2000 rpm for 15 min., and then the supernatant layer was read at 534 nm.

D- Calculation:
The concentration of MDA (nmol/ml) was calculated by using the following formula:

Concentration of MDA (nmol/ml) = \( \frac{\text{Abs}_{\text{test}} - \text{Abs}_{\text{blank}}}{1.56 \times 10^5} \)

1.56 x 10^5 is the molar extinction coefficient

Results

The results of the present study include the followings:

Physical Characteristics of the Newborns:

The general physical characteristics of the diseased and the apparently healthy neonates included in this study are shown in the table (1). The data obtained shows that there is a significant statistical difference between the NBW and the LBW neonates regarding the birth weight and the gestational age at a P value of less than 0.001, while there is no significant difference concerning the age of the newborns. Also there is a significant statistical difference (P value
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< 0.001) between the LBW neonates and the LBW+BA and LBW+RDS neonates regarding the gestational age, age, and birth weight. The table also reveals that there is no statistical difference between the LBW+BA and the LBW+RDS regarding the gestational age, age, and the birth weight.

Serum Malondialdehyde (MDA):

Table (2) illustrates the mean ± SD, range, standard error of the mean, t-test, and the probability of the serum level of MDA (mg/dl) in the studied neonates. This table reveals that the mean serum level of MDA is higher in LBW in comparison with the NBW, which is statistically significant at a P value of less than 0.001. The mean serum level of MDA in LBW+BA is significantly (P < 0.001) higher than the mean values in LBW and NBW neonates. As well as the LBW+RDS has a mean serum level of MDA, which is significantly (P < 0.001) higher than the mean level in LBW and NBW neonates. It is also evident that the mean serum level of MDA in LBW+BA is significantly (P < 0.01) higher than the mean value in LBW+RDS.

Figure (1) illustrates the correlation between serum MDA levels and birth weight, which is a strong positive correlation (r = +0.31, significant at the 0.01 level) in NBW neonates.

There is a strong positive correlation (r = +0.32, significant at the 0.01 level) between serum MDA levels and the birth weight in LBW+BA, as demonstrated in figure 2.

The predictive values of serum MDA levels in LBW+BA and LBW+RDS as compared to NBW neonates were done by using cut-off value of 0.69 nmol/ml. Sensitivity, specificity, positive predictability, negative predictability, and efficiency test of serum MDA are demonstrated in table 3.

Discussion

The neonatal period is a highly vulnerable time for an infant, who is completing many of the physiologic and biochemical adjustments required for extra uterine existence. The initial examination of a newborn infant should be performed as soon as possible after delivery to detect abnormalities and to establish a baseline for subsequent examination.

In studies on newborns, it is necessary to know the birth weight to decide whether the neonate is a normal or a low birth weight because most of the biochemical parameters differ according to maturity and the birth weight. Therefore, each newborn included in this study was assessed carefully for the body weight at birth. The assessments of the birth weight were performed by using 2 digital baby scales in order to avoid the instrumental errors.

Low birth weight neonates are either preterm or small for gestational age newborns. The problems for each group were differing because of the difference in the maturity of organs function and structure.

Diseased new born (weather newborn with birth asphyxia or RDS) have some degree of hypoxia depends on the severity of the disease which may affect the oxygenation of tissue cells which may affect the level of MDA in these patients as compared with normal newborns weather they were normal or low birth weight.

The data obtained shows that there is a significant statistical difference between the NBW and the LBW neonates regarding the birth weight and the gestational age at a P value of less than 0.001, while there is no significant difference concerning the age of the newborns. This is due to the fact that preterm are those newborns that delivered before 37 week completed weeks while full term is the newborn that born between 38-42 weeks gestation and
this explains the difference in the birth weight between the two groups due to the fact that fat deposition is mainly occur at the 3rd trimester so if the baby born prematurely this leads to decrease the birth weight compared with that who born at full gestation. (3) Also there is a significant statistical difference (P value < 0.001) between the LBW neonates and the LBW+BA and LBW+RDS neonates regarding the gestational age, age, and birth weight. This due the fact that RDS and BA is a disease of premature and extreme prematurity which characterized by decreased gestational age and decreased birth weight. (2) The table also reveals that there is no statistical difference between the LBW+BA and the LBW+RDS regarding the gestational age, age, and the birth weight.

The mean serum MDA level in LBW is significantly higher (P < 0.001) than that in NBW neonates, also the mean serum MDA levels in LBW+BA and LBW+RDS are significantly higher (P < 0.001) than the mean values in LBW and NBW neonates. These findings are in agreement with the findings of Kose, Yazici, and Assioglu (2001) (6) who measured serum MDA levels spectrophotometrically in 100 diseased LBW neonates and also in 200 healthy controls, they found a higher serum MDA level in diseased LBW in comparison with the healthy controls. Also these findings are in agreement with the findings of Berger (1999) (14) who found an increase in serum level of MDA induced by lipid peroxidation that happen in premature infants with lung disease.

The formation of MDA (end product of lipid peroxidation) is enhanced in the plasma by the effect of oxidative stress that takes place during premature delivery. (7,15)

Kondoh and Sato (2002) (16) found that hypoxia causes an oxidative tissue injury, which leads to an increase in lipid peroxidation product (MDA). This finding is most likely to be the cause behind the higher serum levels of MDA in LBW+BA and LBW+RDS in this study.

Khaw, et al (2002) (17) found that oxygen therapy during caesarean section increased the fetal oxygenation and causing a concomitant increase in oxygen free radical activity in fetal circulation, which leads to an increase in lipid peroxidation product (MDA). Also this finding explains the elevated serum levels of MDA in LBW+BA and LBW+RDS in the present study.

The mean serum level of MDA in LBW+BA is significantly higher (P < 0.01) than the mean serum level in LBW+RDS. This could be attributed to that, the stress of hypoxia on tissues in BA is more than that in RDS, (18) therefore a greater amount of MDA is synthesized from the lipid peroxidation in tissues and liberated to the circulation.

Suttnar, Masova, and Dyr (2001) (19) found that there was a significant difference in plasma MDA mean concentration between samples collected in tubes containing different types of anticoagulants, these differences was attributed to different antioxidant properties of different anticoagulant used for blood collection. While in this study, MDA was measured in sera which were separated from the blood samples, in other wards, the effect of the anticoagulants was bypassed in this study.

Mohanty, et al (2002) (20) found that both fat and protein intakes stimulate reactive oxygen species generation, which leads to lipid peroxidation and an increase in the lipid peroxidation products (MDA) for 1 to 3 hrs after the intake. While in this study, blood samples were collected before feeding started.

Pratico, et al (2002) (21) found that moderate brain injury induced by hypoxia leads to a widespread brain lipid peroxidation, which is a reliable marker
of brain oxidative damage. These findings also explain the findings in this study, where the neonates with BA or RDS underwent a state of hypoxia which affected their brains with over production of MDA which was released to the circulation and caused higher serum levels of MDA in those neonates. These findings clarifies that newborn infants with normal serum MDA levels are most likely to be delivered with a mature, well developed, intact brain. In other words that serum MDA is a reflection of brain maturity.

The positive correlation between serum MDA levels and serum glucose levels in NBW neonates, may be attributed to that, erythrocytes consume glucose directly from the circulation more than white blood cells and platelets since it is of a higher number and contains no mitochondria therefore it depends on anaerobic glycolysis for energy supply, (22) at the same time the stress of labor which induces lipid peroxidation in erythrocyte membrane with the production of MDA which is usually librated to serum, (6) therefore, the damage in erythrocytes causes an increase in serum glucose and MDA levels.

The positive correlations between serum MDA levels and birth weight in NBW and LBW+BA could be attributed to the body mass, in which a higher birth weight leads to a higher serum level of MDA.

As a conclusion:
1. The serum levels of malondialdehyde (MDA) in low birth weight with birth asphyxia (LBW+BA) and low birth weight with respiratory distress syndrome (LBW+RDS) are significantly higher than the serum levels in normal birth weight (NBW) and low birth weight (LBW) neonates.
2. The birth weight is an important factor in relation to the serum levels of the studied biochemical parameters.
3. The present observations suggest a narrow range of variations in serum levels of MDA in sera of the studied neonates.

References
8. Hong YL, Yeh SL, Chang CY, and Hu ML: Total plasma malondia-
aldehyde levels in 16 Taiwanese college students determined by various determined by various Thiobarbituric acid tests and an improved high–performance liquid chromatography-based method.

**Table (1):** Physical Characteristics of Newborns.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Birth weight (g) mean ± SD</th>
<th>Gestational age (wks) mean ± SD</th>
<th>Age (min) mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NBW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>3176.53 ± 386.60</td>
<td>39.26 ± 0.94</td>
<td>49.82 ± 26.57</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>3135.6 ± 411.85</td>
<td>39.15 ± 0.98</td>
<td>48.40 ± 28.09</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>3158.65 ± 396.38</td>
<td>39.22 ± 0.95</td>
<td>49.2 ± 27.12</td>
</tr>
<tr>
<td><strong>LBW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>49</td>
<td>2116.85 ± 206.53</td>
<td>34.08 ± 1.81</td>
<td>40.10 ± 22.11</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>2117.82 ± 221.49</td>
<td>34.23 ± 2.12</td>
<td>37.94 ± 22.71</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>2117.35 ± 213.21</td>
<td>34.16 ± 1.96</td>
<td>39.0 ± 22.33</td>
</tr>
<tr>
<td><strong>LBW+BA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>1775.53 ± 495.23</td>
<td>32.35 ± 3.36</td>
<td>54.1 ± 24.03</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>1802.95 ± 346.70</td>
<td>31.9 ± 2.98</td>
<td>55.22 ± 26.16</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>1787.6 ± 432.25</td>
<td>32.16 ± 3.03</td>
<td>54.6 ± 24.74</td>
</tr>
<tr>
<td><strong>LBW+RDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>1914.27 ± 451.45</td>
<td>32.65 ± 3.41</td>
<td>63.44 ± 25.84</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>1966.42 ± 413.99</td>
<td>32.85 ± 2.93</td>
<td>52.85 ± 24.82</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>1936.18 ± 432.54</td>
<td>32.74 ± 3.19</td>
<td>59.0 ± 25.71</td>
</tr>
</tbody>
</table>
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**Table (2):** Biostatistical Calculation and Students t-test for MDA

Levels in Sera of NBW, LBW, LBW+BA, and LBW+RDS.

<table>
<thead>
<tr>
<th>Serum levels of MDA (nmol/ml)</th>
<th>NBW</th>
<th>LBW</th>
<th>LBW + BA</th>
<th>LBW+RDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.49 ± 0.1</td>
<td>0.63 ± 0.11</td>
<td>0.85 ± 0.11</td>
<td>0.78 ± 0.08</td>
</tr>
<tr>
<td>Range</td>
<td>0.26 – 0.71</td>
<td>0.41 – 0.91</td>
<td>0.64 – 1.06</td>
<td>0.64 – 1.0</td>
</tr>
<tr>
<td>Standard error of mean</td>
<td>0.01</td>
<td>0.011</td>
<td>0.015</td>
<td>0.012</td>
</tr>
<tr>
<td>Confidence interval of mean:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>0.47</td>
<td>0.61</td>
<td>0.82</td>
<td>0.76</td>
</tr>
<tr>
<td>Upper</td>
<td>0.51</td>
<td>0.66</td>
<td>0.89</td>
<td>0.81</td>
</tr>
<tr>
<td>t-test</td>
<td></td>
<td>9.07</td>
<td>19.27</td>
<td>17.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.43</td>
<td>8.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.58</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td>0.000*** (S)</td>
<td>0.000*** (S)</td>
<td>0.000*** (S)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.000*** (S)</td>
<td>0.001** (S)</td>
</tr>
</tbody>
</table>

**= P < 0.01
*** = P < 0.001
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**Table (3): The Predictive Value for MDA.**

<table>
<thead>
<tr>
<th>Tests</th>
<th>LBW+BA</th>
<th>LBW+RDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98%</td>
<td>88%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>92.4%</td>
<td>91.6%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>98.9%</td>
<td>94.1%</td>
</tr>
<tr>
<td>Efficiency</td>
<td>96.6%</td>
<td>93.3%</td>
</tr>
</tbody>
</table>

**Figure (1):** The correlation of serum malondialdehyde (MDA) levels to birth weight in normal birth weight (NBW) neonates.

**Figure (2):** The correlation of serum malondialdehyde (MDA) levels to birth weight in low birth weight with birth asphyxia (LBW+BA).