A study of Serum Malondialdehyde and Free Iron levels in Pregnant Women

Najat Zaid Mohammad¹, Jian Lateif Hussein², and Ziyad Ahmed Shareef³.

1- Department of Chemistry, College of Science, Salahadeen University, Erbil, Kurdistan Region, Iraq.
2- Department of Chemistry, College of Science, Salahadeen University, Erbil, Kurdistan Region, Iraq.
3- Azadi Teaching Hospital – Duhok, Kurdistan Region, Iraq.

ARTICLE INFO

Article History:
Received: 03/07/2017
Accepted: 06/09/2017
Published: 21/05/2018

Keywords:
Iron, free radical, oxidative stress, malondialdehyde, Pregnancy.

* Corresponding Author:
Najat Zaid Mohammad
najat.mohammad@su.edu.krd

ABSTRACT

The aim of the current study to find relationship between oxidative stresses that illustrated by serum iron and malondialdehyde for women during the gestation period and after delivery, comparing within the subject in healthy non-pregnant women. The result obtained from the analysis of blood for 111 subjects aged (20 – 40) years, divided into two groups; the first group included 25 healthy subjects. While the second group divided to four sub groups, the first trimester which included (24 subjects), the second trimester of pregnancy included (13 subjects) and the third trimester included (38 subjects) and the last fourth subgroup for those women after delivered were included (11 women). All the chosen subjects in Erbil city. Free serum iron level showed a slight reduce at the first trimester of pregnant (449 ± 65 µg/dl) as compared with the normal control subjects (449.5 ± 65 µg/dl). While in the second trimester showed a significant increase (903.31 ± 108 µg/dl) compared to the same normal value. Also an increase of MDA concentration observed in the blood (6.527 ± 1.8 µmol/L) for the gestation women in the second trimester when compared to the normal control subjects which are (4.562 ± 0.7 µmol/L). These results improve the relationship between free serum iron and oxidative stress in pregnant women in one hand; on the other hand, free serum iron and MDA are increasing more obvious in the second trimester of pregnancy.

1. INTRODUCTION

There are many definitions of free radicals. It can be described as molecular fragments or molecules have one or more unpaired electrons. The unpaired electrons present usually considerable high degree of reactivity. In vivo which causes changing biochemical compounds, and killing cells. It means technically, free radicals are extremely unstable and very active molecules. These radicals or unpaired electron that derived from oxygen are representing the most important type of such species produced in living systems. Recently during the last two decades, there has been high interest in the function and role of oxygen-free radicals, they generally known as “reactive oxygen species” (ROS) in experimental, practical and clinical medicine (Halliwell and Gutteridge 1989), reactive oxygen species” ROS can be created (i) during X-rays irradiation or by UV light irradiation and with exposure to
gamma rays; (ii) are produced by reactions of metal-catalyzed; (iii) are found as pollutants in the atmosphere; (iv) are generated by macrophages and neutrophils during inflammation; (v) are by-products of electron transport reactions by mitochondria-catalyzed and other mechanisms (Cadenas et al., 1989).

Superoxide anion, that produces by physical irradiation increase their level either by following oxygen “activation” or with metabolic operations, Superoxide anion is represented the “primary” ROS, and can further lead to interact with other molecules to created “secondary” ROS, either directly or indirect by through metal-catalyzed processes or enzymes (Fridovich, 1986). Superoxide radical ion does not react directly with proteins, sugars, or nucleotide, and its ability to peroxidase lipids is and exciting to inquire controversially. Superoxide is undergoing to a mutation reactions (Desideri and Falconi, 2003).

\[ 2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \]

Super oxide dismutase (SOD) enzymes are increasing this reaction in biological systems more than four orders of magnitude. It was found that SOD enzymes do not work alone, but it was in conjunction with enzymes that removing \( H_2O_2 \), such as peroxidases, catalases, and glutathione (Marcel, ZaÁmocky Æand Franz Koller, 1999). The redox-active metals closely linked with the participation of the generation of different free radicals (Michiels et al., 1994), it has been suggested and expected that iron regulation level ensures the absence and nonexistence of free intracellular iron; So in vivo, under stress conditions, an excess of free radical and superoxide are generated “free iron” from molecules that containing iron. The release of iron by superoxide has been illustrated with enzymes of the dehydratase, lyase family for [4Fe−4S] cluster-containing enzymes (Liochev and Fridovich, 2002). The Fenton reaction can participate with the released Fe (II), producing highly reactive hydroxyl radical (Fe (II) + \( H_2O_2 \rightarrow Fe \) (III) + \( OH^- + OH \)). The end product of the lipid peroxidation process being malondialdehyde (MDA), as well as to the MDA there are 4-hydroxy-2-nonenal (HNE) (Marnett et al., 1999). The malondialdehyde (MDA) is one of the high reactive three carbonal aldehyde produced from lipid hydroperoxidation. It can be also derived from the hydrolysis of deoxyribose, pentose, hexoses, from some amino acids and DNA. The MDA has most been measured by the thiobarbituric acid reaction (TBA test) (Pryor, 2000).

2. MATERIALS AND METHODS

1. Subjects:

The study comprised 111 healthy pregnant women subjects, which were divided into two groups, the first group included (25) healthy normal control, while the second group divided into four subgroup: the first trimester included (24) of pregnant women, then the second trimester included (13) and the third trimester included (38) and the last subgroup included (11) subject after delivery, all the subjects were in the age range of (20-40) years, and were attending for antenatal examine at Erbil city.

2. Statistical Analysis

Statistical analysis was done by using GraphPad prism program to find ANOVA and \( t \)-test study. All the data were
presented as mean ± standard deviation. \( P \) values < 0.05 were considered significant.

3. Materials:

Used Chromogen, ascorbic acid, Buffer is Acetate buffer, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) made from BDH laboratory reagent Product England.

4. Instruments:

The U.V visible spectrophotometry, Cecil CE 3021, 3000 series used, Centrifuge, Hermle labortechnic Z 200A, Made in Germany and thermostatic, Con .tem. Plate, England.

5. Method:

The collection of blood sample:
- For serum collection tubes were used without anticoagulants. All samples were centrifuged at 3000 rpm for around 15 min and then serum was collected from each sample separately.
- Determination of serum iron:
  - A spectrophotometric method was used for serum iron determination. It is based on comparing the color that develops when the (ferrous) iron in serum is treated by a chromogen reagent, with that which develops from a standard solution. The kit was purchased from (Randox–United Kingdom) (Cerriotti and Ceriotti 1980).

- Determination of serum malondialdehyde (MDA):
  - The MDA serum levels were assessed by spectrophotometric methods based on measuring the concentration of pink chromogenic color product, which produce when MDA reacted with thiobarbituric acid. The lipid peroxidation was expressed as \( \mu \) moles MDA per dl serum. All chemicals were used in high purity commercially available. One molecule of MDA reacts stoichiometrically with two molecules of 2-thiobarbituric acid, the reaction occurs at a pH of 2 - 3 but excess acid (pH < 2) inhibits the color development (Sinnhurber, R.O. and Yu 1958) and (Yu et al., 1986).

The Method of determination of serum MDA:
- The level of serum MDA was determined spectrophotometrically with a TBA solution. In brief, to 150 \( \mu \)L serum samples added the followings chemicals:

3. RESULTS AND DISCUSSION

Normally, pregnant women need more food in normal cases to fill the shortfall of food during pregnancy that associated high metabolic byproducts (Arimond, et al., 2017). This leads to reduced macro and micronutrients in normal pregnancy, provoking oxidative stress, lipid peroxidation process is one of a mechanism of cell can be used as the parameter from of oxidative stress reaction in vivo and vitro study. Lipid peroxide is the product of an oxidation reaction of polyunsaturated fatty acids, that are very reactive and unstable, that typically lead to decompose and produce a various series of compounds. These include reactive carbonyl compounds, which is the considerable malondialdehyde. Therefore measurement of malondialdehyde is very widely used to providing specific indicator about lipid peroxidation. The MDA measured by the thiobarbituric acid reaction (TBA test) was used, but unfortunately, this method is not highly specific and accurate (Esterbauer et al., 1991).

Concerning Free Iron concentration levels, changes were detected in iron level during different trimesters of pregnancy. As shown in figure (1) there is a slight decrease in iron
during the first trimester (449.5 ± 65.55 µg/dl) compared with normal women level (460.1 ± 45.9 µg/dl). The results may be due to the time of embryonic development which requires Iron for hemoglobin synthesis and body cell growth (Marnett et al., 1999).

Figure 1: The Serum iron concentration during three trimesters for pregnancy, after delivery and normal control level.

The serum iron level was increased during the second trimester (903 ± 108 µg/dl). Ferrous Sulphate intake by the pregnant women during this period may have caused these results. Other factors may also contribute to the increase in iron levels at this stage. However, further future studies are required in order to hypothesize about. Serum iron declined after the second trimester reached (579.9 ± 57 µg/dl) as well as after delivery table (1). The results may be explained by the decrease in intake of iron supplements during this trimester and subjects not taking proper care of their health or failing to follow their doctor’s directions. And the main reason is bleeding also contribute to the reduction of serum iron after delivery. As shown statically the second trimester is high significant with control subject p value = 0.0004, and the second trimester period also has significant relationship with other periods trimesters and after delivery as shown in figure (1).

Table 1: The Bio statistic ANOVA calculation for serum iron (µg/dl) level for pregnant women.

| Groups        | Control | First trimester | second trimester | Third trimester | After delivery |
|---------------|---------|-----------------|------------------|-----------------|----------------|
| No.           | 25      | 21              | 10               | 45              | 10             |
| Mean(µg/dl)   | 460.1   | 449.5           | 903.3            | 579.9           | 319.0          |
| Std. Error of Mean | 45.91  | 65.55           | 108.3            | 57.53           | 46.96          |
| P vale to control | NS     | P value = 0.0004 | NS               | NS              | NS             |

NS .non-significant

The malondialdehyde concentration was physiologically raised but statically non-significant as shown in table 2. The results shows an increase in MDA level in second trimester (6.5 ± 1.79 (µmol/L) if compared with other groups figure (2), this attributed to an increase in serum iron levels which is obtained in the present results as shown in
Figure (1), because Fe^{+2} participate in reduction of H_2O_2 to form \cdot OH, these enhance lipid peroxidation increased by OH free radical (hydroxyl radical) according to the Fenton reaction (Bhasin et al., 2002).

Table 2- The Bio statistical ANOVA calculation for serum MDA level for pregnant women.

| Groups        | Control | First trimester | second trimester | third trimester | After delivery |
|---------------|---------|----------------|------------------|-----------------|---------------|
| No.           | 25      | 24             | 13               | 38              | 11            |
| Mean (µmol/L) | 4.562   | 3.611          | 6.527            | 5.421           | 5.048         |
| Std. Error of Mean | 0.7015  | 0.5453         | 1.798            | 0.6665          | 0.8020        |
| P value       | NS      | NS             | NS               | NS              | NS            |

NS = non-significant

According to these and other investigators results, recommend pregnant to take care and not intake excess amount of iron (ferrous ion Fe (II) drug) due to high level of iron lead to oxidative stress which have great role in the many diseases (Abraham, et al., 1999) and (Gratacos et al., 2000).

Also there are many other factors that affect oxidative stress, where our society (especially the pregnant woman) does not take it seriously and consider on these factors. The most important factor is food, one the important factors antioxidant intake, omega-3- fatty acid and vitamins antioxidants as reported by other investigators (Taghizadeh et al., 2016). Other research Published in 2016 for woman with and without iron supplementation, when comparing with the group of pregnant women with iron supplementation shows decreased the level of oxidative stress (De Lucca et al., 2016), these improve that the high iron drug supplementation cause oxidative stress while the normal iron level has not effect.

Figure 2: The relationships and comparing of MDA concentration during pregnancy semesters, after delivery and control (not pregnant woman).
4. CONCLUSIONS

the Conclusion of this research is shows in the second trimester of pregnant woman appears high Iron concentration level and high MDA level, these results attributed to high Iron intake more than her requested and unhealthy diets which are necessary and important for pregnant woman's health.

REFERENCES

Abraham, S. C., J. H. Yardley and T. T. Wu 1999. "Erosive injury to the upper gastrointestinal tract in patients receiving iron medication: an underrecognized entity." Am J Surg Pathol 23(10): 1241-1247.

Arimond, M., B. S. Vitta, Y. Martin-Prével, M. Moursi and K. G. Dewey 2017. "Local foods can meet micronutrient needs for women in urban Burkina Faso, but only if rarely consumed micronutrient-dense foods are included in daily diets: A linear programming exercise." Maternal & Child Nutrition: e12461-n/a.

Anne Rehema, Kersti Zilmer, Ursula Klaar1, Helle Karro1, Tiu Kullisaar, Mihkel Zilmer. 2004. "Ferrous iron administration during pregnancy and adaptational oxidative stress (Pilot study)." Medicina (Kaunas) 40(6): 547-552.

Bhasin, G., et al. 2002. "Iron augments stage-I and stage-II tumor promotion in murine skin." Cancer Lett 183(2): 113-122.

Cadenas, E., (1989). Biochemistry of oxygen toxicity, Ann. Rev. Biochem. 58, 79–110.

Ceriotti, F. and G. Ceriotti 1980. "Improved direct specific determination of serum iron and total iron-binding capacity." Clin Chem 26(2): 327-331.

De Lucca, L., F. Rodrigues, L. B. Jantsch, W. S. Neme, F. M. P. Gallarreta and T. L. Gonçalves 2016. "Oxidative Profile and 8-Aminolevulinate Dehydratase Activity in Healthy Pregnant Women with Iron Supplementation." International Journal of Environmental Research and Public Health 13(5): 463.

Desideri, A. and M. Falconi 2003. "Prokaryotic Cu,Zn superoxide dismutases." Biochem Soc Trans 31(Pt 6): 1322-1325.

Esterbauer, H., et al. 1991. "Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes." Free Radic Biol Med 11(1): 81-128.

Fridovich, I. 1986. "Biological effects of the superoxide radical." Arch Biochem Biophys 247(1): 1-11.

Gratacos, E. 2000. "Lipid-mediated endothelial dysfunction: a common factor to preeclampsia and chronic vascular disease." Eur J Obstet Gynecol Reprod Biol 92(1): 63-66.

Halliwell, B. and J. M. C. Gutteridge (1989). Free radicals in biology and medicine, Clarendon Press.

Leonard, S.S., Harris, G.K., Shi, X.L., 2004. Metal-induced oxidative stress and signal transduction, Free Rad. Biol. Med. 37, 1942-1921.

Liochev, S.I., Fridovich, I., (2002). The Haber-Weiss cycle - 70 years later: an alternative view, Redox report 7, 55–57.

Marnett L.J., 1999. Lipid peroxidation — DNA damage by malondialdehyde, Mut. Res.-Fund. Mol. Mech. Mutagen. 424, 83–95

Michiels, C., M. Raes, O. Toussaint and J. Remacle 1994. "Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress." Free Radic Biol Med 17(3): 235-248.

Pryor, W. A. 2000. "Vitamin E and heart disease." Free Radical Biology and Medicine 28(1): 141-164.

Sinnhuber, R.O. and Yu, T.C.E. (1958). Characterization of the red pigment in the Z-thiobarbituric acid determination of oxidative rancidity, Journal of Food Science 23, 626 - 634.

Taghizadeh, M., M. Jamilian, M. Mazloomi, M. Sanami and Z. Asemi 2016. "A randomized-controlled clinical trial investigating the effect of omega-3 fatty acids and vitamin E co-supplementation on markers of insulin metabolism and lipid profiles in gestational diabetes." Journal of Clinical Lipidology 10(2): 386-393.

Weinstein, T., Avry C., Asher K., Mona B., Yaacov O., Michal H., Tsipora M., and Uzi G., 2000. Haemolysis in haemodialysis patients: evidence for impaired defense mechanisms against oxidative stress. Nephrol Dial transplant; 15, 883-887.

Yu, L. W., et al. 1986. "High-performance liquid chromatography analysis of the thiobarbituric acid adducts of malonaldehyde and trans,transmuconaldehyde." Analytical Biochemistry 156(2): 326-333.

Zámocký, M. and F. Koller 1999. "Understanding the structure and function of catalases: clues from molecular
evolution and in vitro mutagenesis." Progress in Biophysics and Molecular Biology 72(1): 19-66

JOHNSTONE, T., VAN REEKUM, C. M., URRY, H. L., KALIN, N. H. & DAVIDSON, R. J. 2007. Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. J Neurosci, 27, 8877-84.

MATSUMOTO, K., PUIA, G., DONG, E. & PINNA, G. 2007. GABA(A) receptor neurotransmission dysfunction in a mouse model of social isolation-induced stress: possible insights into a non-serotonergic mechanism of action of SSRIs in mood and anxiety disorders. Stress, 10, 3-12.

MOYSES, Z. P., NAKANDAKARI, F. K. & MAGALDI, A. J. 2008. Fluoxetine effect on kidney water reabsorption. Nephrol Dial Transplant, 23, 1173-8.

ÖZDEN, H., BILDIRICI, K. İ., ÜSTÜNER, D., ÜSTÜNER, C., CENGIZ, B. P., TÜLAY, A. & YıLMAZ, V. 2005. Histopathologic examination of rat liver after experimental application of fluoxetine. Türkiye Ekopatoloji Dergisi, 11, 9-15.

TEN HOLT, W. L., VAN IPEREN, C. E., SCHRIJVER, G. & BARTELINK, A. K. 1996. Severe hyponatremia during therapy with fluoxetine. Arch Intern Med, 156, 681-2.

TIRADENTES, R. V., PIRES, J. G., SILVA, N. F., RAMAGE, A. G., SANTUZZI, C. H. & FUTURO NETO, H. A. 2014. Effects of acute administration of selective serotonin reuptake inhibitors on sympathetic nerve activity. Braz J Med Biol Res, 47, 554-9.

UNGVARI, Z., PACHER, P. & KOLLER, A. 2000. Serotonin reuptake inhibitor fluoxetine decreases arteriolar myogenic tone by reducing smooth muscle [Ca2+]i. J Cardiovasc Pharmacol, 35, 849-54.

WERNICKE, J. F. 2004. Safety and side effect profile of fluoxetine. Expert Opin Drug Saf, 3, 495-504.

YILMAZ, A., ELBEY, B., YAZGAN, U. C., DONDER, A., ARSLAN, N., ARSLAN, S., ALABALIK, U. & ASLANHAN, H. 2016. Protective Effects of Caffeic Acid Phenethyl Ester on Fluoxetine-Induced Hepatotoxicity: An Experimental Study. Biomed Res Int, 2016, 1247191.