

Oxidative Stress Role in Aborted Women with Cytomegalovirus infection in Thi-Qar Governorate, Iraq

Asst. Prof. Dr. Alaa Hussein Ali Al Nasir¹

1. Department of Obstetrics and Gynecology, College of Medicine – University of Thi-Qar, Iraq.

*Corresponding author: Alaa Hussein Ali Al Nasir

Abstract:

Background: termination of pregnancy resulting in expulsion of an immature fetus of less than twenty weeks called as abortion. Where aborted fetus is weighing less than 500 g. Human cytomegalovirus infections is one of several factors related to spontaneous abortion.

Aim: To determine the levels of oxidative stress (malondialdehyde) (MDA), (ceruloplasmin, transferrin, Vitamin C) (Cp, Tf, Vit.C) in aborted women with cytomegalovirus (IgG, IgG positive).

Material and method: A case control study include (60) women aborted with CMV (Patients), (60) healthy women (positive controls) and (60) women aborted without CMV (negative controls). at the period between (November, 2017) to (May, 2018). Spss version 23 had been used for data analysis, P value <0.05 was considered as significant

Results: There was a significant increase in the serum levels of (MDA, Tf, Cp) in patient group, and a significant decrease in the serum levels of (Vit.C) in patient group throughout the general comparison among the patients group, negative control group and positive control group. Age has a big role, with significant statistical difference in detected levels of oxidations. According to age, the results show a presence of a significant increase in (MDA) patient older in comparison with young patients. ($p \leq 0.05$). We found significant increase in concentration of serum (Cp, Tf, Vit.C, Cu) in younger patient in comparison with other age groups ($p \leq 0.05$).

Conclusion: lipid peroxidation and the antioxidant system associates with abortion with CMV and abortion without CMV levels, the age effects positively on the levels of (MDA, NO) and effects negatively on the levels of (Cp, Tf, Vit.C), levels of IgG associate with (MDA, Cp, Tf, Vit.C) positively.

Key word: Oxidative Stress, Abortion, Cytomegalovirus

How to cite this article: Al Nasir AHA (2020): Oxidative stress role in aborted women with cytomegalovirus infection in Thi-Qar Governorate, Iraq, *Ann Trop Med & Pub Health*; 23:S395. DOI:

<http://doi.org/10.36295/ASRO.2020.2319>

Introduction

Pregnancy is an integral stress condition in which many metabolic and physiological functions are changed to considerable extent. Profound physiological changes occur during pregnancy (Saladin A and Kenneth S, 2012). Anemia, hyperlipidemia, hypertension, diabetes and preeclampsia may occur as pregnancy complications which also had a role in the oxidative stress (Mathai, 2005; Goonewardene and Shehata, 2011 and Okojie *et al.*, 2011). Recurrent spontaneous abortion (RSA) is one of the most common obstetrical complications. It has been shown that the total rate of spontaneous pregnancy loss is 31.0% out of the total abortion cases (Raghupathy R, 2003). The commonest reasons of presentation acutely to gynecologists is spontaneous miscarriage, which occurs in 10.9–30% of all early pregnancies (Nybo Andersen AM *et al.*, 2000; Farquharson, RG Farquharson, RG *et al.*, 2005). Cytomegalovirus and Rubella virus can leading to recurrent spontaneous abortion transmissible in uterus at various stage of gestation can be caused by a wide array of organisms (Stegmann BJ and Carey JC, 2002; Abdul-Karim ET *et al.*, 2009). Cytomegalovirus (CMV) is herpes virus and a leading biological factor causing congenital abnormalities, intra-uterine death of the fetus and recurrent spontaneous miscarriage (Munro SC and Hall B, 2005). During pregnancy, in fact, CMV can stretch the placenta from the cervix or following both primary and recurrent maternal infection, with subsequent vascular inadequacy, tissue damage, and transmission to the fetus (Yamamoto-Tabata T *et al.*, 2004; Nigro G, 2009). Cytomegalovirus (CMV) infection during pregnancy is far more complex than other infections,

due to the ability of the virus to be frequently reactivated during the childbearing age and be transmitted to the fetus through maternal immunity (Stagno S *et al.*, 1977). During pregnancy, women may have either a primary (first) CMV infection or non-primary infection, in which a earlier infected woman experiences reactivation of a latent virus or re-infection with a new viral strain (Mussi-Pinhata *et al.*, 2009; Wang *et al.*, 2011; De Vries *et al.*, 2013). The incidence of infection in pregnancy is supposed to be as high as 1 to 200 pregnancies, of which around 40% will result in fetal infections (Philip, 2006).

Congenital cytomegalovirus occurs in 0.05%-1.5% of births. Higher rate of infection among populations with lower economic standard of living. The risk of fetal infection is greatest with maternal primary cytomegalovirus infection 30% and much less likely with recurrent infection <1% (Clarncce & Gowen, 2011). Rate of transmission of cytomegalovirus infection are 3-7 times greater in infant born to adolescent mothers (Clarncce & Gowen, 2011). Perinatal transmission is common, accounting for occurrence of 10-60% through the 1st 6 months of life. The most important perinatal sources of virus are genital tract secretions at delivery and breast milk. Among Cytomegalovirus seropositive mothers, virus is perceptible in breast milk in 96%, with postnatal transmission occurring in approximately 38% of infant (Joana & Gillhan, 2004). About 90% of infected infants are asymptomatic (Philip, 2006).

Aims of Study:

- 1- Investigation of the possible effect of cytomegalovirus on serum oxidative stress.
- 2- Investigation of the role of age on oxidative stress.
- 3- Studying the possible relationships between the value of IgG and each one of the studied parameters

Materials and Methods:

Design of Study: A case control study is conducted at the Bent Al-Huda hospital in Thi-Qar and in the biochemistry laboratory in college of science, at the period between (November, 2017) to (May, 2018). The study included (180) women, (60) aborted women with cytomegalovirus (patients) aged (15-40) and (60) aborted women without cytomegalovirus (negative control) and (60) healthy women (positive control). However, the mentioned above women were divided according to age into five groups (15-20, 21-25, 26-30, 31-35, 36-40) years. Exclusion criteria: Cases excluded from this study: pregnant women, smokers, patients with chronic diseases such as diabetes, hypertension, women with any infected other pathogen such as toxoplasmosis, women with positive IgM (CMV) and women which use oral contraceptives or any other drugs.

Collection of Blood Sample:

About (5mL) of blood was obtained from women by venipuncture. The blood was allowed to clot at 37°C room temperature, and then centrifuged at 3000xg for 10 min. the serum samples was removed and stored at (- 20°C) for later measurement biochemical parameters, unless used immediately.

Patient profile

Name:

Age:

Number of previous abortion:

Data of last abortion: Period of marriage: Smoking:

Other disease:

Number of previous birth:

Address:

Biochemical tests

Anti-CMV antibodies: (IgG, IgM) Lipid peroxidation: (MDA)

Antioxidants: (CP, Tf, Vitamin C)

Determination of anti-CMV

antibodies (IgG, IgM) by ELISA:

The CMV IgG, IgM ELISA (sigma - aldrich, USA) Kits are intended for the detection of IgG, IgM antibody to cytomegalovirus (CMV) in human serum or plasma. Diluted patient serum is added to wells coated with purified antigen. IgG, IgM specific antibody, if present, binds to the antigen.

Calculations:

-Calculate the cut-off value: Calibrator absorbance x Calibrator Factor (CF).

-Calculate the Ab (Antibody) Index of each determination by dividing the absorbance value of each sample by cut-off value.

Sample absorbance value × 10

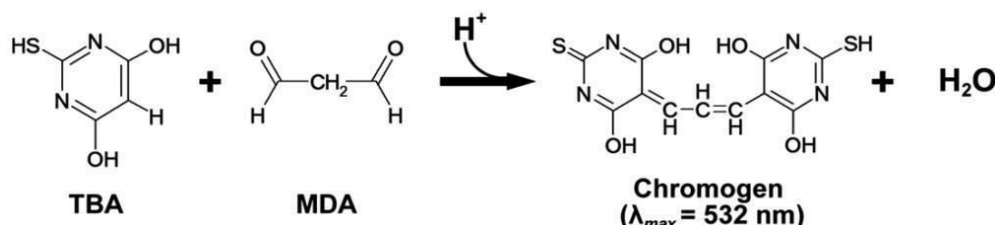
Cut – off

=

Ab (Antibody) Index

Determination of Serum Malondialdehyde ($\mu\text{mol/L}$) concentration: Lipid peroxidation was determined by using the thio barbituric acid method (Fong *et al.*, 1973). (Figure2-1). MDA concentrations were calculated using the extinction coefficient of MDA (ϵ_{MDA}) equal to $1.56 \times 10^5 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ (Wills, 1969). (MDA) formed from breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction.

Figure 1: Scheme of the adduct MDA-(TBA)2 (Souza *et al.*, 2012).



. The concentration of MDA calculated as follow:- (Muslihet *et al.*, 2002)

$$\text{MDA} \left(\frac{\mu\text{mol}}{\text{I.}} \right) = \frac{\text{absorbance at } 532 \text{ nm}}{L \times E_n} \times D \times 10^6$$

L: light path (1cm)

E₀: Extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

D: Dilution factor = 1 ml Vol. Used in ref./0.15 = 6.7

Determination of Ceruloplasmin (mg/dl) Concentration: Principle:

Plasma (Cp) concentration was measured by the method of (Ravin, 1961). Calculation :

$$\text{Cp (mg/L)} = \frac{A_{\text{test}} - A_{\text{blank}}}{\epsilon_{\text{cp}}} \times 10 = \frac{\Delta A \times 10}{0.68}$$

ϵ_{cp} = the extinction coefficient of Cp equal to 0.68

Determination of Transferrin (g/L) Concentration: Total Iron Binding Capacity ($\mu\text{g/L}$) Concentration Principle: (Ramsay, 1958; Burtis *et al.*, 1999).

T.I.B.C. was determined by addition of sufficient Fe^{+3} to saturate iron binding sites on apotransferrin. The excess Fe^{+3} was removed by adsorption with basic magnesium carbonate powder. After centrifugation, bound iron remaining in supernatant was measured with direct method REF 92108 (Ferene) or with deproteinization method REF180008 (SFBC).

Calculation:

$$\text{Iron conc. } \mu\text{mol/l} = \frac{(A_2 - A_1)_{\text{Assay}}}{(A_2 - A_1)_{\text{Standard}}} \times \text{Conc. of standard (35.80)}$$

Calculate Transferrin Concentrations:

$$\text{Tf(g/L)} = \text{TIBC}(\mu\text{g/L}) \times 0.007$$

Determination of Serum Vitamin C Concentration (mg/dl)

Calculation: (Shohag *et al.*, 2012).

$$\text{Serum vitamin C(mg/dl)} = \frac{\text{A test} - \text{A blank}}{\text{A standard}} \times 2$$

Ethical consideration:

A verbal consent from all participant been obtained, with identification of the objectives and work procedure had been pronounced to all.

Statistical analysis:

The statistical analysis was done by using Microsoft Excel 2010, SPSS version 23. The results were expressed as mean \pm standard deviation (mean \pm SD) with LSD. One-way ANOVA was used to compare parameters in different studied groups. Pearson's coefficient (r) was applied to determine the relationship among the present study parameters. P-values ($P \leq 0.05$) were considered statistically significant. Correlation analysis was calculated using Pearson's correlation coefficient.

Results:

The comparison is based on age group(A1) between(15-20) years old and group(A2) between(21-25) years old and (A3)between(26-30) years old and (A4)between(31-35) years old and group(A5) between(36-40) years old. According to age, the results show a presence of a significant increase in (MDA) patient A5 in comparison with patient each (A4, A3, A2 and A1) groups($p \leq 0.05$). We found significant increase in concentration of serum (Cp, Tf, Vit.C, Cu) in patient A1 in comparison with (A2, A3, A4 and A5) group ($p \leq 0.05$).

Lipid Peroxidation Status(Malondialdehyde):

Table (1) shows a significant increase in concentrations of serum MDA in patient group in comparison with positive control group and negative control group, shows a significant increase in concentrations of serum MDA in negative control group in comparison with positive control group ($p \leq 0.05$).

Table (1) Serum MDA concentration in studied groups

Groups	No.	MDA ($\mu\text{mol/L}$) Mean \pm SD
Patient	60	5.08 \pm 0.89 ^a
-ve control	60	3.79 \pm 0.76 ^b

+ve control	60	1.72 ±0.36 ^c
LSD		0.21

Moreover this study found a positive relationship between serum MDA levels and IgG levels as show in figure (3-1).

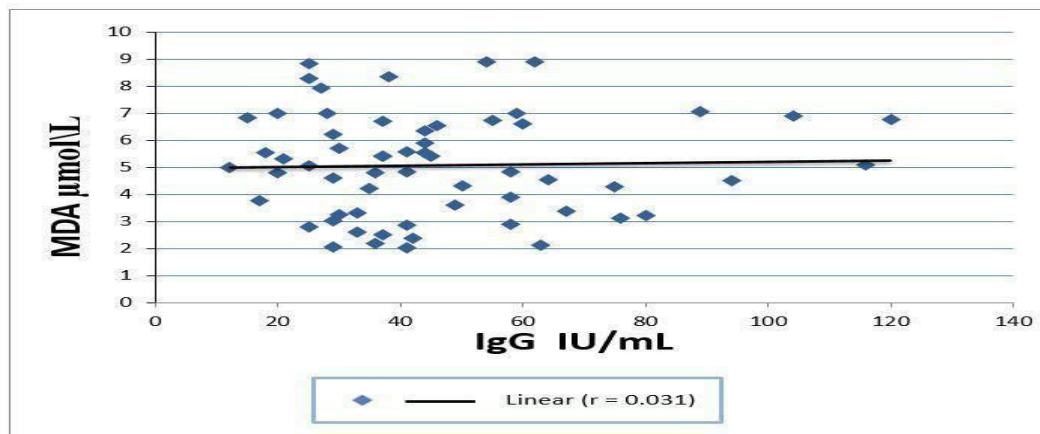


Figure 2: Relationship between IgG and MDA

AntioxidantSystem:

Serum Ceruloplasmin Concentration:

Table (2) show a significant increase in concentrations of serum Cp levels in patient group and negative control in comparison with positive control group ($p \leq 0.05$).

Table (2) Serum ceruloplasmin concentration in studied groups

Groups	No.	CP Concentration (g/L) Mean ± SD
Patient	60	3.34 ±0.91 ^a
-ve control	60	3.27±0.95 ^a
+ve control	60	2.46±0.86 ^b
LSD		0.27

There are positive relationship between serum Cp levels and IgG levels as show in figure (4).

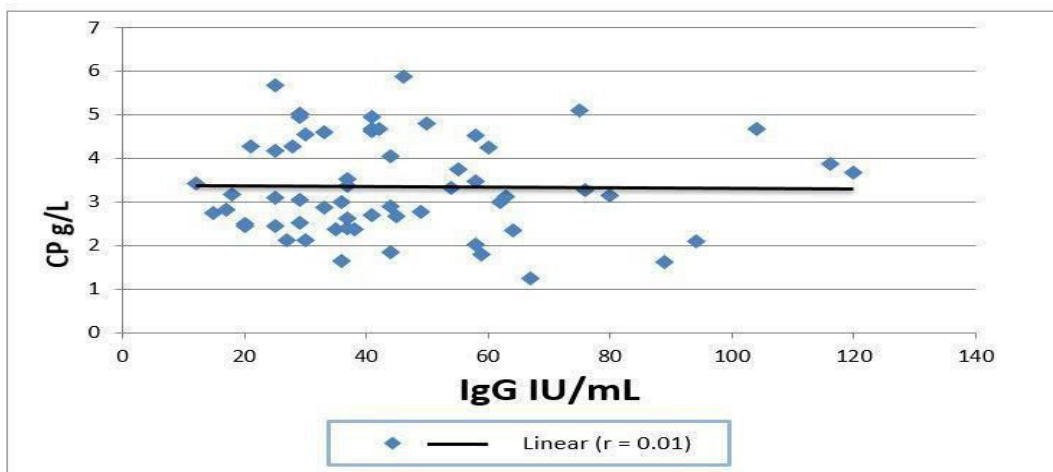


Figure 3: between Correlation IgG and Cp

Serum Transferrin Concentration:

Table (3-5) show a significant increase in concentrations of serum Tf levels in patient group in comparison with positive control group and negative control group, show a significant increase in serum Tf in positive control group in comparison with negative control group ($p \leq 0.05$).

Table(3) Serum transferrin concentration in studied groups

Groups	No.	Tf concentration (g/L) Mean \pm SD
Patient	60	4.39 ± 0.89^a
-ve control	60	3.32 ± 0.87^c
+ve control	60	3.77 ± 0.83^b
LSD		0.26

There are positive relationship between serum Tf levels and IgG levels as show in figure (4).

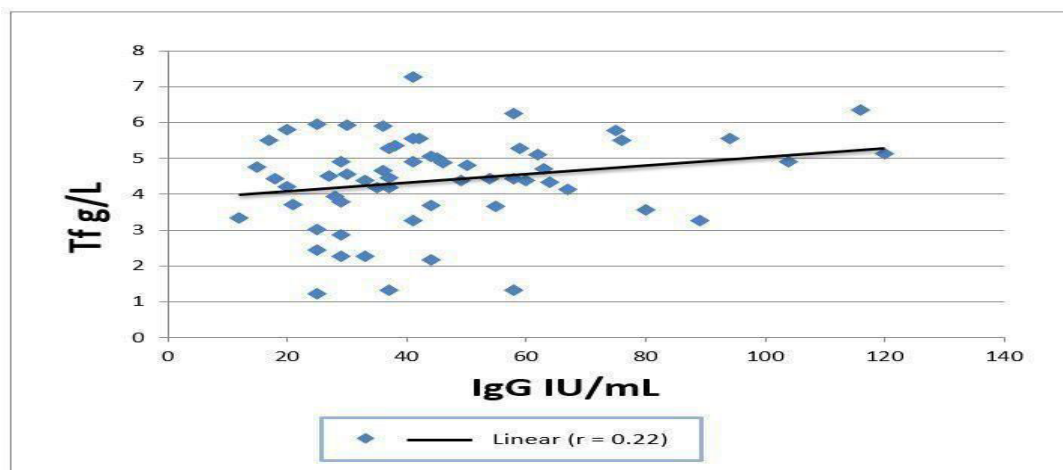


Figure 4: Relationship between IgG and Tf

Serum Vitamin C Concentration:

Table (3-6) shows a significant decrease in concentrations of serum vitamin C levels in patient group in comparison with negative control and positive control group ($p \leq 0.05$), and there is a significant decrease in the concentrations of vitamin C levels in negative control group in comparing with positive control group ($p \leq 0.05$).

Table(4) :Serum vitamin C concentration in studied groups

ps		concentration (mg/dl) Mean \pm SD
it		$\pm 0.01^c$
ontrol		0.02^b
ontrol		$\pm 0.03^a$

This study showed a positive relationship between serum vit.C levels and IgG levels as show in figure (5).

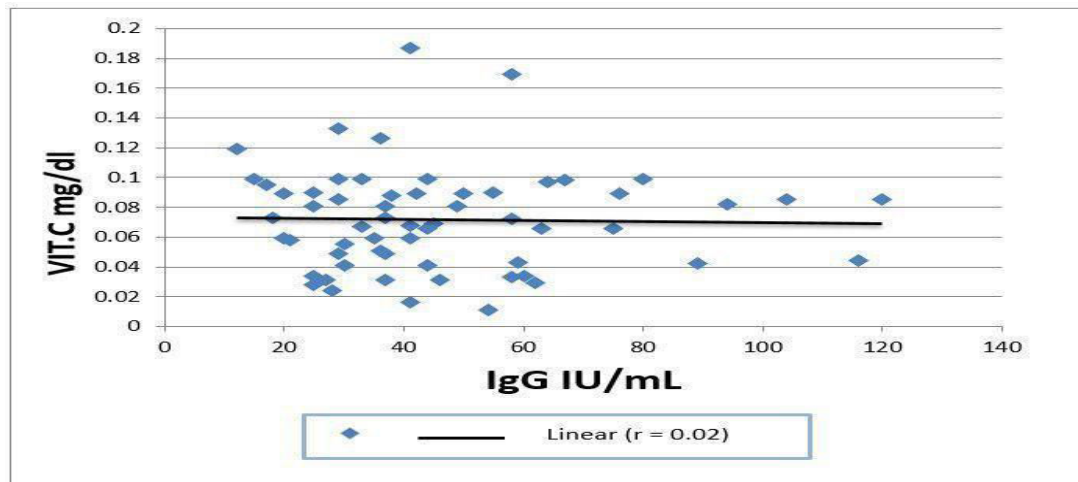


Figure 5: Relationship between IgG and Vit. C

Discussion

Our study shows a significant increase in concentrations of serum MDA in patient group in comparison with positive control group and negative control group, shows a significant increase in concentrations of serum MDA in negative control group in comparison with positive control group($p \leq 0.05$). The increased MDA levels is known to be due to increased production of reactive oxygen species, these results show that systemic oxidative stress, of which lipid peroxidation represents a major manifestation, plays an important role in usual abortion . These results were consistent with those reported by (Jauniauxet *al.*,2000; Agarwal *et al.*,2005). MDA is a byproduct of lipid peroxidation, therefore , an elevation in MDA levels may reflect an overproduction of lipid peroxides and/or impaired antioxidant defense mechanism. These lipid peroxides are produced mainly in the placenta due to membrane disruption by ROS (Routledge MN,2000). Successful pregnancy requires the development of an adequate uteroplacental circulation. Abnormal placentation leads to placental oxidative stress and syncytiotrophoblast dysfunction, and it has been proposed as a cause of early abortion (BurtonGJ *et al.*, 2003). Moreover this study found a positive relationship between serum MDA levels and IgG levels. To prevent ROS induced damage, cells have evolved antioxidant systems. As a result, there is a delicate balance between ROS production and antioxidant activity that maintains a physiologic balance leading to cellular homeostasis. When this balance is perturbed by an excess of ROS generation, a state of oxidative stress leads to cell damage and cell dysfunction (Agarwal and Allamaneni,2004; Agarwal *et al.*,2005). For ceruloplasmin antioxidant activity, including the protecting the organism as a whole form within the possible ill effects caused by the release of free radical oxidation products. This suggests that the organism might respond by raising the antioxidant efficiency of plasma by elevating ceruloplasmin levels (Winyard PG *et al.*, 1989)Our study also show a significant increase in concentrations of serum Cp levels in patient group and negative control in comparison with positive control group($p \leq 0.05$). Ceruloplasmin concentration increase are identified in diverse pathological conditions as an important defense mechanism reflecting the body's resistance to an oxidant insult (Kozlov *et al.*, 1984). Ceruloplasmin increases in blood serum during copper exhaustion, this will contribute to the total ceruloplasmin assay(DP Oparinde *et al.*,2011). Also in our study there is positive relationship between serum Cp levels and IgG levels as show in figure (3-4). Our study show a significant increase in concentrations of serum Tf levels in patient group in comparison with positive control group and negative control

group, show a significant increase in serum Tf in positive control group in comparison with negative control group ($p \leq 0.05$). The high plasma transferrin concentration found in humans with decrease iron stores may be due to a positive feedback of storage iron levels on transferrin synthesis (Aisen, 1984). Transferrin is the major carrier protein of iron. Iron ions are delivered in the blood by transferrin protein; each transferrin molecule can carry two iron ions (Attardo *et al.*, 2006). The antioxidant property of transferrin is its ability to bind with iron ions and store it as a ferritin and prevent the oxidative role of iron which allows to generate free radicals by Fenton and Haber-Weiss reactions (De Feo *et al.*, 2001). Our study shows significant decrease in concentrations of serum vitamin C levels in patient group in comparison with negative control and positive control group ($p \leq 0.05$), and there is a significant decrease in the concentrations of vitamin C levels in negative control group in comparing with positive control group ($p \leq 0.05$). In a report based on the women with recurrent abortion by (Vuralet *et al.*, 2000), they have found that antioxidant elements including vitamin C decreased in case group in comparison with healthy pregnant women. There are the negative relationships between vitamin C and MDA, the negative relationship between vitamin C and MDA may be due to the exhaustion of vitamin C when the oxidant burden is increased (Padayatty *et al.*, 2003).

Conclusion

1. Serum lipid peroxidation can be considered a feature of abortion with CMV and abortion without CMV.
2. Disorder in the antioxidant system in aborted women with CMV can be detected according to the levels of (ceruloplasmin, transferrin and vitamin C).
3. Levels of IgG associate with (MDA, Cp, Tf, and Vit.C.) positively.
4. The age effects positively on the levels of (MDA, NO) and effects negatively on the levels of (Cp, Tf, Vit.C, Cu and Fe).

Reference

- Abdul-Karim, E.T., Abdul-Muhymen, N. and Al-Saadie, M. (2009). Chlamydia trachomatis and rubella antibodies in women with full-term deliveries and women with abortion in Baghdad. *East Mediterr Health J.* 15: 1407-1411.
- Agarwal, A., Gupta, S., & Sharma, R. K. (2005). Role of oxidative stress in female reproduction. *Reproductive biology and endocrinology*, (3):28-35
- Agarwal, A., & Allamaneni, S. S. (2004). Role of free radicals in female reproductive diseases and assisted reproduction. *Reproductive biomedicine online*, 9(3), 338-347.
- Agarwal, A., Gupta, S., & Sharma, R. K. (2005). Role of oxidative stress in female reproduction. *Reproductive biology and endocrinology*, 3(1), 28.
- Aisen, P. (1984). Transferrin metabolism and the liver. *Semin. Liver Dis.* 4:193-206.
- Attardo, G. M., Guz, N. (2006). Molecular aspects of viviparous reproductive biology of the tsetse fly (*Glossina morsitans morsitans*): regulation of yolk and milk gland protein synthesis. *J Insect Physiol.* 52:1128–1136.

Burton GJ, Hempstock J, Jauniaux E .2003. Oxygen, early embryonic metabolism and free radical-mediated embryopathies. *Reprod Biomed Online* .6:84–96 .

Clarncce, W. &Gowen, J. (2011). Congenital Infection, Cytomegalovirus. *Nelson Essentials of Pediatrics*, 6th ed. Philadelphia, Saunders: 260-261.

De Feo, T. M., Fargion, S., Duca, L., Cesana, B. M., Boncinelli, L., Lozza, P., ... &Fiorelli, G. (2001). Non-transferrin-bound iron in alcoholabusers. *Alcoholism: Clinical and Experimental Research*, 25(10), 1494-1499.

De Vries, J. J., van Zwet, E. W., Dekker, F. W., Kroes, A. C., Verkerk, P. H., &Vossen, A. C. (2013). The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model. *Reviews in medical virology*, 23(4), 241-249.

D. L., de Carvalhoe Oliveira, P. F., Boppana, S., & Britt, W. J. (2009). Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. *Clinical infectious diseases*, 49(4), 522-528.

Farquharson, R. G., Jauniaux, E., &Exalto, N. (2005). Updated and revised nomenclature for description of early pregnancy events. *Human Reproduction*, 20(11), 3008-3011.

Fong, K. L., McCay, P. B., Poyer, J. L., Keele, B. B., &Misra, H. (1973). Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. *Journal of Biological Chemistry*, 248(22),7792-7797.

Goonewardene, M., Shehata, M. (2011). Anemia in pregnancy. *Best Practice and Research clinic Obstetric Gynecology*,26(1), 3-24.

Jauniaux, E., Gulbis, B., & Burton, G. J. (2003). The human first trimester gestational sac limits rather than facilitates oxygen transfer to the foetus—a review. *Placenta*, 24,S86-S93.

Joana, C. &Gillhan,C.(2004). Infections. . *Obstetrics and Gynecology an evidence-based text for MRCOG*, 1st ed. Arnold, London; 2004: 158- 159.

Mathai, M. (2005). Reviewing maternal deaths and complications to make pregnancy and childbirth safer. In *WHORegional Health Forum* (Vol. 9, No. 1, pp.27-29).

Menden, C. , Boian, J. , Murthy, L. and Petering, H.G. (1977). Plasmaantioxidant. *Anal Lett* .10: 197.

Munro, S.C. and Hall, B. (2005). Cytomegalovirus infection. *J. Clin. Microbiol*,47(2): 40-46.

Nigro G, Mazzocco M, Mattia E, et al.(2011).Role of infections in r ecurrentspontaneousabortion. *J Matern Fetal Neonatal Med*;24:983–9.

Nybo Andersen AM,Wohlfahrt J, Christens P, et al.(2000). Maternal ageand fetal loss: population based register linkage study. *BMJ*;320:1708–12.

Okoji ,F.O., Idonije, O., Blessing, Eseigbe Mabel ,A., Okhiai, O., Unuabonah Faith and Dike Magdalene. (2011): Comparative study of lipid profile of normalpregnant women in the different trimester. *Archives of Applied Science Research*, 3(3): 528-532.

Oparinde, D. P., Ezeogu, C. I., Okesina, A. B., Adeniji, A. O., Iyanda,A. A., &Atiba, A. S. (2011).Copper to Ceruloplasmin Ratio During Normal Pregnancy In Osogbo, Nigeria. *Tropical Journal of Health Sciences*, 18(1).

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., ... & Levine, M. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American college of Nutrition*, 22(1),18-35.

Philip, N. B.(2006).Perinatal infection Cytomegalovirus *Obstetrics by Ten Teachers*. 18th ed. BookPower : 203-204.

Plonka, A. , and Metadiewa, D. ; Biochem. Biophys.(1989). Res. Commun.,95:978.

Ravin, H. A. (1961). An improved colorimetric enzymatic assay of ceruloplasmin. *The Journal of laboratory and clinical medicine*, 58(1), 161-168.

Ramsay, W. N. M.(1958).Advances in Clinical Chemistry, H. SobotkaetC.P. Steward Ed., Academic press . 1(1).

Routledge, M. N. (2000). Mutations induced by reactive nitrogen oxide species in the supF forward mutation assay. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 450(1),95-105.

Saladin, A., S. Kenneth.(2012). "Anatomy and Physiology: the Unity of Form and Function", 6th Ed., New York NY, McGraw-Hill.

Saladin, A., S. Kenneth.(2012). "Anatomy and Physiology: the Unity of Form and Function", 6th Ed., New York NY, McGraw-Hill.

Stegmann, B.J. and Carey, J.C.(2002).TORCH Infections:Toxoplasmosis, Other (syphilis, varicella zoster, parvovirus B19, Rubella, Cytomegalovirus (CMV), and Herpes infections. *Curr. Womens Health Rep* 2:253-258.

Stirrat GM.(1999)." Recurrentmiscarriage". *Lancet* 336:673-75. **Stagno, S., Reynolds, D. W., Huang, E. S., Thames, S. D., Smith, R. J., & Alford Jr, C. A. (1977).** Congenital cytomegalovirus infection: occurrence in an immune population.*New England journal of medicine*, 296(22),1254-1258.

Vural, P., Akgül, C., Yildirim, A., &Canbaz, M. (2000). Antioxidant defence in recurrent abortion. *Clinicachimicaacta*, 295(1-2), 169-177.

Wang, C., Zhang, X., Bialek, S., & Cannon, M. J. (2011). Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. *Clinical infectious diseases*, 52(2),e11-e13.

Wills, E. (1969). Role of fatty acid in the pathogenesis of insulin resistance and NIDDM. *Biological Chemistry Journal* ,113, 315.

Yamamoto-Tabata, T., McDonagh, S., Chang, H. T., Fisher, S., & Pereira, L. (2004). Human cytomegalovirus interleukin-10 downregulates metalloproteinase activity and impairs endothelial cell migration and placental cytotrophoblast invasiveness in vitro. *Journal of virology*, 78(6),2831-2840.