

# The Role of Oxidative Stress and Vitamin D in Aborted Women with Cytomegalovirus

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## Abstract

Abortion is one of the commonest reasons for acute presentation to gynecologists, recurrent spontaneous abortion due to maternal infections transmissible in uterus at various stage of gestation can be caused by a wide array of organisms including cytomegalovirus. This study included (25) aborted women, (25) non- aborted women with cytomegalovirus seropositive test as a patients groups and (25) non- aborted women with cytomegalovirus seronegative test as a control group. The study was designed to evaluate the concentration of malondialdehyde (MDA) as an oxidative stress marker, the concentration of ceruloplasmin (Cp) and transferrin (Tf) as an antioxidant marker and total vitamin D in cytomegalovirus infected women and investigate the relation between oxidative stress and total vitamin D. The results show a significant decrease in levels of serum MDA in aborted group with CMV in comparison to non-<sup>\*</sup>-aborted and control groups ( $p > 0.05$ ), serum ceruloplasmin and transferrin significantly decrease in aborted group in contrast to non-aborted and control groups ( $p > 0.05$ ) and total vitamin D was decreased in all study groups but there was a significant difference between studied groups ( $p > 0.05$ ). The results of this study show disorder in levels of (MDA, Cp, Tf and VD) aborted group with cytomegalovirus.

**Keywords:** Abortion, Cytomegalovirus, Vitamin D, Malondialdehyde, Ceruloplasmin and Transferrin.

## Introduction

Miscarriage or spontaneous abortion is defined as expulsion or extraction of the embryo or fetus weighing <500 g, which is equivalent to approximately 20–22 weeks of gestation. It is the most common complication of early pregnancy [1]. It was estimated that 6–15% of all clinically detected pregnancies end with spontaneous abortion [2].

Spontaneous abortion is a multifactorial disorder resulting from genetic factors, anatomic factors, autoimmune disorders, endocrine dysfunction, thrombophilia, life style factors, and maternal infections. However, the underlying cause remains undetermined in up to 50% of cases [3]. Embryo–fetal infections have been reported to cause spontaneous abortions (SAs) at a rate lower than 4%. The possible mechanisms include production of toxic metabolic byproducts, fetal or placental infection [4]. Viruses appear to be the most frequently involved pathogens and cytomegalovirus is a common virus that can reach

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the placenta by viremia, following both primary and recurrent infection, or by ascending route from the cervix, mostly [5].

Reactive species are frequently formed after viral infections. Antioxidant defenses, including enzymatic and non-enzymatic components, protect against reactive species. An imbalance in the production of reactive species and the body's inability to detoxify these reactive species is referred to as oxidative stress [6].

Vitamin D is a secosteroid hormone that plays a vital role in bone metabolism and mineral homeostasis [7]. In addition to this physiological function, vitamin D modulates the immune system [8]. During pregnancy, low vitamin D status may increase the risk of obstetrical complications [9]. A high proportion of RSA patients reportedly have vitamin D deficiency [10], and low concentrations of vitamin D have been associated with an increased risk of first trimester miscarriage [11]. This study was aimed to evaluate the concentration of malondialdehyde (MDA) as an oxidative stress marker, the concentration of ceruloplasmin (CP) and transferrin (TF) as an antioxidant marker and total vitamin D in cytomegalovirus infected women and investigate the relation between oxidative stress and total vitamin D.

## **Material and Method**

**Subjects :** 25 aborted women with CMV seropositive and 25 non-aborted women with CMV seropositive with age range from (16-43) years were selected from those attending the Bint Al-Huda Teaching Hospital at Thi-Qar province during the period between October 2020 and January 2021 were included in this study as a patient groups. Another group of 25 apparently healthy individuals with age range from (16-43 years) was included in this study

as a control group.

**Exclusion criteria:** Women who smoke and have chronic diseases such as diabetes and cardiovascular disease, women with unknown cause of abortion have been excluded from this study.

From each subject; 3-5 ml of peripheral blood was collected by vein puncture. The collected samples were placed in gel vacuum tube and allowed to clot at room temperature, and then the samples were centrifuged at 3600 round per minute (min) (RPM) for 10 min for serum separation. The collected sera were transferred to the different tube and frozen at -20 °C to be analyzed later, unless used immediately. Hemolysis samples were discarded.

### **Determination of anti-cytomegalovirus IgG and IgM antibodies**

CMV IgG and IgM were measured using enzyme linked immunosorbent assay (ELISA) kits by Bioactiva Diagnostica.

### **Determination of total vitamin D**

Total vitamin D was measured using enzyme linked immunosorbent assay (ELISA) kit by Monobind Inc.

### **Determination of malondialdehyde**

Malondialdehyde (MDA) was measured using enzyme linked immunosorbent assay (ELISA) kit by Sunlong.

### **Determination of Ceruloplasmin**

Ceruloplasmin (Cp) was measured by biochemical method that based on the Cp-catalyzed oxidation of colorless paraphenylene diamine (PPD) to blue-violet oxidize form.

**Determination of transferrin**

Transferrin was measured by measuring both iron concentration and total iron binding capacity (TIBC) and using the following formula

$$TF \left( \frac{g}{l} \right) = TIBC(\mu g/L) \times 0.007$$

**Statistical analysis:** Statistical package for social

sciences (SPSS) version 22 was used. The significance of the difference in the mean of normality distributed variables was assessed by one way ANOVA. Pearson and correlation coefficient analysis was used to assess the correlation between continuous variables. P-values were less than or equal to 0.05 ( $p \leq 0.05$ ) is considered significant.

**Result**

**Table (1) Classification of the study groups**

Total patients infected with CMV						Healthy control group
Aborted women with CMV			Non – aborted women with CMV			
IgG positive	IgM positive	IgG +IgM positive	IgG positive	IgM positive	IgG +IgM positive	
21	0	4	25	0	0	
NO.=25			NO.=25			NO.=25

Table 2 showed the malondialdehyde concentration in total study groups. The results in the table below showed (56%) of aborted women with CMV were had low malondialdehyde concentration while (0%) had high MDA concentration and mean for this group ( $21.74 \pm 5.6$ ), (20%) of non-aborted

women with CMV were had low of malondialdehyde concentration while (12%) had high MDA level with mean equal to ( $28.19 \pm 6.8$ ) and (24%) of control had low level of MDA while (32%) had high MDA concentration with mean equally to ( $28.82 \pm 4.8$ ).

**Table (2): comparison of malondialdehyde concentration in total study groups**

Study Groups/Test	Malondialdehyde Concentration				
	Frequency (%)				Mean Titer (ng/ml)±SD
	Low	Normal	High	Total	
(n=25) Aborted	56	44	0	100	21.74 ± 5.6 a
Non-aborted (n=25)	20	68	12	100	± 6.8 b 28.19
Control (n=25)	24	44	32	100	± 4.8 b 28.82
Total (n=75)	33.3	52	14.7	100	26.25 ± 5.6
LSD	0.645				
p-value	For FR%:CMV+ (Aborted) X CMV+(Non - aborted) 0.04, CMV+(Aborted) X Control 0.03 and CMV+(Non-aborted)X Control 0.3				

(%): percent, SD: standard deviation, n: number, CMV<sup>+</sup>: Cytomegalovirus positive

Note: Each value represents (mean ± SD) values with non-identical superscript (a, b or c ...etc.) were considered significantly different ( $P \geq 0.05$ ).

Table 3 showed the comparison of ceruloplasmin concentration in total study groups. The results in the table below showed (52%) of aborted women with CMV had low ceruloplasmin concentration and only

(4%) had high Cp level with mean for all cases (20.440 ±4.6). Among non-aborted group with CMV (28%) had low level of CP while (12%) had high level of Cp concentration and had mean equal to (23.880 ±7.0) .In control group (32%) had low Cp concentration while (8%) had high level of ceruloplasmin with mean equal to (23.508 ± 18).

**Table (3): comparison of ceruloplasmin (Cp) concentration in total study groups**

Study Groups/Test	Ceruloplasmin Concentration				
	Frequency (%)				Mean Titer (mg/dl)±SD
	Low	Normal	High	Total	
Aborted (n=25)	52	44	4	100	±4.6 a 20.440
Non-aborted (n=25)	28	60	12	100	±7.0 a 23.880
Control (n=25)	32	60	8	100	± 6.4 a 23.508

**Cont... Table (3): comparison of ceruloplasmin (Cp) concentration in total study groups**

Total (n=75)	37.3	54.7	8	100	± 18 22.60
LSD	0.344				
p-value	For FR%:CMV+ (Aborted) X CMV+(Non - aborted) 0.04, CMV+( Aborted) X Control 0.03 and CMV+(Non - aborted) X Control 0.5				

(%): percent, SD: standard deviation, n: number, CMV<sup>+</sup>: Cytomegalovirus positive

Comparison of Transferrin (Tf) concentration in total study groups showed in table 4. The results in the table below showed that (28%) of total aborted group had high transferrin level and means for this group was (3.4180 ± 0.98). Non- aborted group showed the higher mean (3.6568 ± 1.210) and about (36%) were showed high level of transferrin while in control group (40%) had high transferrin level and mean for this group (3.5277. ± 0.94).

**Table (4): comparison of Transferrin (Tf) concentration in total study groups**

Study Groups/Test	Transferrin Concentration				
	Frequency (%)				Mean Titer (g/l)±SD
	Low	Normal	High	Total	
Aborted (n=25)	0	72	28	100	± 0.98 <sup>a</sup> 3.4180
Non-aborted (n=25)	0	64	36	100	± 1.21 <sup>a</sup> 3.6568
Control (n=25)	0	60	40	100	± 0.94 <sup>a</sup> 3.5277
Total (n=75)	0	65.33	34.7	100	± 1.08 3.535
LSD	0.238				
p-value	For FR%:CMV <sup>+</sup> X CMV <sup>+</sup> (Non - aborted) 0.05, CMV <sup>+</sup> (Aborted)X Control 0.03 and CMV <sup>+</sup> (Non-aborted) X Control 0.05				

(%): percent, SD: standard deviation, n: number, CMV<sup>+</sup>: Cytomegalovirus positive

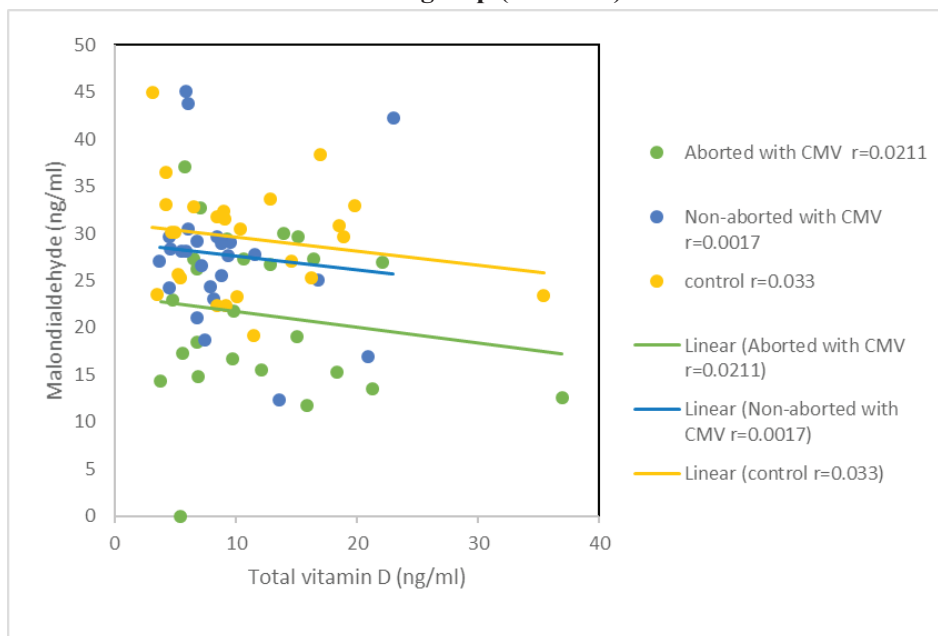
A comparison of total vitamin D concentration in total study groups is illustrated in table 2. In aborted group with CMV (44%) of cases had sever vitamin D deficiency with equal to (11.467 ±4.9) in contrast to non-aborted and control groups which (60%),(36%) of cases had sever VD deficiency with mean equal to (7.916 ± 2.5), (8.452 ± 4.0) respectively.

**Table (5): comparison of total vitamin D concentration in total study groups**

Study Groups/Test	Vitamin D Concentration Total					Mean Titer (ng/ml)±SD
	Frequency (%)					
	Very sever deficiency	Sever deficiency	Deficiency	Normal	Total	
Aborted (n=25)	8	44	36	12	100	11.467 ±4.9 a
Non-aborted (n=25)	16	60	16	8	100	± 2.5 b 7.916
Control (n=25)	20	36	40	4	100	± 4.0 b 8.452
Total (n=75)	14.7	46.7	30.6	8	100	± 3.8 9.278
LSD	3.015					
p-value	For FR%: CMV+ (Aborted) X CMV+( Non -aborted) 0.05, CMV+ (Aborted) X Control 0.05					

(%): percent, SD: standard deviation, n: number, CMV<sup>+</sup>: Cytomegalovirus positive

**Figure (1) shows the negative correlation between vitamin D and malondialdehyde in aborted with CMV, control groups and positive correlation in non-aborted with CMV group. Aborted with CMV with coefficient correlation (r = 0.0211), non-aborted with CMV with coefficient correlation (r = 0.0017) and control group (r = 0.033).**



**Figure (1): Correlation between vitamin and malondialdehyde in all study groups**

## Discussion

Precisely regulated balance between the production of reactive oxygen species (ROS) and the activity of the components of antioxidant protection is required for the maintenance of integrity and function of all cells and tissues in normal pregnancy [12]. Any disruption in the balance between antioxidants and oxidants during pregnancy may cause serious problems, such as abortion. In my current study, we have investigated the oxidative status by measuring the malondialdehyde (MDA) as lipid oxidative damage marker and antioxidants (ceruloplasmin and total transferrin) in aborted and non- aborted women and compared that with healthy women who had normal delivery. In agreement with the previous research [13], aborted women had experienced lower levels of oxidative damage measured by serum MDA in as compared with non-aborted and control groups . The exact reason for such decreasing of MDA level is , one possible mechanism that lipid peroxidation products may affect viral replication through modulation of the activation state of cells, regulation of host inflammatory and immune responses, and by causing oxidative damage to host tissues and viral components. Oxidative damage of infected and adjacent cells may also limit viral spread [6]. Other previous researches had reported that viral infection induce server oxidative stress that disagree with my current study [14], [15].

Ceruloplasmin is protein composed of 1,046 amino acids and has potential role in scavenging reactive oxygen radicals, CP is also recognized as a part of plasma antioxidant system of. The decreased level of lipid damage was seen to be paralleled with low levels of serum ceruloplasmin among aborted women infected with CMV that show low means level for ceruloplasmin and in matching to non-aborted

and control groups, suggesting that the subjects may have been under non-stressed physiological state. The previous studies that correlate the level of CP and CMV infection that agree with my current study Sato et al., (2007) who find that CP decreased in patients with CMV infection. Another studies that correlate the level of CP and CMV infection and they find insignificant differences between the normal and infected patients [16], [17] which approved results different to results obtained in this study. The other possible explanation for decreasing the concentration of CP among aborted women infected with CMV is effectiveness of antioxidant as CMV infection inhibitor that approved by Kotelnikova et al., (2011) that worked on human embryonic fibroblast cell culture had reported that introduction of antioxidant decreases the concentration of virus proteins in the cells to values approaching the protein concentration in the non-infected cell culture [18]. Transferrin is the major carrier protein of iron. Iron ions are delivered in the blood by the protein transferrin, each transferrin molecule can carry two iron ions. The antioxidant property of transferrin is its ability to bind with iron ions and storage it as a ferritin and prevent the oxidative role of iron which allows to generate free radicals by Fenton and Haber-Weiss reactions [19]. Because both the host and pathogen require iron, the innate immune response carefully orchestrates control over iron metabolism to limit its availability during times of infection to prevent oxidative stress or microbial proliferation during infection. In the present study both the iron and transferrin were low in contrast to non –aborted and control groups in agreement with other study performed AL-Salih et., (2019) that reported iron level was decreased in aborted women with CMV[20] . In most cases, iron is retained within macrophages of the reticuloendothelial system, leading to inappropriately low availability of iron

bound transferrin required for erythropoiesis. In these inflammatory states, release of cytokines leads to elevated hepcidin expression, diminishing ferroportin on the surface of enterocytes, recycling macrophages and hepatocytes, sequestering iron in storage sites and diminishing iron uptake from the diet [21], [22]. Hypovitaminosis D, a highly prevalent health problem among pregnant females. In current study, all studied groups had low level of vitamin D concentration. Of total 75 studied cases 97% have low level of Vitamin D and 55% of them was sever deficient for vitamin D. This was similar to previous studies among pregnant women in Iran [23]. Considering the diversity of factors which affect vitamin D level in different countries including sunshine exposure, wearing habits, diet, season of study, variation of vitamin D deficiency prevalence is expected. But it is notable that the place of our study (Thi-Qar province) is sunny place most of the days and living in apartment is not as common as other countries. So maybe clothing habits, harmful effects of sun on skin and unawareness of the necessity of sunlight for synthesizing vitamin D are the most affecting factors which determine vitamin D levels in women. The placenta specifically the maternal decidua which forms the interface with newly implanted fetal placental trophoblast cells considered as a site of immune privilege that may act as an important target for vitamin D3. It has previously been hypothesized that D3 may help to support successful implantation by attenuating decidual T-cell function [24] so low level of vitamin D can leads to abortion. A cross – sectional study performed in Egypt had reported that 30% of pregnant studied women that receive VD3 supplementation their pregnancy ended with miscarriage versus 45% of the control group that don't receive VD3 supplementation. Risk of miscarriage decreased by 15% in the study group compared to the control group [25]. Vitamin D may contribute to the

immune system's ability to limit viral shedding and therefore play a role in limiting perinatal and early postnatal CMV transmission. Bearden et al., (2020) approved that lower levels of maternal bioactive vitamin D, were associated with increased congenital transmission of CMV and consequently abortion that agree with my current study [26]. Vitamin D is one of the key controllers of systemic inflammation, oxidative stress. In turn, molecular and cellular actions form of VD slow down oxidative stress, cell and tissue damage. On the other hand, hypovitaminosis D impairs mitochondrial functions, and enhances oxidative stress and systemic inflammation [27]. This study show a significant negative correlation between MDA as an oxidative stress maker and total vitamin D in agreement with other previous studies [28], [29] .

### **Conclusion**

Total vitamin D, malondialdehyde, ceruloplasmin and transferrin is significantly decreased in aborted group with CMV. The exact reason for such decreasing of these is , one possible mechanism that lipid peroxidation products may affect viral replication through modulation of the activation state of cells, regulation of host inflammatory and immune responses, and by causing oxidative damage to host tissues and viral components. Vitamin D have a negative correlation with malondialdehyde.

### **Ethical Clearance and financial support**

Lastly the ethical approval for this study was issued by the ethical committee of college of science of Thi-Qar University. Moreover there was a financial support from college of science in Thi-Qar University.

### **Conflict of Interest: Nil**

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