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A STUDY THE RELATION BETWEEN NITRIC OXIDE AND SOME TRACE ELEMENTS IN SERA OF ABORTED WOMEN WITH CYTOMEGALOVIRUS

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ABSTRACT

Abortion is one of the commonest reasons for acute presentation to gynaecologists, 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(60) patients (females) aborted with cytomegalovirus and (60) negative control (females) aborted without cytomegalovirus and (60) positive control (healthy women).The study was designed to determine and compare the levels of (NO, Fe, Cu)in study groups and evaluate of the relationship between nitric oxide and (Cu, Fe) instudy groups.The results show a significant increase in levels ofserum NO in patient group in comparing withnegative control groupand positive control group($P > 0.05$), asignificant increase in levels of serum Cu in negative control group in comparing with patient group and positive control group($P > 0.05$)and significant decrease in levelsof serum Fe levels in patient groupin comparison with negativecontrol group and positive control group. ($P > 0.05$).The results of this studyshowdisorder in levels of(NO, Fe, Cu)patients group (females) aborted with cytomegalovirus.

KEYWORDS: Abortion, Cytomegalovirus, Nitric oxide, Cupper, Iron.

INTRODUCTION

Abortion is one of the commonest reasons for acute presentation to gynaecologists, which occurs in 10.9–30% of all early pregnancies (Nybo Andersen AM*et al.*, 2000; Farquharson, RG *et al.*, 2005). About half of all early miscarriages occur due to a genetic problem within the ova or sperm. In addition to other factors such as immune system problems and serious infections can increase the risk of miscarriage. The chance of happening a miscarriage also increases with age (Christopher F *et al.*, 2010; Baker PN *et al.*, 2006). Recurrent spontaneous abortion due to maternal infections transmissible in uterus at various stage of gestation can be caused by a wide array of organisms which include (Cytomegalovirus, Rubella virus) (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, 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. The frequency of vertical transmission and severity of the consequence is reported to be much greater for primary maternal infection (Fowler *et al.*, 1992). Nevertheless, non-primary infection is more common than primary infections and thus likely contributes more total cases of congenital CMV infection and related disability(Wang *et al.*, 2011; Mussi-Pinhata*et al.*, 2009; de Vries *et al.*, 2013). Although the detection of maternal anti-CMV antibodies is easy to accomplish, there is still no consensus about serological screening during pregnancy (Johnson *et al.*,2013). Normally positive immunoglobulin class M (IgM) antibodies indicate an acute and recent infection, whereas positive immunoglobulin class G (IgG) antibodies indicate past infection; In addition, IgM can remain positive for several months making it difficult to establish the time of infection (Duff P *et al.*, 2007). Diagnosis of primary maternal CMV infection dependson detection of virus-specific Immunoglobulin G(IgG) antibody in the blood of a pregnant womanwho was earlier seronegative,or on detectionof specific Immunoglobulin M (IgM) antibodyassociated with low IgG avidity (Sonoyama *et al.*,2012). Nitric oxide (NO) is a free radical, an uncharged molecule with an unpaired electron. NOplays multiple roles in both intracellular and extracellular signaling mechanisms (Moncada *et al.*, 1991). NO is perhaps the most important among the group of early mediators produced by cells of the innate immune system. Phagocytes constitute the first line of microbial defence and they function by sensing the presence of different types of infectious agents (Carneiro-Sampaio & Coutinho,2007). Fallopian tube relaxation and contraction is regulated by NO. In addition, it is cardinal for the maintenance of blood flow to the reproductive organs, particularly the uterus by vasodilatation (Randy, 2013 ; Krassas *et al*., 2013). Trace elements are present in human body at a very low concentration values at amounts of microgram (µg) to milligrams (mg) that are essential for certain biomedical processes (Stephen, 1992). Trace elements form part of daily diet, which are well known to play vitally important roles in the perpetuation of health (Hasan, 2013). Every one of trace element contributes differently in many important physiological and biochemical processes in the body ,the interaction between trace elements in biological processes play a role in mediating biological and chemical reactions which could be used to the management of human health (Rasdi F *et al*.,2013). Deficiencies of trace elements such as zinc, copper, selenium and magnesium have been implicated in various reproductive events like infertility, pregnancy loss, congenital anomalies, preeclampsia, placental abruption, premature rupture of membranes, still births and low birth weight (Chang SC *et al*.,2003). Iron is the most abundant transitional metal in the body. The critical role of iron in the pathophysiology of disease is derived from the easiness with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical(Mohammed, 2013; Swaminathan *et al*., 2007). Copper is an essential trace element, an important catalyst for heme synthesis and iron absorption. Following zinc and iron, copper is the third most abundant trace element in the body. Its role as a cofactor component of cytochrome oxidases, superoxide dismutase, tyrosinase, uricase, dopamine ß-hydroxylase, lysyl oxidase and ceruloplasmin make it a key micronutrient for oxidative pathways (Siddiqui *et al*., 2006). The aim of this study is to evaluate levels of nitric oxide serum and levels of some trace (Cu, Fe) and evaluate of the relationship between nitric oxide and (Cu, Fe) inStudy groups.

MATERIAL AND METHODS

This study is conducted at the Bent Al-Huda hospital in Thi-Qar from Biochemistry Laboratory in College of Science, at the period between (November, 2017) to (May, 2018).The study included (180) subjects,(60) patients (females) aborted with cytomegalovirus aged(15-40) and (60) negative control (females) aborted without cytomegalovirus and (60) positive control (healthy women). Women who smoke and have chronic diseases such as diabetes and cardiovascular disease have been excluded from this study. About (5mL)of blood was obtained from aborted women and control group, the blood was allowed to clot at 37ºC room temperature, and then centrifuged at 3000rpm for 10 min. the serum samples was removed and stored at(- 20ºC).

Determination of anti-CMV antibodies (IgG,IgM): Samples were tested for CMV (IgG,IgM) by ELISA kits commercially available from sigma - aldrich, USA.

Determination of Serum nitric oxide Concentration: Used the method of the researcher (Dervisevic *et al*.,2012). This method includes the measurement of nitrite oxide (NO^{-2}), which is the most stable oxide of nitrogen oxides and the addition of zinc sulphate to the serum sample, which works on the deposition of proteins first and reduction of nitrate oxide (NO^{-3}) to nitrite oxide (NO^{-2}).

Standard Curve: Preparation of nitrate standards: Prepare sodium Nitrate solution (1M) with concentrations between 1-100 µM by deionized the nitrite standard solution with deionized water and addition zinc sulphate to reduce Nitrate to nitrite then deionized water was used as a blind probe with addition of Griess reagent Plot a standard curve of nitric oxide concentration (x-axis) against absorbance (y-axis). By using standard curve of NO the unknown NO is detected by through equation explained in Figure (1).

$$\{y = 0.0022x + 0.0062\}$$

y= Absorbance sample.
x= concentration NO the unknown.

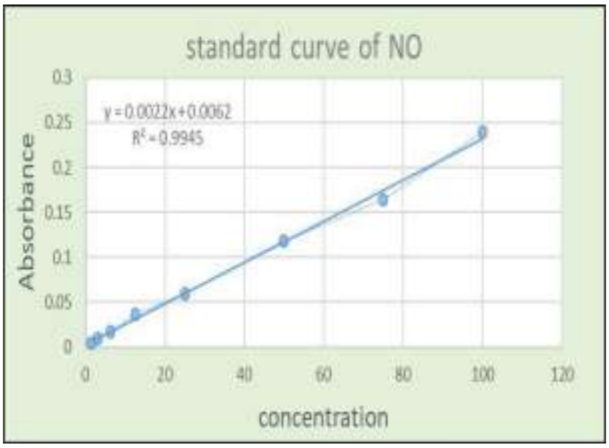

Figure (1): Standard curve of NO.

Determination of Serum Iron and Copper concentration

Digestion of serum: The digestion of serum was being by adding Nitric acid (2ml,70%) and Perochloric acid (1ml,70%) to serum (0.5ml). The mixture was heated for one hour at 160 C by using oil path in abayrex tube. After cooling the solution was compeleted to 10ml with Hydrochloric acid (0.3N) (Sameerah, A *et al*., 2016).

Flame atomic absorption spectrophotometer: To measure the concentration of elements(Fe ,Cu), used method was Flame atomic absorption spectrophotometerwhich is the most sensitive and method of working followed the manufacturer's instructions, The levels of elements (Cu^{+2} ,Fe^{+3}) were determined along a wavelength of (324nm,248nm) Respectively, and expressed their concentrations by ppm.

Statistical analysis

The statistical analysis proceeded in all groups of study, descriptive statistics analyzed by using one-way analysis of variance (ANOVA) were performed using means and standard deviations (SDs) with LSD test for continuous variables and correlation coefficient between parameters. ($p > 0.05$) was considered to be significant. All analyses were performed with the Statistical Package for the Social Sciences SPSS for Windows (version 17.0, SPSS Inc, Chicago, III).

RESULTS

In this study, we estimated the levels nitric oxide, serum iron and copperamong (Patient) aborted women with cytomegalovirus, (negative control)aborted women without cytomegalovirusand (positive control)healthy women. The results shows a significant increase in concentrations of serum NO in patient group and negative control group in comparing with positive control group($P > 0.05$)(Table 1). The results shows a significant increase in concentrations of serum Cu in negative control group in comparing with patient group and positive control group($P > 0.05$), shows a significant increase in concentrations of serum Cu in patient group in comparing with positive control group($P > 0.05$)(Table 2).The resultsshows a significant decrease in concentrations of serum iron levels in patient groupin comparison with negativecontrol groupand positive control group ($P > 0.05$), shows a significant decrease in concentrations of serum iron levels innegativecontrol group in comparison withpositive control group ($P > 0.05$)(Table 3).

Table 1: Serum NO concentration in studied groups.

Groups	No.	NO concentration(µmol/ml) Mean±SD
Patient	60	5.51 ±1.12 [a]
-ve control	60	5.37 ± 1.05[a]
+ve control	60	4.23 ± 1.14[b]
LSD		0.33

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

No: Number of subjects.

-ve control: negative control(Aborted women without CMV).

+ve control: positive control(Healthy women).

Table 2: Serum copper concentration in studied group.

Groups	No.	Cu concentration(µmol/L) Mean±SD
Patient	60	0.29 ± 0.06[b]
-ve control	60	0.36 ± 0.09[a]
+ve control	60	0.23 ± 0.05 [c]
LSD		0.02

Table 3: Serum iron concentration in studied group.

Groups	No.	Fe concentration(µmol/L) Mean±SD
Patient	60	1.26 ± 0.13[c]
-ve control	60	1.30 ±0.14[b]
+ve control	60	1.35 ± 0.15[a]
LSD		0.02

Correlation relationship between NO and (copper, iron) in patient, positive control group and negative control group.

Figure(2) shows the correlation relationship between NO and copperin patient, positive control group and negative control group, patient with coefficient correlation (r =0.21), positive control with coefficient correlation (r = -0.12) and negative control (r= 0.07).

Figure(3) shows thenegative correlation relationship between NO and iron in patient, positive control group and negative control group, patient with coefficient correlation (r = -0.05), positive control with coefficient correlation (r = -0.28) and negative control group(r = -0.31).

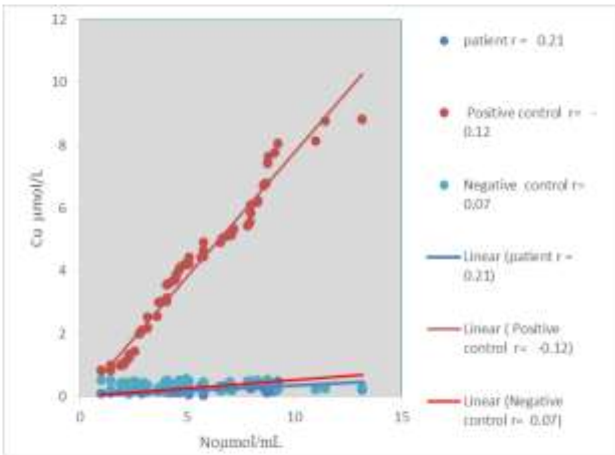

Figure (2): Correlation between NO and Cu in patient and positive control and negative control groups.

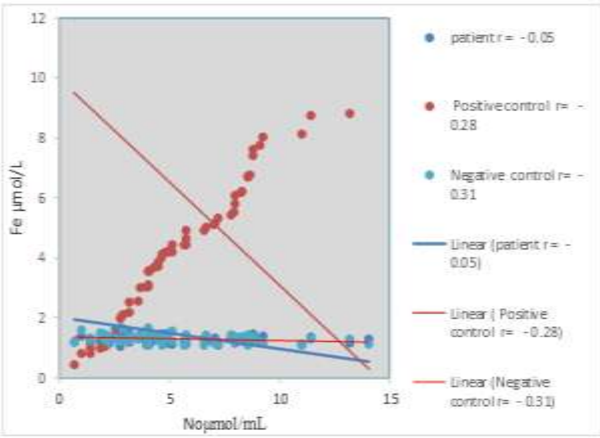

Figure (3): Correlation between NO and Fe in patient and positive control and negative control groups.

DISCUSSION

High levels of NO, such as those produced by macrophages, can negatively influence fertility and can cause pregnancy complications such as abortion. Macrophages stimulate endothelial NO synthase to release NO (Imlay JA and Linn S,1986; Halliwell B and Aruoma OI, 1998; Tsuboi H *et al*., 1998). These abnormal immune responses might eventually stimulate macrophages and/or endometrial cells to persistently produce a large amount of NO and increase its levels(Marcus S *et al*., 2003). Within the female reproductive processes, NO takes part in various events, one of the most established being cervical ripening (Chwalisz K. et al.,1997; Thomson A. J. et al.,1997; Chwalisz K., & Garfield R. E., 1998) by a dose-dependent reduction of spontaneous contractile activity of the uterine cervix early in pregnancy(Ekerhovd E. et al., 1998). Furthermore, NO may increase the vasodilatoreffects of the uterine vascular bed, inhibit the endometrial platelet aggregation, and regulate spontaneous contractile myometrial activity(Rosselli M., 1997). Copper contributes to the production of hemoglobin and contributes to metabolism because it allows many critical enzymes to function properly(Osredkar J and Sustar N, 2011; Harris E,2001). It also acts as a pro-oxidant and an antioxidant, therefore copper has a role in reducing the damage of free radicals that are generated naturally in the body by sweeping or neutralizing free radicals and thus reduce the damage of free radicals on the walls of cells(Bonham M *et al*.,2002;Davis C,2003); increased serum level of Cu may be explained to its form of serum which is formed in response to inflammation associated with the disease (Mitreski A *et al*., 2003; Rajeswari S and Swaminathan S, 2014). Iron (Fe) is an primary transition metal required for the synthesis of two important functional proteins such as hemoglobin and myoglobin, which are involved in the transport of molecular oxygen during respiration (Ganz T and Nemeth E, 2006). There are several causes ofiron deficiency could be a lack of iron in diet, inadequate absorption of iron, or some from of blood loss, such as from menstruation or slow internal bleeding. Iron deficiency can also occur with pregnancy. It can develop at any age (Mayo Clinic Staff, 2004;WHO, 2004).

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