

A STUDY OF OXIDATIVE STRESS, IN PATIENTS WITH HYPEREMESIS GRAVIDARUM

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ABSTRACT

Hyperemesis gravidarum continue to be a major health problem among pregnant women. Lipid peroxidation can be an adverse factor in human pregnancy .The presented study was carried out from the beginning of December 2005 till September 2006 on one hundred females with hyperemesis gravidarum; ninety pregnant women were apparently healthy as control group.The studied females were classified into three groups according to their age, parity and family history. The biochemical parameter measured was Malondialdeyde (MDA) by using stander method. The presented study showed there was a significant increase ($P<0.05$) in serum Malondialdehyde MDA levels in pregnant patients as compared to control groups. The present study consider as an important study which showed how much exposure of pregnant women with hyperemesis gravidarum to oxidative stress as measured by increase serum MDA levels.

INTRODUCTION

Hyperemesis Gravidarum simply means a severe form of nausea and vomiting with onset at usually less than 16weeks of estimated gestational age, have clinical features include persistent vomiting, dehydration, ketosis, electrolyte and metabolic disturbances, weight loss (more than 5 percent of body weight) and nutritional deficiency that may require hospitalization⁽¹⁾. Free radical production (superoxide, hydroxyl and nitric oxide, and other reactive oxygen species (ROS)) occurs as a consequence of the endogenous reactions and plays an important role in the cell. Increased formation of ROS and/or decreased antioxidant defense can be defined as oxidative stress, which is widely recognized as an important feature of many diseases such as diabetes mellitus, cancer, and renal failure. Superoxide dismutase (SOD) and catalase (CAT) are some important endogenous antioxidants, which protect the cells from oxidative stress⁽²⁾. Lipid

peroxidation (LPO) is one of the most important expressions of oxidative stress induced by ROS. MDA is an indicator of LPO which increases in various diseases. Pregnancy, mostly because of the mitochondria-rich placenta, is a condition that favors oxidative stress⁽³⁻⁵⁾. Oxygen free radicals are incriminated in the causation of several neonatal diseases including bronchopulmonary dysplasia, retinopathy of prematurity, persistent ductus arteriosus, necrotizing enterocolitis, intracranial hemorrhage and hypoxic ischemic encephalopathy⁽⁶⁻⁹⁾. The role of oxidative stress is also clear and well known in the pathogenesis of acquired malnutrition⁽¹⁰⁻¹²⁾. Malnutrition involves deficiency of not only the macronutrients *i.e.* fats, proteins, carbohydrates but also results in subphysiological concentration of most micronutrients. Many antioxidant defense systems depend on micronutrients themselves⁽¹³⁾. Proteins provide amino acids for synthesis of antioxidant defense enzymes, reduced

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glutathione and albumin (as sacrificial antioxidant protein). Therefore, one would expect a gross derangement of the antioxidant defense mechanisms in malnutrition. Small for gestational age babies born at term to undernourished mothers provide a unique opportunity to have an insight into the mechanism and implications of the fetal growth retardation, secondary to intrauterine malnutrition. No study is available on the role of oxidative stress in term neonates born out of intrauterine growth retardation as a consequence of maternal malnutrition⁽¹⁴⁾.

MATERIALS & METHODS

A prospective case-control study during the period collect from 1/12/2005 to 1/9/2006 during which 100 pregnant women with sever nausea and vomiting (hyperemesis gravidarum) were collected from obstetric clinic of Basrah Maternity and Children Hospital and Basrah General hospital, their age range between 15-40 years. The pregnant females with hyperemesis gravidarum were allocated into three groups according to their age 15-24 years, 25-34 years, 35-40 years. And also classified according to their number of pregnancies into three groups primigravida, P2-P5, > P5. Also classified according to their family history into two groups: Positive family history, and Negative family history.

Ten (ml) of blood samples were collected by veni puncture using a sterile disposable syringe in plain plastic tubes. The serum was separated immediately after withdrawal and stored in plain tubes at -4°C until used or immediately analyzed. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give red species absorbing at 535 nm⁽¹⁵⁾.

Reagents:

- 1- 75ml of 20% trichloroacetic acid (TCA).
- 2- 2 ml of concentrated hydrochloric acid (37%).

- 3- 0.375 gm of thiobarbituric acid (TBA).

All were mixed and the final volume was completed to 100 ml with distilled water to form TCA- TBA- HCl reagent, the concentration in the final reagent:

15% w/v TCA, 0.375% w/v (TBA), 2% v/v concentrated hydrochloric acid.

This solution may be mildly heated to assist in the dissolution of the barbituric acid.

Procedure:

- 1- 1ml of serum was combined with 2 ml of TCA-TBA-HCL solution and mixed thoroughly, then heated for 15 min. in boiling water bath.

- 2- After cooling the precipitate was removed by centrifugation at 3000 rpm. For 10 min.

- 3- The absorbency was determine at 535 nm against reagent blank, which was containing all the reagent minus the serum.

$$\text{MDA } (\mu\text{mol/liter}) = \frac{\Delta A}{1.56} \times 10$$

All values were expressed as means \pm standard deviation. Data were analyzed by one- way analysis of variance (ANOVA) for equal sample size and independent sample T- test to difference between two groups, using the computerized SPSS program. $P < 0.05$ considered to be lowest limit of significance.

RESULTS

The basic clinical characteristics of all studied females were presented in (Table 1). There were no significant differences in clinical characteristics ($P > 0.05$) between the patients and control groups. All participants were classified according to their age, parity, and family history. In (Table 2) The serum MDA level was significantly increase in patient in comparison to the control ($P < 0.05$), The serum MDA level was significantly increase ($P < 0.05$) in the first, second and third age groups of patients as compared to the same match age groups of the control females,

however there were no significant alteration between the different age group of studied female patients ($P>0.05$). The serum MDA concentration was significantly increase ($P<0.05$) in the first, second and third groups of patients as compared to the same match parity group of the control female. However there was no significant alteration between the different groups of the parity of the studied female patients ($P>0.05$). The serum concentration of MDA was significantly increase in the patient groups in comparison with control groups of the same family history ($P<0.05$).

DISCUSSION

Lipid peroxidation is autocatalytic process where by polyunsaturated fatty acids and phospholipids undergo degradation by a chain reaction to form primary products known as lipid hydroperoxides in cell membranes, body fluids, etc.⁽¹⁶⁾ and variety of secondary metabolites MDA which is established as breakdown product and its measurement an indicator for lipid peroxidation⁽¹⁷⁾.

One of the sources of lipid peroxide is the placenta which are secreted toward the maternal side of the placenta rather than fetal side⁽¹⁸⁾ Pregnancy is a physiological state accompanied by a high energy demand of many bodily functions and an increased oxygen requirement. Because of the increased intake and utilization of oxygen, increased levels of oxidative stress would be expected⁽¹⁹⁾. In this study we revealed a significant increase in the serum MDA level in patients with hyperemesis gravidarum in comparison with healthy pregnant control females. These results are in agreement with

other study published else where in the world⁽²⁰⁾.

The increase in serum MDA level in patients with hyperemesis gravidarum could be due to changes in the balance system of oxidants and antioxidants, pregnancy causes oxidative stress from the first trimester to the third trimester; and hyperemesis gravidarum may aggravate oxidative stress during pregnancy and causes a decrease in nutrient antioxidant, and oxidative disorders occur, when that low values of plasma glutathione in hyperemesis gravidarum patients suggest that oxidative stress is associated with this condition^(5, 21) Also liver disease, usually consisting of mild serum transaminase elevation, occurs in almost 50% of patients with hyper emesis gravidarum, impairment of mitochondrial fatty acid oxidation has been hypothesized to play a role in the pathogenesis of maternal liver disease associated with hyperemesis gravidarum. It has been suggested that women heterozygous for fatty acid oxidation defects develop hyperemesis gravidarum associated with liver disease while carrying fetuses with fatty acid oxidation defects due to accumulation of fatty acids in the placenta and subsequent generation of reactive oxygen species and this could be lead to increase oxidative stress, and then increase in the serum MDA level⁽²²⁾.

CONCLUSION

From this study we can concluded the following:

- 1- A significant rise in serum MDA levels in different groups of patients as compared to control groups.
- 2- No significant alteration was observed in the studied biochemical parameter between groups of patients in regard to family history.

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TABLES:

(Table: 1) characteristics of all studied females

Parameters		Control		Patients	
		No.90	%	No.100	%
Age	15-24	30	33.33	32	32
	25-34	35	38.88	44	44
	35-40	25	27.77	24	24
parity	Primi	25	27.7	38	38
	P2-P5	41	45.55	32	32
	> P5	24	26.66	30	30
Family history	+ve	25	27.7	62	62
	-ve	65	72.22	38	38

Table 2 The serum concentration of MDA in studied females.

Parameters	Controls			Patients		
	15-24years (30)	25-35years (35)	35-40 years (25)	15-24years (32)	25-35years (44)	35-40years (24)
Age	1.179 ± 0.44	1.218± 0.41	1.20± 0.493	1.523± 0.51 ^{a*}	1.48 ± 0.495 ^{a*}	1.56 ± 0.52 ^{a*}
Parity	Primi (25)	P2-P5 (41)	> P5 (24)	Primi (38)	P2-P5 (32)	> P5 (30)
	1.126 ± 0.444	1.255 ± 0.468	1.186 ± 0.390	1.491±0.534 ^{a*}	1.552 ± 0.435 ^{a*}	1.501 ± 0.54 ^{a*}
Family history	1.162± .46	1.22±0.437		1.497± 0.50 ^{a*}	1.543± 0.511 ^{a*}	
	+ve (25)	-ve (65)		+ve (65)	-ve (65)	

- a- Significant between patients and control.
- b- Significant among the three groups of patients.
- * P< 0.05 **P< 0.001

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دراسة مستويات الجهد المؤكسد عند مرضى فرط الحمل

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لازالت حالات (فرط الحمل) تمثل مشكلة صحية كبيرة عند النساء الحوامل وان اكسدة الدهون يمكن أن تشكل عاملاً ضاراً في الحمل عند الانسان. أجريت الدراسة الحالية ابتداءً من شهر كانون الأول لسنة ٢٠٠٥ ولغاية شهر أيلول لسنة ٢٠٠٦ على مائة من النساء الحوامل المصابات بفرط الحمل تراوحت اعمارهن بين (١٥_٤٠) سنة و تسعون من النساء الحوامل ممن كانوا ظاهرياً سليمات كمجموعه ظابطه تراوحت اعمارهن ايضاً بين

(١٥_٤٠) سنة. قسمت المجاميع التي شملتها الدراسة الى ثلاث مجموعات وذلك حسب اعمارهن و عدد الولادات وتاريخ الاسره المرضي. وقد تم قياس المعايير الكيمائية المالونداي الديهايد باستخدام الطرق القياسية. أظهرت الدراسة الحالية أن هنالك زياده معنويه في تراكيز المالونداي الديهايد ($P < 0,05$) في مصل الدم عند النساء الحوامل المصابات مقارنة بالمجموعة الضابطة. أن الدراسة الحالية تعتبر دراسه مهمه والتي أظهرت مدى تعرض النساء الحوامل المصابات بفرط الحمل للجهد المؤكسد وذلك من خلال زياده مستويات المالونداي الديهايد وأنها كذلك تعتبر الدراسة الأولى من نوعها في مدينة البصرة لقياس الجهد المؤكسد عند المرضى المصابين بفرط الحمل.

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