Study the Levels of oxidative stress in Normal Pregnant Women in Ramadi City

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ARTICLE INFO
Received: 6 / 2 /2012
Accepted: 18 / 9 /2012
Available online: 30/11/2013
DOI: 10.37652/juaps.2013.83094
Keywords:
lipid peroxidation, oxidative, antioxidants, pregnancy.

ABSTRACT
The present study aimed at examining the levels of antioxidants in pregnant women (in their 3rd trimester) in Ramadi city. It included collecting blood samples after a 12 hours fasting period from 25 pregnant women in week 36 of their pregnancy at Ramadi Hospital for Maternity and Childhood for the period extending from the 1st of December, 2010 to the 1st of May, 2011. The levels of Malondialdehyde (MDA), Glutathione (GSH), Superoxide Dismutase (SOD) and Vitamins A and C were measured. Results have shown a significant increase in the levels of Malondialdehyde (MDA) and Body mass index (BMI), which were 3.376 (µmol/l) and 29.504(Kg/m²), respectively in the experiment group in comparison with the control group which were 1.795(µmol/l) and 24.68(Kg/m²), respectively. Superoxide Dismutase (SOD) show significant decrease 0.013 in the experiment group in comparison with the control group which was 0.082. Nonenzymic antioxidants namely; Glutathione and vitamins A and C, on the other hand, revealed a significant decrease, 3.616(µmol ), 0.361(mg/l) and 0.457(mg/l), respectively in the experiment group in comparison with the control group, which were 10.591(µmol), 0.668(mg/l) and 1.283(mg/l), respectively.

Introduction
Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent(1). In recent years, the role of decreasing antioxidants and increasing superoxide is gaining importance as these are a threat to normal pregnancy. Certain biochemical indices are useful in assessing the progress of pregnancy. The generation of free radicals is a normal physiological process and free radicals act on lipids to cause lipid peroxidation(2). The cells have evolved a number of counteracting antioxidant defences. These antioxidant defense mechanisms can be categorized under the heads of free radical scavenging and chain breaking antioxidants. Reduced glutathione, alpha tocopherol, ascorbic acid and retinol are nonenzymatic chain breaking antioxidants which limit the cellular concentration of free radicals and prevent excessive oxidative damage(3). Oxidative damage has been implicated in pathogenesis of many diseases and tissue injury in animals and humans.

The presence of reactive oxygen species (ROS) in cells using oxygen as an energy source led to the formation of protective mechanisms. Recent evidence suggests that these act as a form of an integrated antioxidant system(4). The antioxidant system is composed of a number of components including : enzymes, proteins and small molecules. The antioxidant system comprises antioxidants inhibiting the formation of free radicals, free radical scavengers, and a series of mechanisms involved in the recovery of damages caused by free radicals. A healthy organism maintains equilibrium among production, use and neutralization of ROS. Disruption of this equilibrium leads to an oxidative stress. The present study was undertaken to assess the role of antioxidants, lipid peroxidation and superoxide generation in normal pregnancy.

Material and methods
The study comprised 10 healthy non-pregnant women representing the control group and 25 normal pregnant women (in 3rd trimester) as subjects. The subjects and the control groups were in the age ranging between 16 to 30 years, and were attending for
anténatal checkup at Ramadi Hospital for Maternity and Childhood, Iraq. Body height and weight of the subjects were measured to calculate their body mass index (BMI). Blood samples were collected from the women in the morning of the fasting overnight. 5 ml of blood was collected in white tubes from each woman by venipuncture to separate the serum and it was stored at 4°C until being processed. Lipid peroxidation (MDA) was determined in the serum using the method proposed by Guidet& Shah (5). The concentration of glutathione (GSH) was determined in the serum by the method proposed by Sedlak & Lindsay(6,7). The activity of Superoxide Dismutase in blood was determined using the Modified Photochemical Nitroblue Tetrazolium (NBT) Method (8). Vitamin A was estimated following Wootton's procedure, (9). Vitamin C concentration was determined in the serum using Stanley's method (10).

The results were statistically analyzed using students t-test and values were expressed as percentage and mean± standard deviation.

**Results and discussion**

Free radicals by their unstable and transient nature are difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. In the present study, BMI was increased significantly in pregnant women (29.5 Kg/m²) (Table 1), when compared to non-pregnant (24.68 Kg/m²) because, at birth, a baby weighs about 3.3kg, the placenta, which keeps the baby nourished, weighs 0.7kg and the amniotic fluid, which supports and cushions the baby, weighs 0.8kg (11). BMI was widely accepted as a better measure of over or underweight(12). Many studies have shown that pregnancy weight gain within the Institute of Medicine (IOM)recommended ranges is associated with the best outcome for both mothers and infants (13).

**Table 1:** Oxidative and antioxidant parameters in pregnant women as compared to controls

| Parameter       | pregnant Mean (SD) | Control Mean (SD) | p     |
|-----------------|--------------------|-------------------|-------|
| BMI (kg/m²)     | 29.5(0.04)         | 24.68(0.08)       | 0.004 |
| MDA (µmol/l)    | 3.372              | 1.795             | 0.001 |
| GSH (µmol/l)    | 0.457              | 0.143             | 0.001 |
| SOD (µmol/l)    | 0.488              | 0.428             | 0.001 |
| Vitamin A (mg/l)| 1.328              | 0.428             | 0.001 |
| Vitamin C (mg/l)| 0.361              | 0.238             | 0.001 |

Markers of lipid peroxidation (MDA) has increased during the progression of normal pregnant subjects (3.372µmol/l) as compared to non-pregnant controls (1.795 µmol/l p<0.001).Such alteration suggests an easier membrane lipo-peroxid ability and, consequently, easier membrane damage during gestation. (14, 15).

A significant decrease of glutathione was observed in pregnant women (3.617 µmol/l ) as compared to non-pregnant controls (10.591 µmol/l (p<0.001). The decrease in the levels of glutathione may be attributed to the increased turnover; preventing oxidative damage in these patients suggests an increased defense against oxidant damage (16). SOD significantly decreased in pregnant women (0.013 (p< 0.01)when compared to non-pregnant (0.082) (p< 0.01). SOD is the important antioxidant enzyme having an antitoxic effect against super oxidation. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide(16). Vitamin A is the most important chain breaking antioxidants and they protect polyunsaturated fatty acids from oxidative damage by donating hydrogen to the lipid peroxy radical. The present study has indicated a significant decrease of vitamin A (0.457mg/l) in pregnant as compared to non-pregnant women (1.283 mg/l p<0.001), Vitamin C experienced a significant decrease in pregnant women (0.361 mg/l ) as compared to non-pregnant women.
Antioxidant vitamins, with the ability to stabilize highly reactive free radicals, act as the first line of defense against free radical attacks and lipid peroxidation. Vitamin C is an important aqueous phase antioxidant. Antioxidants may act synergistically, for instance, when vitamin C regenerates α-tocopherol from the tocopherol radical, the ‘sacrificial’ antioxidant acts more actively by sparing vitamin E than by recycling it. The important role of vitamin C in gestational suggests that changes in its concentration may influence susceptibility of vascular endothelium to oxygen toxicity. Thus, our present study on vitamin C concentration may provide a means of assessing the total capacity of chain-breaking antioxidants to prevent lipid peroxidation in plasma and it might be important to evaluate the effectiveness of potential antioxidant defense systems in a limited scale (17, 18).

A similar observation was made by Patil et al (19) which revealed decreased levels of glutathione, vitamin C and Vitamin A. This may be due to the increased lipid peroxidation. Kumar and Das found a decreasing trend in the levels of vitamin C throughout the gestational phase but the decrease was not significant compared to the levels in controls(20). Sharma et al found a raising oxidative stress and low antioxidant status during pregnancy (21). In contrast to our observation, some studies have reported that there is no evidence of increased lipid peroxidation in pregnancy(22). The present study has revealed decreased levels of glutathione, superoxide dismutase, vitamin A, vitamin C and increased levels of malondialdehyde. This may be due to the increased lipid peroxidation.

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