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Adiponectin, apelin, and the Role of Oxidation and Antioxidants in Pregnant Women in Samarra City

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Abstract: The aim of the research was to find the relationship between some physiological and biochemical variables in pregnant women and their effect on certain vital functions in the body before and during pregnancy. Samples were collected from women visiting Samarra General Hospital and the outpatient medical clinics in Samarra city during the period from mid-September 2023 to early November 2023. A total of 90 blood samples were collected and divided into three groups: (30) control samples from married women who are not pregnant and do not use contraceptives, (30) samples from pregnant women in the period of 4 to 6 months of pregnancy, and (30) samples from pregnant women in the period of 7 to 9 months of pregnancy.

The results showed a significant increase ($P \leq 0.05$) in the concentration of apelin in pregnant women during the period of 7-9 months of pregnancy (33.615 ± 110.332) compared to the groups of pregnant women during the 4-6 months period (12.557 ± 85.967) and the healthy control group (5.565 ± 50.045), with a significant difference between the pregnant women in the 4-6 months period and the control group. A significant increase ($P \leq 0.05$) was also observed in the concentration of adiponectin in pregnant women during the 7-9 months period (0.682 ± 4.904) compared to the groups of pregnant women during the 4-6 months period (1.345 ± 4.194) and the healthy control group (0.461 ± 3.005), with a significant difference between the pregnant women in the 4-6 months period and the control group. Additionally, a significant decrease ($P \leq 0.05$) was found in the concentration of catalase in pregnant women during the 7-9 months period (1.813 ± 4.465) compared to the groups of pregnant women during the 4-6 months period (0.880 ± 5.824) and the healthy control group (1.545 ± 10.272), but there was a significant increase in the control group compared to pregnant women in the 4-6 months period. A significant increase ($P \leq 0.05$) was also observed in the concentration of MDA in pregnant women during the 7-9 months period (6.820 ± 24.016) compared to the groups of pregnant women during the 4-6 months period (11.600 ± 19.623) and the healthy control group (1.518 ± 8.813), with a significant difference between the pregnant women in the 4-6 months period and the control group.

1 Introduction

Pregnancy is the condition that occurs as a result of the fertilization of the egg by the sperm, leading to changes at the level of the fertilized egg, which ultimately results in the formation of a fully developed embryo ready for birth. Pregnancy lasts for about nine months, during which the pregnant woman undergoes many physiological changes to accommodate the growth and development of the fetus (1). The growth of one or more embryos occurs inside the mother's womb, known as the embryo during the first eight weeks of pregnancy. After that, it is referred to as the living fetus until birth (2). Pregnancy can be single or multiple, as in the case of twins or triplets, and it is accompanied by hormonal and metabolic changes (3).

Pregnancy is considered a severe and comprehensive stress condition associated with deep hormonal, biochemical, anatomical, and psychological changes that affect metabolic processes as well as hepatic excretory functions (4). Pregnancy lasts approximately 40 weeks and can be divided into three time periods. The first period starts from the beginning of pregnancy to 13 weeks, and is

characterized by the highest risk of miscarriage. The second period spans from 13 to 26 weeks, during which fetal growth and development occur. The third period begins from 26 to 42 weeks, marking the start of the fetus's life (5). During pregnancy, women undergo numerous metabolic and hormonal changes, with metabolic processes in the female body adapting to the physiological demands of pregnancy, including the needs of the fetus for growth and development until birth (3). Pregnancy is a period of profound hormonal and metabolic changes in the body that lasts for a relatively short time, during which various changes in the skin, nails, and hair occur (6). Maternal milk provides nutrition for the baby and is associated with long-term health benefits for both infants and mothers. A healthy diet is an important means for breastfeeding mothers to support optimal health for themselves and their children (7).

2. Materials and methodes

1.1 Study design:

The study sample consisted of 90 women, divided into three groups as shown in Figure (3-1), as follows:

1- 30 control samples: Married women who are not pregnant and do not use contraceptives.

2- 30 pregnant women in the 4-6 months period of pregnancy.

3- 30 pregnant women in the 7-9 months period of pregnancy.

Samples were collected from women visiting Samarra General Hospital and the outpatient medical clinics in Samarra city, from mid-September 2023 to early November 2023.

1.2. Physiological and biochemical test:

A set of physiological and biochemical parameters was measured and included for the studied groups

APLN Determination

The ADP concentration was measured using a ready-to-use kit specifically designed for ADP estimation, employing a competitive enzyme reduction assay for the quantitative in vitro measurement of adiponectin in human serum, plasma, and other biological fluids.

The ready-to-use kit involves an ELISA test, where the test plate is pre-coated with an antibody specific to ADP, with 96 wells.

The microplate provided in this kit is pre-coated with antibodies specific to adiponectin. Standards or samples are added to the appropriate wells, along with a biotin-conjugated antibody specific to adiponectin. Then, avidin conjugated with Horseradish Peroxidase (HRP) is added to the wells for further reaction. After adding the TMB substrate solution, color changes will occur only in the wells containing adiponectin, the biotin-conjugated antibody, and the HRP-conjugated avidin. The enzyme reaction is stopped by adding sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm with a precision of ±10 nm. The adiponectin concentration in the samples is determined by comparing the optical density of the samples with the standard curve **.**

ADP Determination

The ADP concentration was measured using a ready-to-use kit specifically designed for ADP estimation, employing a competitive enzyme reduction assay for the quantitative in vitro measurement of adiponectin in human serum, plasma, and other biological fluids.

The ready-to-use kit involves an ELISA test, where the test plate is pre-coated with an antibody specific to ADP, with 96 wells.

The microplate provided in this kit is pre-coated with antibodies specific to adiponectin. Standards or samples are added to the appropriate wells, along with a biotin-conjugated antibody specific to adiponectin. Then, avidin conjugated with Horseradish Peroxidase (HRP) is added to the wells for

further reaction. After adding the TMB substrate solution, color changes will occur only in the wells containing adiponectin, the biotin-conjugated antibody, and the HRP-conjugated avidin. The enzyme reaction is stopped by adding sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm with a precision of ±10 nm. The adiponectin concentration in the samples is determined by comparing the optical density of the samples with the standard curve.

CAT Determination

The CAT concentration was determined using a ready-made kit specifically designed for CAT estimation. This is a competitive enzyme reduction assay technique for the quantitative measurement of catalase in human serum, plasma, and other biological fluids in vitro.

The kit consists of an ELISA test, with a test plate pre-coated with an antibody for CAT at a concentration of 96 wells.

The microplate in this kit is pre-coated with a catalase-specific antigen. Standards or samples are added to the appropriate wells along with a biotin-conjugated antibody for catalase. Then, avidin conjugated with horseradish peroxidase (HRP) is added to each well and allowed to react. After adding the TMB substrate solution, wells containing catalase, the biotin-conjugated antibody, and the avidin-HRP conjugate undergo a color change. The enzyme-substrate reaction is stopped by adding sulfuric acid, and the color change is measured using a spectrophotometer at a wavelength of 450 nm ± 10 nm. The catalase concentration in the samples is determined by comparing the optical density values of the samples with the standard curve.

MDA Determination

The MDA concentration was determined using a ready-made kit specifically designed for MDA estimation. This is a competitive enzyme reduction assay technique for the quantitative measurement of malondialdehyde (MDA) in human serum, plasma, and other biological fluids in vitro.

The kit consists of an ELISA test, with a test plate pre-coated with an antibody for FE concentration, with 96 wells.

This method uses an enzyme-linked immunosorbent assay (ELISA) based on competitive inhibition. The microplate is pre-coated with monoclonal antibodies specific to malondialdehyde. A competitive inhibition reaction begins between biotin-labeled malondialdehyde and unlabeled malondialdehyde (either standards or samples) with the antibodies bound to the plate. After an incubation period, the unbound complex is washed away. Then, avidin conjugated with horseradish peroxidase (HRP) is added to each well and incubated. The amount of HRP bound is inversely proportional to the malondialdehyde concentration in the sample. After adding the substrate solution, the intensity of the resulting color is inversely proportional to the malondialdehyde concentration in the sample

3. Statistical analysis

The results of the current study were analyzed using the SAS 2001 statistical software. ANOVA was used to estimate the variance between groups, and significant differences between the means were tested using the Duncan Multiple Range Test to compare four groups at a significance level of 0.05, in order to determine the degree of significant differences between these groups.

4. Results and Discusstion

4.1. Results:

Table (1) shows a significant increase ($P \leq 0.05$) in the concentration of apelin in pregnant women during the period of 7-9 months of pregnancy (33.615 ± 110.332) compared to the two groups of pregnant women in the 4-6 months of pregnancy period (12.557 ± 85.967) and the healthy control group (5.565 ± 50.045), with a significant difference observed between the 4-6 months pregnancy group and the control group. Similarly, a significant increase ($P \leq 0.05$) in the concentration of

adiponectin was observed in pregnant women during the 7-9 months period (0.682 ± 4.904) compared to the 4-6 months pregnancy group (1.345 ± 4.194) and the healthy control group (0.461 ± 3.005), with a significant difference found between the 4-6 months pregnancy group and the control group.

Table (1) APLN and ADP concentration in the studied groups.

Pregnancy Period	Apln Levels (Mean ± SD)	ADP Levels (Mean ± SD)
7-9 Months	(110.332 ±33.615)	(4.904 ± 0.682)
4-6 Months	(85.967±12.557)	(4.194 ± 1.345)
Control Group	(50.045 ± 5.565)	(3.005 ± 0.461)

The results presented in Table (2) show a significant decrease ($P \leq 0.05$) in catalase concentration in pregnant women during the 7-9 months of pregnancy period (1.813 ± 4.465) compared to the two groups of pregnant women during the 4-6 months period (0.880 ± 5.824) and the healthy control group (1.545 ± 10.272). However, a significant increase was observed in the control group compared to the 4-6 months pregnant women group.

Additionally, a significant increase ($P \leq 0.05$) in MDA concentration was observed in pregnant women during the 7-9 months period (6.820 ± 24.016) compared to the two groups of pregnant women during the 4-6 months period (11.600 ± 19.623) and the healthy control group (1.518 ± 8.813), with a significant difference between the 4-6 months pregnant women group and the control group.

Table (2) CAT and MAD concentration in the studied groups.

Pregnancy Period	CAT (mean ± SD)	MAD (mean ± SD)
7-9 Months	± 1.813) (4.465	± 6.820) (24.016
4-6 Months	± 0.880) (5.824	± 11.600) (19.623
Control Group	± 1.545) (10.272	± 1.518) (8.813

4.2. Discussion:

The current study showed a significant increase in apelin concentration in pregnant women during the 7-9 months period compared to the two groups in the 4-6 months period and the healthy control group. The increase in apelin levels in the later stages of pregnancy may be attributed to its role in stimulating uterine contractions, and its mechanism that links apelin to promoting cell proliferation and preventing programmed cell death in osteocytes in humans. This could represent a potential mechanism connecting apelin with normal fetal development and preventing Intrauterine Growth Restriction (IUGR) (8). Moreover, the increase in the apelin receptor (APJ) and associated peptides such as Elabela / Toddler during pregnancy regulates the adipoinsular axis and supports the function of both the cardiovascular and central nervous systems. The apelin system is essential for the formation of the fetal heart and blood vessels, placental growth and function, and plays a role in the initiation of labor. Administration of exogenous apelin has been shown to increase the weight of low-birth-weight infants (9). Since apelin's effect increases as the placenta develops, it is considered a hormonal marker for vascular cells during angiogenesis, as it is present in high

concentrations in placental tissues such as cytotrophoblasts, syncytiotrophoblasts, and stromal cells. Apelin also affects the integrity and metabolism of stem cells (10).

The results for adiponectin showed an increase in its levels during the 7-9 months period compared to the 4-6 months groups and the healthy control group. Adiponectin is a hormone derived from adipose tissue that circulates in the body and regulates various physiological processes, including energy metabolism, insulin sensitivity, and is associated with body mass index (11). The regulation of the adiponectin gene by DNA methylation is a genetic factor influenced by both genetic and environmental factors (12). Adiponectin levels significantly increase in the blood during pregnancy as a response to physiological changes in the body, including the relative insulin resistance that accompanies pregnancy. This stimulates the body to produce more adiponectin to enhance insulin sensitivity and improve glucose metabolism. Additionally, hormonal changes, such as increased pregnancy hormones, play a crucial role in regulating adiponectin levels. Adiponectin also contributes to protecting both the mother and the fetus by reducing inflammation and improving vascular function (13).

Catalase levels showed a decrease throughout pregnancy to its end in all stages, as pregnancy is a physiological process that requires high energy and more oxygen for the body to function. There is a correlation between pregnancy and the activity of some oxidative enzymes, including catalase, which delays oxidative stress (OS), as pregnancy naturally increases this stress. It is a condition that triggers a systemic inflammatory response due to elevated levels of reactive oxygen species (ROS) and nitrogen oxygen species (NOS) in the bloodstream. An increase in ROS and RNS can lead to the depletion of antioxidants in the body during pregnancy and result in a series of harmful outcomes such as underdevelopment, abnormal placental function, and a range of pregnancy complications, including pre-eclampsia and fetal developmental anomalies due to restricted intrauterine growth (14).

It has been shown that MDA levels increase with the progression of pregnancy due to the coordinated rise in the concentration of reactive oxygen species (ROS), which acts as an indicator of the severity of oxidative stress caused by pregnancy complications. This is linked to the systemic inflammatory response during pregnancy, which results from the activation of peripheral granulocytes and lymphocytes during the third trimester, leading to the production of large amounts of ROS, causing oxidative stress (14). MDA levels can peak in the second and third trimesters in pregnant women with pre-eclampsia (15). The increase in MDA may be attributed to a reduction in antioxidants (16). The significant rise in MDA and ROS has a negative impact on fetal and placental function, uterine growth, red blood cells, and DNA (17).

5. Conclusion:

The current study found a significant increase in the levels of apelin (APLN) and adiponectin (ADP) in the later stages of pregnancy.

Pregnancy is a state of oxidative stress, as the study results showed an increase in the oxidative stress marker (MDA) and a decrease in the activity of the antioxidant enzyme catalase (CAT) during the three months of pregnancy.

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