# Clinical Study for Testosterone, Progesterone and Oxidative Stress During Phases of Menstrual Cycle in Women with Bronchial Asthma

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## Abstract

This study aims to evaluate the hormones (testosterone (T) and progesterone (P)) and oxidative stress malondialdehyde (MDA), ceruloplasmin (CP) and transferrin (Tf) during Phases of menstrual cycle (follicular and luteal phase) in females with asthmatic. Included (70) women patients with bronchial asthma without taking contraceptive pills aged (14-45 years). Included (70) women as a control group supposed healthy without taking contraceptive pills with the same age in this study. The results showed a significant decline in levels of the Progesterone (P) and Testosterone (T) and significantly higher in levels of the (MDA) and (CP) in all patients in both follicular phase as well in the luteal phase in compared with control group in the follicular and luteal phase respectively ($P \leq 0.05$).We did not notice any significant difference in concentration of serum TF in all patients in both follicular phase as well in the luteal phase in comparison with control group in the follicular phase and luteal phase respectively ($P \leq 0.05$).Showed serum levels of Testosterone and Progesterone significantly higher in all patients in the luteal phase when comparison with all patients in the follicular phase ($P \leq 0.05$). We did not notice any significant difference in (MDA, CP and TF) levels in all patients in the luteal phase when comparison with all patients in the follicular phase ($P \leq 0.05$).

***Keywords:*** *Asthma, menstrual cycle, Testosterone, Progesterone, Lipid peroxidation, Ceruloplasmin, transferrin.*

## Introduction

Bronchial asthma is a common persistent inflammatory disease of airways, which leads to variable or even persistent airflow limitation. The essential symptoms are chest tightness,persistent cough, wheezing and dyspnoea. The prevalence of bronchial asthma in humans varies around the world affecting 1 to 18% of any investigated population [1]. Women are more often affected by asthma. The mechanisms underlying the gender differences in asthma prevalence are still under investigation but refer mostly to hormonal differences and differences in lung capacity [2].

The spread and severity of bronchial asthma are often linked with moments are necessary for the reproductive life of a female. As a result, it has been speculated that the changes hormonal in the menstrual could play a vital in the pathophysiology of bronchial asthma, leading to worsening of disease severity. Women's seem to experience an increase of bronchial asthma symptoms through the menstrual phases of or premenstrual[3].

A response to the gonadotropins, sex hormones will be secreted testosterone, Progesterone, andestradiol is excreted and, thus, feedback on the level of the pituitary and hypothalamusto control natural reproductive mission[4]. Progesterone hormone is produce to a lesser extent in the placenta through the pregnancy and the adrenal glands. Thus, cyclical hormone exposure she started at menstruation and ends at menopause occurs monthly both differentiation and growth are regulated through specialized tissue within the reproductive system and breast tissue[5]. Testosterone is also produced, to a smaller degree, in the ovaries and adrenal cortex in women. The central nervous system stimulates the hypothalamus to begin testosterone production, which suggests a strong link between the nervous and endocrine systems[6].

Oxidative stress is as a disorder in equilibrium between (ROS), freeradicals ($FR_s$), and endogenous

antioxidant defencemechanisms [7], or more simply, it is a disturbance in the equilibriumbetween oxidant-antioxidant states, favouring the oxidant environment[8]. **Oxidative stress results because of an imbalance between neutralization and the formation of** ROS/ RNS**. Or on the other hand, oxidative stress can arise when cells are unable to destroy the overabundance of free radicals resulting.[9].**

Malondialdehyde is appreciatedto impair lots of physiological mechanisms in the human body during its ability to react with molecules, for example, proteins and DNA. MDA is used as a biomarker in an organism to measure the level of oxidative stress.[10].

Ceruloplasmin (Cp) is one of the main proteins taking part in copper metabolism by distributing it in the human body. It is responsible for carrying more than 95% of copper in blood serum[11]. Transferrin a glycosylated $Fe^{3+}$ binding protein, which is found in blood plasma, lymph, and other body fluids and it is synthesized in the liver, central nervous system, testes, ovaries, spleen, mammary glands, and kidneys.

## Materials and Method

This study was conducted at "AL Hussein Teaching Hospital in Dhi-Qar", especially, in respiratory counselling, Biochemistry Laboratory at the College .of Science, University of Dhi-Qar at the period between 1/9/2017 to 1/6/2018.

The study included (140) subjects, (70) controls and (70) patients of women only. It is notable that the smokers were excluded.

**The controls and patients were divided into two groups:**

1. **Control group:** Included (70) supposed healthy subjects of women without taking contraceptive pills aged (14-45 years).
2. **Patient group:** Included (70) patients with bronchial asthma of women without taking contraceptive pills aged (14-45 years).

**Blood Samples Collection:** About 8mL of blood samples from bronchial asthma controls and patients two phases. The first sample was taken from the females in the follicular phase and the second sample was taken from the same females in the luteal phase. This applies to all patients and controls. The serum was separated immediately in order to allow clotting at room temperature. The blood was centrifuged at 3000rpm for 10 minutes and stored in plain pipes at (-20ºC) until used or immediately analysed.

**Serum Hormones (Progesterone and Testosterone):** Serum progesterone (P4) and Testosterone concentrations are determinedby enzyme-linked through enzyme linked fluorescence test using the Mini-VIDAS Automatic immunofluorescence assay system (bioMerieux, Marcy letoile, Lyon, France.

**Measured of Serum Malondialdehyde (MDA):** The level of serum malondialdehyde (MDA)was measured spectrophotometrically through the method of Muslih *et al*[12]. It concentrations were calculated using the extinction coefficient of MDA (εMDA) equivalent (0.156 x18 nmol/ml).

**Measured of Serum Ceruloplasmin (CP):** Serum Cp concentration was determined through the method of Menden *et al*[13] which using the extinction coefficient of ceruloplasmin (εCp) equivalent (0.68) to calculate its concentration.

**Measured of Serum Transferrin (Tf):** The Serum Tf concentration was measuredthrough colorimetric method Burtis *et al*[14]. Where excess iron is added to the saturated serum the transferrin. Unregulated iron has been accelerated with basic magnesium carbonate. The iron in the supernatant is determined by centrifugation. Where the concentration of residual iron is tested and the result expressed as (Total Iron Binding Capacity). Where Tf concentration is calculated for the following equation.

(Serum Tf (g/L) = TIBC (μmol/L)/25.1)[15]

**Statistical Analysis:** Statistical analysis was done using the software [SPSS] the "results were expressed" as mean ± SD with LSD. Way analysis of variance [ANOVA] test was used to compare parameters different studied groups. A"*P* values ≤ 0.05" was considered statistically significant".

## Result and Discussion

**General Comparison for all Studied Parameters:**

**Serum Testosterone Concentration:** The mean of serum testosterone level in the patients groups which was found to be significantly lower (P≤0.05) than that control group in both two phases as in the table (1).

That certain systemic chronic inflammatory disorders and a few pulmonary diseases affect testosterone (T) biosynthesis[16]. Testosterone and its metabolites contribute to the physiological balance between autoimmunity and protective immunity by maintaining regulatory T cells. Testosterone has immunosuppressive effects and is probably also protective against immuno-inflammatory processes that trigger asthma[17].

**Table 1: Serum Testosterone levels in control and patient groups in the menstrual phases**

| Testosterone (ng/mL) Mean±SD | | | |
|---|---|---|---|
| **Group** | **No.** | **Follicular** | **Luteal** |
| Control | 70 | 0.82±0.06A | 0.90±0.15A |
| Patient | 70 | 0.50±0.09B | 0.71±0.10B |
| L.S.D | | 0.03 | 0.15 |

**Note:** "Each value represents mean ± S.D values with non identical superscript (A, B or C... etc.) were considered as significant differences (P≤ 0.05)."

**No:** Number of subjects., **SD:** Standard deviation., **LSD:** Least Significant Difference.

**Serum Progesterone Concentration:** The mean of serum progesterone level in the patients groups which was found to be significantly lower (P≤0.05) than that control group in both two phases as in the table (2).

Women report more pronounced symptoms, which seem to change with the various life stages such as menstruation, pregnancy and menopause and in association with female sex hormone levels[2]. These hormones cause differences in the clinical manifestation of asthma. Thus, oestrogen promotes bronchial hyperreactivity, and both FEV1 and exhaled nitric oxide (NO) show a cycle-dependent course. Twenty to forty percent (20-40%) of premenopausal women suffer from pre- or peri-menstrual asthma (PMA) and experience an exacerbation in the week preceding menstruation, based on increased inflammation in the bronchi[1].

**Table 2: Serum Progesterone levels in control and patients groups in the menstrual phases**

| Progesterone (ng/mL) Mean±SD | | | |
|---|---|---|---|
| **Group** | **No.** | **Follicular** | **Luteal** |
| Control | 70 | 0.57±0.07A | 8.80±1.11A |
| Patient | 70 | 0.38±0.06B | 6.65±0.77B |
| L.S.D | | 0.02 | 0.32 |

**Serum Malondialdehyde Concentration:** Table (3) shows significantly higher in concentrations of serum malondialdehyde (MDA) in all patients in both follicular phase as well in the luteal phase in comparison with a control group in the follicular phase and luteal phase respectively (P≤0. 05).

There is a growing lot of evidence indicating the oxidative stress in the pathogenesis of bronchial asthma. In allergic inflammation exist overproduction of (ROS). Reactive oxygen species may induce symptoms characteristic of bronchial asthma: bronchial hyperresponsiveness, bronchospasm, mucus hypersecretion, beta-adrenergic receptor, dysfunction and activation of an arachidonic acid cascade, bronchial epithelial damage and increased permeability[18].

Increasing the arachidonic acid in airway inflammatory cells in asthma patients. This arachidonic acid may lead to oxidation produce an end product of (LPO). So MDA is increased in bronchial asthma due to generation by decomposition of arachidonic acid and larger PUFA through enzymatic and nonenzymatic processes[19].

**Table 3: Serum MDA levels in control and patients groups in the menstrual phases**

| MDA (µmol/L) Mean±SD | | | |
|---|---|---|---|
| **Group** | **No.** | **Follicular** | **Luteal** |
| Control | 70 | 2.07±0.29B | 2.26±0.33B |
| Patient | 70 | 3.14±0.53A | 3.06±0.49A |
| L.S.D | | 0.14 | 0.14 |

**Serum Antioxidant Concentrations:** Table (4) shows significantly higher in the concentration of serum Ceruloplasmin (CP) in all patients in both follicular phase as well in the luteal phase when comparison with a control group in the follicular phase and luteal phase respectively (p≤0. 05).

The same Table shows no a significant difference in concentration of serum Tf in all patients in both follicular phase as well in the luteal phase incomparison with a control group in the follicular phase and luteal phase respectively (P≤0. 05).

Ceruloplasmin is known for the preservation of considerable peroxidase activity and capable of suppression superoxide radicals. This is in charge of restricting the damage caused through these radicals[20].

Note that the elevation of CP is part of the increase in iron metabolism proteins and part of bronchial asthma and chronic obstructive pulmonary disease,

probably as an anti inflammatory response to the airway inflammation which characterizes these conditions[21]. Ceruloplasmin can be used as non-invasive biomarkers for evaluation of chronic airway inflammation and can be useful in determining the severity of asthma[22].

**Table 4: Serum antioxidant levels in control and patients groups in the menstrual phases**

| | | CP (g/L) Mean±SD | | TF (g/L) Mean±SD | |
|---|---|---|---|---|---|
| **Group** | **No.** | **Follicular** | **Luteal** | **Follicular** | **Luteal** |
| Control | 70 | $2.26\pm0.37^{B}$ | $2.21\pm0.35^{B}$ | $4.08\pm0.76^{A}$ | $3.96\pm0.71^{A}$ |
| Patient | 70 | $3.62\pm0.71^{A}$ | $3.55\pm0.66^{A}$ | $4.09\pm0.85^{A}$ | $3.97\pm0.78^{A}$ |
| L.S.D | | 0.18 | 0.17 | 0.31 | 0.29 |

Comparison for all Studied Parameters in patients According to the follicular and luteal phase:

**Serum Hormones Concentrations:** Table (5) shows significantly higher in concentrations of Testosterone and Progesterone in all patients in the luteal phase when comparison with all patients in the follicular phase (P≤0. 05).

Women of reproductive age face periodic changes in concentrations the sex hormones. Through the follicular phase of menstrual cycleremain testosterone hormone level and progesterone hormone level low, while (FSH) hormone and (LH) atthe highest level. Finally, by the luteal phase, (FSH) level and (LH) level are low, While the level of each of the following hormones 17-β-estradiol, testosterone, and progesterone are moderately high[23].

Patients who suffer from premenstrual asthma (PMA) are frequently affected by the periodic changes in serum levels for progesterone hormone.[24]Indicate earlier studies that an increase in the progesterone hormone secretion possibility it has a role for hyperventilation in the luteal phase[25].

**Table 5: Levels serum Testosterone and Progesterone during the follicular and luteal phase in patients.**

| **Menstrual Phase** | **No.** | **Testosterone (ng/mL) Mean±SD** | **Progesterone (ng/mL) Mean±SD** |
|---|---|---|---|
| Follicular | 70 | $0.50\pm0.09^{B}$ | $0.38\pm0.06^{B}$ |
| Luteal | 70 | $0.71\pm0.10^{A}$ | $6.65\pm0.77^{A}$ |
| L.S.D | | 0.03 | 0.19 |

**Serum Oxidative Stress Concentrations:** Table (6) shows no significant difference in concentrations of serum (MDA, CP and TF) in all patients in luteal phase in comparison with all patients in follicular phase (P≤0.05).

Sex hormones work on the production of reactive oxygen species (ROS) via neutrophils which may have immune functions, and these reactive oxygen species (ROS) are reported to be involved in the occurrence of ovulation in the ovaries[26]. After ovulationthe corpus luteum is created and progesterone hormone is secreted, a process of functional corpus luteum regression begins if pregnancy is not established. Reactive oxygen species (ROS) are also thought to play a role important in promoting this functional corpus luteum regression[26] and reactive oxygen species (ROS) production may be regarded as important in maintaining homeostasis in ovarian function[27].

**Table 6: Levels serum Oxidative Stressduring the follicular and luteal phase in patients.**

| **Menstrual phase** | **No.** | **MDA (µmol/L) Mean±SD** | **CP (g/L) Mean±SD** | **TF (g/L) Mean±SD** |
|---|---|---|---|---|
| Follicular | 70 | $3.14\pm0.53^{A}$ | $3.62\pm0.71^{A}$ | $4.09\pm0.85^{A}$ |
| Luteal | 70 | $3.06\pm0.49^{A}$ | $3.55\pm0.66^{A}$ | $3.97\pm0.78^{A}$ |
| L.S.D | | 0.16 | 0.20 | 0.29 |

## Conclusion

**From the data in this study we can conclude the following points:** Bronchial asthma decreases the levels of both testosterone and progesterone. Lipid peroxidation associates with bronchial asthma. Disorder the antioxidant system in patients with bronchial asthma, according to the levels of (ceruloplasmin and transferrin). Phases of menstrual cycle have effect on (testosterone, progesterone).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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