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Article

# Study of Norepinephrine Hormone in Patients with Secondary Infertility in Salah Al-Din Province and Assessment of Some Biochemical Parameters

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**Abstract:** Objective: This study was to evaluate norepinephrine levels among patients with secondary infertility in Salah Al-Din province and among BMI/age groups as a cause of a tool influencing physiological and hormonal homeostasis. The study comprised 80 samples of a 1:1 ratio of patients and control subjects of both sexes and various age groups. The results showed that patients with secondary infertility had lower levels of norepinephrine compared to healthy controls, indicating a problem in the development of the sympathetic nervous system or loss of regulation for catecholamine secretion. However, although there were numerical differences in the norepinephrine levels among the groups studied, these differences did not reach statistical significance with BMI. In terms of age, there was a degree of heterogeneity in norepinephrine levels across different age bands suggesting a modulatory effect of age on norepinephrine secretion, the effect was not the same in all groups.

**Keywords:** Infertility, Secondary Infertility, Norepinephrine, Body Mass Index (BMI).

## Introduction

Infertility is clinically defined as the failure to achieve pregnancy after 12 months or more of regular, unprotected sexual intercourse, and it is considered a significant global health challenge [1]. It is estimated to affect approximately 15% of couples worldwide, with the male factor contributing as a primary or associated cause in nearly half of all cases [2]. Fallopian tube disorders represent another important etiological factor, while about 15% of cases remain unexplained and are classified as "unexplained infertility." Lifestyle and environmental factors, such as smoking and obesity, may also negatively affect fertility [3].

Infertility can be classified into two main types, primary and secondary, depending on whether the woman has previously achieved a pregnancy [4]. It is a common reproductive health problem globally, with estimates indicating that it affects between 11–26% of married women in Iraq within the 20–40-year age group [5]. In Iraq, a study conducted at Kamal Al-Samarrai Hospital showed that the highest proportion of infertile women was in the 25–35-year age group. The study also revealed that 36.64% of cases were among uneducated women, while those with secondary and primary education

accounted for 21.7% and 16.6%, respectively. Furthermore, primary infertility was more prevalent than secondary infertility, with rates of 66.9% and 33.1%, respectively [6].

The findings also indicated that 46.1% of infertile women had a normal body weight, compared with 27.8% who were obese and 26.1% who were overweight. In addition, 66.1% of the women reported stability in their family life. A higher prevalence of primary infertility compared to secondary infertility was also observed across different time periods, with a predominance of urban cases (60%) compared with rural areas (40%) [7], [8], [9].

On the other hand, secondary infertility is defined as the inability to conceive following a previous successful pregnancy [10]. Female fertility naturally declines after approximately 30 years of age due to a reduction in both the quantity and quality of oocytes. It is estimated that one in seven women aged 30–34 years experiences difficulty in achieving pregnancy, with the prevalence increasing progressively with advancing age [11].

Norepinephrine is one of the major catecholamine neurotransmitters involved in stress-response signaling pathways and has been implicated in a variety of psychological and physiological disorders associated with stress, including cardiovascular diseases, affective disorders, post-traumatic stress disorder, and certain types of cancer [12]. Emerging scientific evidence also suggests that norepinephrine plays a role in the regulation of ovarian function.

Norepinephrine is associated with key biological processes regulated by reactive oxygen species (ROS) in ovarian physiology, including ovulation [13]. Elevated levels of norepinephrine have been detected in the follicular fluid of patients with polycystic ovary syndrome (PCOS) [14].

Wang et al. reported that both glutamine and norepinephrine present in follicular fluid act synergistically to enhance the antioxidant capacity of human granulosa cells and may contribute to predicting in vitro fertilization and embryo transfer outcomes [15]. Despite the important role of norepinephrine in ovarian physiology and pathology, the effects of chronically elevated levels induced by stress on ovarian function remain incompletely understood. Therefore, this study aimed to establish a norepinephrine-treated rat model to investigate the mechanism by which norepinephrine overexpression induces ovarian dysfunction, as well as to perform a preliminary evaluation of potential protective factors against stress-induced ovarian injury [16].

## Materials and Methods

### Samples Collection

Sampling was performed in private laboratories and from gynecology clinics after diagnosis by a specialist physician. Between March 2024–June 2024, samples were processed in Tikrit District and adjacent areas (private laboratories). This study included a total of 80 samples (40 patients with secondary infertility [29 females and 11 males] and 40 healthy controls [34 females and 6 males]). Participants were all between the ages of 20 and 50.

### Classification of Samples

The samples were separated in two groups:

The control group was composed of healthy males and females in the first group.

The other group of men and women diagnosed with secondary infertility.

### Serum Preparation

Lee et al.The procedure was performed on patients and healthy controls by taking approximately 3-5 mL of venous blood, separating it into two parts. Serum was isolated by transferring 2–4 mL of blood from each sample into an airtight-sealed gel separator tube. It works as a barrier between serum and cellular components of blood for the separation of serum by gel.

Then centrifuge at 408 × g for between 5–10 minutes. After that, the serum was collected from all samples of secondary infertility and healthy controls then put in 2 mL Eppendorf tubes with sealed caps. Serum samples were preserved at −20°C until biochemical analysis.

**The second part:**

Fresh EDTA blood was added into 2 mL sterile airtight tubes specifically designed for use with human tissues (about 1 mL). Anticoagulation was performed using EDTA. Following the collection of samples at time points 0, 1, and 8 weeks post-surgery, they were stored at −20°C until extraction.

Body mass index (BMI)

The standard formula was used to calculate the Body Mass Index (BMI), i.e., BMI = weight (kg)/height2 ($m^2$) in kg/$m^2$ [17]:

$$\text{BMI} = \frac{\text{wieght } (kg)}{\text{length (m)}^2}$$

**Age Group Classification**

Participants were grouped into three categories based on age as below:

Group I: (20–30) years

Group II: (31–40) years

Group III: (41–50) years**Estimation of Norepinephrine Hormone**

Into each well of the norepinephrine microtiter strip plates, there was added 25 μL of enzyme solution.

50 μL of standards, controls and samples were loaded into the appropriate wells.

The plate was immediately transferred to an orbital shaker (around 600 rpm) for incubation at 20–25°C over a time period of 30 minutes.

Add 50 μL anti-adrenaline antiserum to each well, seal the plate with adhesive film

The plate was kept on a shaker (600 rpm) at room temperature (20–25°C) for 2 h.

Afterwards, the adhesive film was peeled off and the contents of the wells were discarded. Each plate was washed three times with 300 μL of washing buffer, and in each cycle the washing buffer was discarded and the plate dried by tapping upside down on absorbent material.

Added 100 μL of the enzyme conjugate into each well.

The plate was shaken for 30 min (600 rpm) at RT (20–25°C).

The wells were emptied and washed again three times with 300 μL washing buffer, followed by drying in the same way as previously described.

100 μL of substrate solution was added to each well and kept at 20–25 °C, on a shaker (600 rpm) for 5–25 minutes in the dark.

Stop solution (100 μL) was added to each well, and the microtiter plate was gently shaken for mixing.

Absorbance was measured using a microplate reader at 450 nm within 10 min.

**Calculations**

Graph paper was used to construct a standard curve, with the concentrations of the standard solutions measured as before on the x-axis and absorbance values measured again based on the information provided in the kit manual on the y-axis directly after reading add to samples. Absorbance values of the samples were then plotted against concentration values on the x-axis, connecting the points to generate a standard curve (Figure 1).

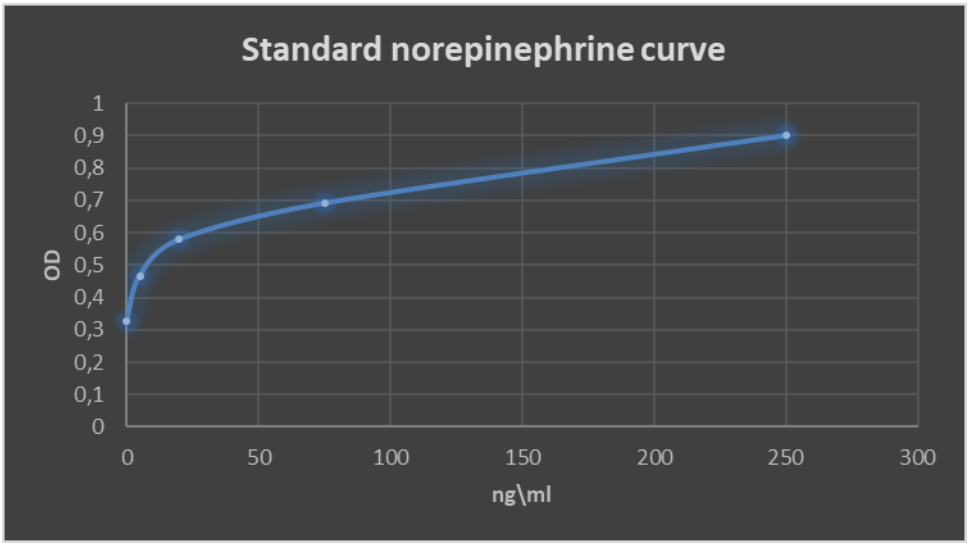


**Figure 1.** Standard Curve of Norepinephrine.

## Results and Discussion

### Norepinephrine Level in Secondary Infertility

Currently, the study showed that compared with control group, norepinephrine was significantly reduced in both sexes of secondary infertility subjects. The norepinephrine on males was (149.449 ± 4.351 ng/mL) in patients vs (175.216 ± 3.342 ng/mL) in the healthy controls It was (143.467 ± 4.787 ng/mL) in patients compared with (152.445 ± 4.971 ng/mL) in controls in females. This evidence addresses malfunction of sympathetic nerves activity related with chronic stress condition.

Norepinephrine is a critical regulator of the hypothalamic–pituitary–gonadal (HPG) axis and its reduction results in disruption of reproductive hormone (GnRH, LH and FSH) secretion, thereby impairing gametogenesis and ovarian function.

Prolonged stress exposure may also lead to catecholamine depletion and consequent neuroendocrine imbalance or oxidative stress as possible explanations for these results. This is in line with current research correlating dysregulation of neurotransmitters to reproductive dysfunction. Specifically, the decline in norepinephrine was significantly greater in males than females, indicating a greater sensitivity of the male reproductive physiology to these shifts.

This finding of the current study correlates with Odetayo [18] who stated chronic stress compromises sympathetic nervous system responses and catecholamine secretion (including norepinephrine), hence affects hypothalamic–pituitary–gonadal axis eventually leading to impaired reproductive function in humans.

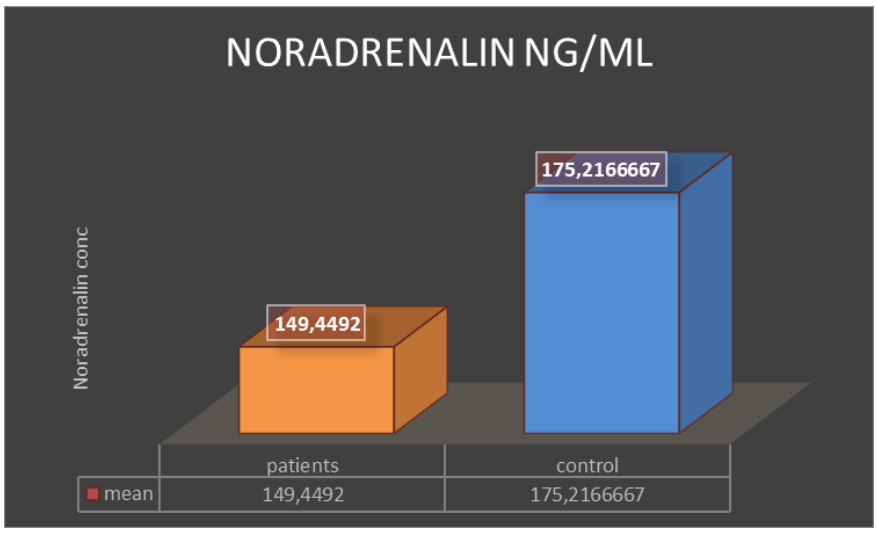


**Figure 2.** Norepinephrine levels in the serum of male patients compared with healthy controls.

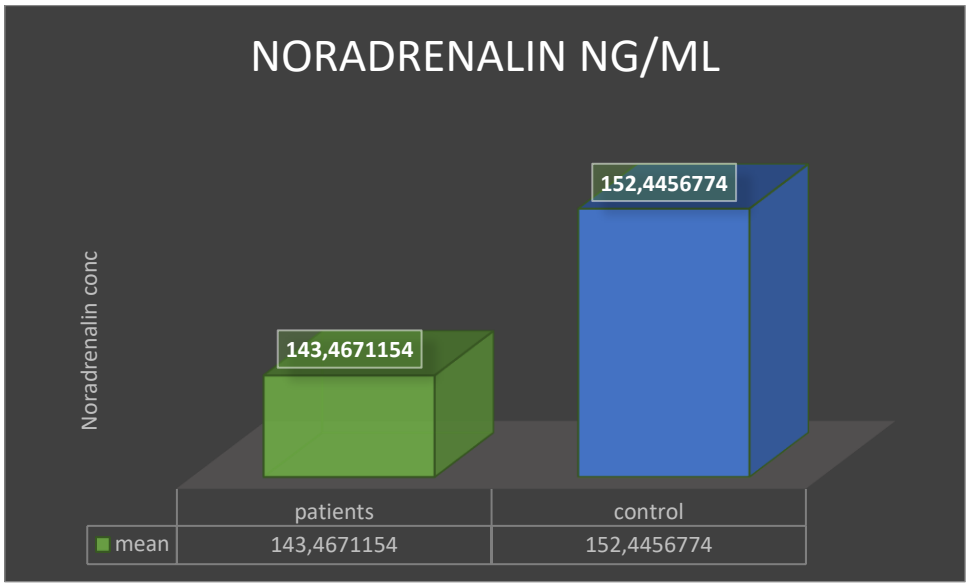


**Figure 3.** Norepinephrine levels in the serum of female patients compared with healthy controls.

**Hormone Norepinephrine and Index Body Mass (BMI)**

In this study, the authors did not find any statistically significant differences in norepinephrine levels among BMI categories between secondary infertility patients and their respective control group. All values recorded belonged to the same statistical letter (a), showing no significant differences in means among groups studied. The results of these experiments are summarized in Table 1.

**Table 1.** Norepinephrine Levels According to Body Mass Index (BMI).

| BMI | Noradrnalin (ng/ml) Mean±S.D | | | |
|---|---|---|---|---|
| | Control | N | Patients | N |
| (18-24)kg/m2 | 186.0±45.7a | 7 | 157.8±32.0a | 7 |
| (25-31) kg/m2 | 137.64±17.88a | 14 | 143.8±32.3a | 7 |
| (32-45) kg/m | 162.65±39.45a | 19 | 145.88±30.03a | 26 |

| | |
|---|---|
| P-value | 0.030 |

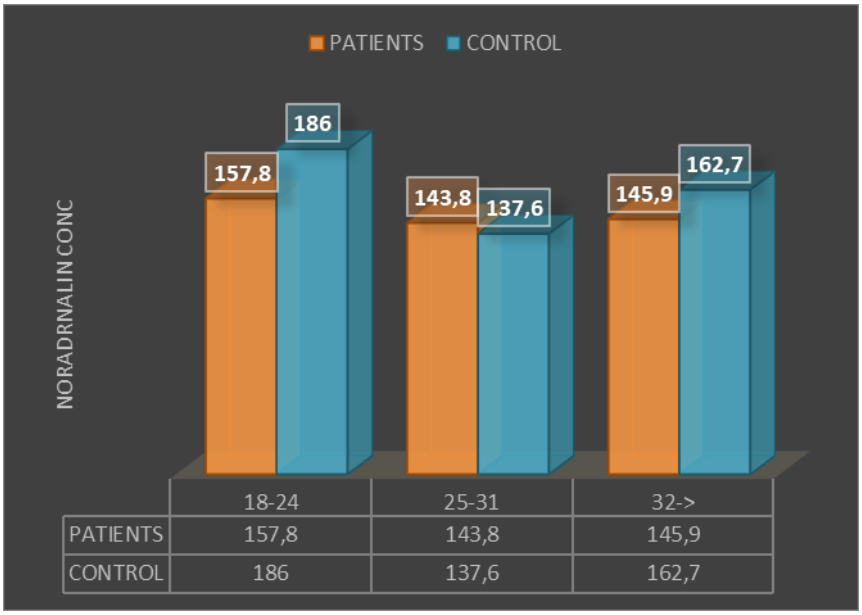


**Figure 4.** Norepinephrine Concentration and Body Mass Index (BMI).

As shown in Figure(4), although there were some slight numerical differences betweenGroup1(G1),Group2(G2) and Group3 (G3) values ,there were no statistically significant differences between the three BMI categories ascenorityphrine levels in both secondary infertility patients and also in control group.

In the patient group, the mean values (in G1/G2/G3) in kg were 157.8/143.8/145.9 as opposed to 186.0/137.6/1627 in controls respectively. The difference in overall was statistically significant (P = 0.030), and pairwise comparisons between the BMI categories of G1, G2, and G3 did not show significant differences between the control group versus secondary infertility group.

The highest norepinephrine concentration was found in BMI category G1 for both patients and controls, perhaps hinting that BMI may only have a small influence during this period. Similar findings have been reported by Hainer V et al. [19], who communicated that the relationship between catecholamines and BMI was not perfectly linear, but modulated by combined physiological and metabolic elements.

**Norepinephrine Measurement Across Age Groups**

Among secondary infertility patients, there was a statistically non-significant decline in norepinephrine levels in age groups G2 and G3: (P-value = 0.522) On the same Reader, norepinephrine in secondary infertility group is G2 (138.86 ± 25.61) and G3 (153.57 ± 33.20), as detailed in the Table (2).

**Table 2.** Norepinephrine Levels According to Age Categories.

| Age Categories | Noradrnalin (ng/ml) Mean±S.D | | | |
|---|---|---|---|---|
| | Control | N | Patients | N |
| (20-30)Y | 146.1±31.6a | 7 | 150.5±33.8a | 7 |
| (31-40)Y | 160.05±39.71a | 14 | 138.86±25.61a | 7 |
| (41-50)Y | 165.8±41.8a | 19 | 153.57±33.20a | 26 |
| P-value | | | 0.522 | |

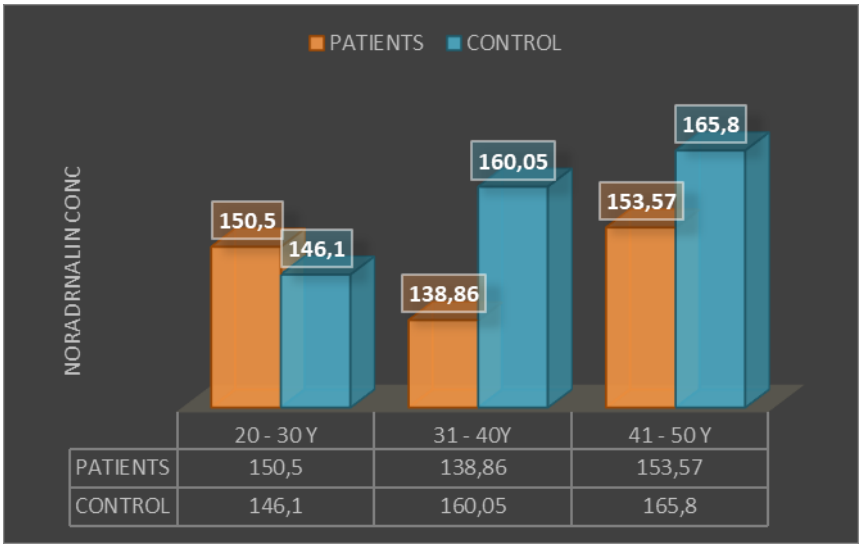


**Figure 5.** Norepinephrine Concentration Across Age Groups.

The results in Figure (5) demonstrate the effect of age on norepinephrine levels within both the secondary infertility patient and control, differences were statistically significant with all three age groups G2 and G3 at higher hormone levels in patients compared to controls.

In secondary infertility group norepinephrine concentrations were: G1 (150.5±33.8), G2 (138.86±25.61), and G3 (153.57 ± 33.20) ng/ml, while control group norepinephrine was: G1(146.1±31.6); G2(160 05±39 71); and (165 80±41 8)ng/ml.Giovanelli et.al., F-7 human female subfertility effect from chronic stressful situations in immune system over a course of years, link to AI -- November2024 The statistical average for all samples was (35.0878).

These results demonstrated a distinctive age-associated variability exhibited between the effect of age and norepinephrine levels with the highest values in specific primary infertility patients were displayed in G2 and G3 against control. It was also mentioned that norepinephrine concentrations increase with age, which may possibly decrease in older ages because of additional comorbid conditions and other physiological changes [10]. These findings are in line with those documented by Himmelfarb et al. [20].

## Conclusion

Findings from this study showed that norepinephrine levels in patients with secondary infertility were lower than those in the healthy control subjects, which may reflect a symptomatic dysfunction of sympathetic nervous system activity or catecholamine regulation. In addition, there was no statistically significant association between norepinephrine levels and body mass index (BMI), suggesting a little or complicated role of this exposure in the condition examined.

For example, differences were seen by age group in hormone levels (although not all reached statistical significance), suggesting that age has some effect on modulating HPG or thyroid hormone levels. As such, we suggest that norepinephrine may serve as a potential biomarker for physiological changes tied to secondary infertility, in hopes of improving its pathological understanding and potentially aiding diagnostic purposes going forward.

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