

# Correlation between oxidative stress and thyroid hormone levels in infertile women

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**Abstract- Background** Female infertility is one of the growing problems in the world and is considered as a multifactorial disease .

**Objective :** The aim of the present study is to investigate the correlation between oxidative stress and thyroid hormones : T<sub>3</sub>, T<sub>4</sub> and thyroid-stimulating hormone TSH in the infertile women .

**Method :** This study was carried out between March 2015 and August 2015 including eighty (80) selected infertile women who attended to Fertility Center in Al-Sadr Medical City in Al-Najaf Al-Ashraf Province . The study also included twenty (20) healthy volunteer fertile women as a control . Hormonal measurement was performed by ELISA technique, while oxidative stress was assessed by determining the level of : malondialdehyde MDA , catalase CAT and glutathione GSH in serum . Student's t-test was used to investigate the significance in the difference between groups .

**Results :** the results have shown a high significant decrease in the level of T<sub>4</sub> and a high significant increase in the level of TSH in the infertile women group as compared to fertile (control) group . The results of the present study also showed a high significant increase in the level of MDA, a significant decrease in the activity of CAT, and a high significant increase in the level of GSH in the infertile women group as compared to control group . The correlation results revealed a significant negative correlation between MDA and TSH , a significant positive correlation between MDA and T<sub>4</sub>, a high significant positive correlation between TSH and each of CAT and GSH, and a high significant negative correlation between T<sub>4</sub> and each of CAT and GSH .

**Conclusions :** It seems that thyroid hormone disorders and increased oxidative stress act synergistically as causative and predisposing factors for female infertility .

**Recommendations :** It is recommended to take in mind the link between thyroid hormones disorders and oxidative stress in the treatment strategies of female infertility .

**Index Terms-** correlation, oxidative stress, thyroid hormones, infertile women

## I. INTRODUCTION

Infertility is a worldwide problem that affects approximately 15% of all couples, and about 33% of the cases of infertility are primarily attributed to the female <sup>(1)</sup> . Numerous medical disorders can contribute to infertility by causing damage to the fallopian tubes, interfering with ovulation and fertilization, or causing hormonal disturbances <sup>(2)</sup> .

Among the multifactorial causes of female infertility, hormonal disturbances have been considered of great importance in the knowledge of causes and diagnosis, as well as the lines of treatment . In the last years, deficiency of thyroid hormones have increasingly been investigated as one of the predisposing factors in causing female infertility <sup>(3)</sup> . Thyroid glands secrete two hormones : thyroxine, which is also called (Tetraiodothyronine or T<sub>4</sub>), and Triiodothyronine or T<sub>3</sub>, they control oxygen use, basic metabolic rate, cellular metabolism, and growth & development; the production and secretion of thyroid hormones are influenced by thyroid-stimulating hormone TSH released by the anterior pituitary gland <sup>(4)</sup> .

Previous literatures indicated a quite deal of link between thyroid hormones and reproductive functions and hormonal changes in female; this may cast a light on the relationship between hyper- or hypothyroidism and reproductive dysfunctions or menstrual disturbances that have been reported in some studies <sup>(5)</sup> . A number of studies have confirmed the effect of hypothyroidism in increasing prolactin and decreasing the concentration of sex hormone-binding globulins SHBG in the fertile female <sup>(6-8)</sup> .

Oxidative stress can be defined as the imbalance between the generation of oxidants and the concentration of antioxidants, in which the generated reactive oxygen species ROS are favored, resulting in harmful effects on the body <sup>(9)</sup> . Many tests have been used as indicators of OS, for instance : malondialdehyde (MDA) which is a natural product formed in all cells as end product of lipid peroxidation ; catalase (CAT) which is an antioxidant enzyme that catalyses the decomposition of H<sub>2</sub>O<sub>2</sub> into water and oxygen ; glutathione GSH which is a non-enzymatic antioxidant compound found in living cells <sup>(10)</sup> .

In the last two decades, there is an increasing data about the role of the highly reactive free radicals in impairing pregnancy and causing primary and secondary infertility, this may be because free radicals are unstable and strongly reactive, so that they have an incredible power to acquire electrons from biological molecules (carbohydrates, proteins, lipids, nucleic acids) resulting in massive damage in the cellular and biochemical (enzymatic hormonal) system of the female reproductive system <sup>(11)</sup> . Many previous studies suggested different roles of reactive oxygen species and reactive nitrogen species in the development and complications of polycystic ovarian syndrome and endometrial damage, which are of the major causes of female infertility <sup>(12-14)</sup> .

It was hypothesized that patients with hypothyroidism have only poor anti-oxidative capacity <sup>(15-16)</sup>, but there are little data to describe the relationship between the levels of thyroid hormones and oxidative stress in infertile females, and this is the main objective of the current study .

II. MATERIALS & METHODS

1. Study subjects :

The study included one hundred (80) selected proved infertile women who attended to Fertility Center in Al-Sadr Medical City in Al-Najaf Al-Ashraf Province in Iraq. The men age of the infertile group was (31±8.9) years. The study also included twenty (20) healthy volunteer fertile women who have one or more than one child, and have no medical history of hypothyroidism or oxidative stress disorders . The men age of the infertile group was (29±11.3) years .

2. Semen and Serum Collection

About 5 ml of venous blood for specific biochemical tests was collected by vein puncture using 5ml disposable syringes. The obtained sera was put then in another disposable tubes and labeled. Serum samples were used to investigate the following biochemical tests :

analysis of MDA as a marker of free radicals, CAT as scavenging enzymes and GSH as nonenzymatic scavengers as well as for analysis of thyroid stimulating hormone (TSH), Triiodothyronine (T<sub>3</sub>) and Tetraiodothyronine (T<sub>4</sub>) .

3. Biochemical tests:

Determination of Malondialdehyde level in serum

Determination of MDA concentration was based on the calorimetric reaction with thiobarbituric acid (TBA) to form a pink color product ,which could be measured by spectrophotometer <sup>(10)</sup> .

Measurement of Catalase Activity in serum

Catalase (CAT) activity was determined by the measurement of the decrease in the absorbance due to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) consumption as described by Aebi <sup>(17)</sup> .

Determination of glutathione level in serum

Determination of GSH level depends on the action of sulfhydryl groups . Sulfhydryl group of GSH could reduce disulfide chromogen of 5,5'-Dithiobisnitrobenzoic acid (DTNB) and change it to an intensely yellow compound, its absorbance can be measured directly by spectrophotometer at 412 nm and it was directly proportional to the GSH concentration <sup>(18)</sup> .

Hormonal tests

The levels of serum thyroid stimulating hormone (TSH), total triiodothyronine (T<sub>3</sub>), and total thyroxine (T<sub>4</sub>) were measured by using enzyme-Linked immune-sorbet assay (ELISA) methods (according to the kits from Biocheck, Inc.).

Statistical Analysis:

The analysis of data was performed by using a Megastat (Version 9.4 2005) computer program . Results were expressed as mean ± standard deviation S.D . Independent unpaired student t-test was used to analyze the differences between groups . Pearson correlative – factor was used for measuring the correlations between indicators .

III. RESULTS

1. Thyroid function tests among infertile and fertile women

The values and statistical difference of thyroid function tests (T<sub>3</sub>, T<sub>4</sub>, TSH) between infertile and fertile women are shown in table (1) . the table indicates that the concentration of T<sub>4</sub> is significantly lower (p <0.05) in the infertile group compared to control fertile group, while the concentration of TSH showed a high significant increase (p <0.01) in the infertile group when comparing with control group .

Table (1) : Thyroid function tests (T<sub>3</sub>, T<sub>4</sub>, TSH) of infertile and control groups .

Parameter	Infertile Women (No. = 80)	Control (No. = 20)	P-Value
T <sub>3</sub> (ng/ml)	1.6 ± 0.43	3.2 ± 1.8	NS
T <sub>4</sub> (µg/dl)	7.8 ± 1.37	19.3 ± 5.8	< 0.0 5
TSH (mIU/ml)	11.4 ± 4.2	2.26 ± 0.71	< 0. 01

2. Oxidative stress tests among infertile and control groups

As illustrated in tables (2) , the level of MDA exhibited a high significant increase (p <0.01) in the infertile women as compared to the fertile women; the activity of CAT showed a significant decrease (p <0.05) in the infertile group; while the level of GSH showed a high significant (p <0.001)

Table (2) : Oxidative stress tests (MDA, CAT, GSH) of infertile and control groups

Parameter	Infertile Women (No. = 80)	Control (No. = 20)	P-Value
MDA (µmol/L)	12.2 ± 3.8	1.8 ± 0.45	< 0.01
CAT (U/ml)	4.3 ± 2.8	8.2 ± 3.4	< 0.05
GSH (µmol/L)	62.4 ± 23.71	132.4 ± 26.2	< 0.001

3. Correlation between thyroid function tests and oxidative stress tests

As shown in table (3) , there is a significant negative correlation (p< 0.05) between MDA and TSH , a significant positive correlation (p< 0.05) between MDA and T<sub>4</sub>, a high significant positive correlation (p< 0.01) between TSH and each of CAT and GSH, and a high significant negative correlation(p< 0.01) between T<sub>4</sub> and each of CAT and GSH .

Table (3) : Correlation between thyroid function tests (T<sub>3</sub>, T<sub>4</sub>, TSH) and oxidative stress tests (MDA, CAT, GSH) among infertile women

	T <sub>3</sub> (ng/ml)	T <sub>4</sub> (µg/dl)	TSH (mIU/ml)
MDA (µmol/L)	r = - 0.148	r = - 0.198*	r = 0.230*
CAT (U/ml)	r = 0.156	r = 0.266 **	r = - 0.377 **
GSH (µmol/L)	r = 0.098	r = 0.321	r = - 0.407 **

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\* Critical (r) value at 0.05 =  $\pm 0.187$

\*\* Critical (r) value at 0.01 =  $\pm 0.234$

#### IV. DISCUSSION

The results of the current study revealed a high significant decrease in the level of  $T_4$  and a high significant increase in the level of TSH in the infertile women group as compared to fertile (control) group (table 1), this can be interpreted as a defect in the function of thyroid gland which stimulate the pituitary gland to response in releasing more quantities of TSH indicating primary hypothyroidism<sup>(19)</sup>.

Many previous studies indicated hypothyroidism as a contributing factor in female infertility<sup>(6-8)</sup>, however, the mechanism by which thyroid hormones affect female reproductability is still under study. It was found that hypothyroidism may results in delayed response of LH to gonadotropin-releasing hormone GnRH and altered estradiol metabolism which, in turn, result in delayed or interrupted ovulation and inadequate corpus luteum<sup>(20)</sup>. Recently, some authors have found that TSH plays a role in successful implantation by directly affecting the status of endometrium<sup>(21)</sup>. Some researchers observed a link between low thyroid hormones and decreased levels of hemostatic factors which may lead to changes in menstrual cycle and increased bleeding. Krassas *et al.* reported that both Gn and  $T_4$  are important in achieving maximum level of success of fertilization and blastocyst development<sup>(22)</sup>. Aghajanova *et al.* referred to the presence of thyroid hormone receptors in human oocytes, where they act together with the LH and human-chorionic- gonadotropin HCG receptor, to enhance a stimulatory action that directly effects on the function of granulosa cell and on differentiation trophoblastic cells<sup>(23)</sup>.

The results of the present study also showed a high significant increase in the level of MDA, a significant decrease in the activity of CAT, and a high significant increase in the level of GSH in the infertile women group as compared to fertile (control) group (table 2), this comes in agreement with many previous literatures that indicated similar results<sup>(12-14)</sup>.

Oxidative stress is considered as an etiologic factor in female infertility; it have negative impacts on female fertility by interfering with ovulation, fertilization, embryo implantation and development. It was found that women with polycystic syndrome have a higher level of oxidative stress indicators than healthy individuals<sup>(24)</sup>. A recent study indicated that oxidative stress can be considered as an independent causative factor in female infertility, and is initiated primarily via ROS overproduction by increased macrophage activity rather than deficiency of antioxidant capacity<sup>(25)</sup>.

It was found that lipid peroxidation, evidenced by increased MDA, had damaging cytotoxic effects on the cell membrane of female reproductive system, especially of the egg cells and even the spermatozoa and the developing embryo. Severe oxidative stress can prevent pregnancy; because of the negative effect on egg-sperm fusion actions such as acrosome reaction and sperm-oocyte fusion<sup>(26)</sup>.

Catalase is antioxidant enzyme; present primarily in the tubal fluid and cervical secretions; it acts to reduce intracellular

and extracellular hydrogen peroxide into water and oxygen. Higher levels of ROS in women with unexplained infertility may lead to increase ROS-scavenging process which, in turn, results in reducing the levels of antioxidants such as catalase enzyme<sup>(27)</sup>.

Previous laboratory investigations have suggested that glutathione GSH is a major factor in protecting body tissues and cells against the damaging effects of ROS. In female genital tract, glutathione is located mainly in the tubal fluid and oocyte/embryo. It equilibrates superoxide anion, reduces hydrogen peroxide and hydroxyl radical, enhances the development of zygotes up to the blastocyst stage, provides a protection to the embryo against ROS and prevents oxygen-induced congenital defects<sup>(28)</sup>. In vitro studies have suggested that the concentration of GSH in the oocyte is necessary to reduce the disulfide bonds during the process of sperm chromatin decondensation, decapitation and pronuclear apposition. GSH protects plasma cellular membrane from lipid peroxidation, neutralizes superoxide and prevents  $O_2$  formation<sup>(29)</sup>.

Previous studies have linked between hypothyroidism and increase in the overall oxidative stress; it was found that high levels on OS may have detrimental effects on the thyroid follicular cells that secrete  $T_3$  and  $T_4$  decreasing their levels in the blood and increasing TSH<sup>(30)</sup>. On the other hand, a study performed on rats revealed that TSH may act to regulate the activity of CAT in liver, with a positive correlation between them<sup>(31)</sup>; Hamid suggested that hypothyroidism may cause tissues to undergo many biochemical and histological changes that make them susceptible to oxidative damage<sup>(32)</sup>; Tullanithi *et al.* found that the antioxidant activity of glutathione peroxidase decrease significantly in hypothyroid patients because of the deficiency of selenium that is considered an important component of the antioxidant system<sup>(33)</sup>.

#### V. CONCLUSIONS

It seems that thyroid hormone disorders and increased oxidative stress act synergistically as causative and predisposing factors for female infertility.

#### VI. RECOMMENDATION

It is recommended to for physicians and health care providers to take in mind the link between thyroid hormones disorders and oxidative stress in the treatment strategies of female infertility, especially in the unexplained infertility.

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