RESEARCH ARTICLE

Role of oxidative stress and altered thyroid hormones in unexplained infertility

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Abstract

Objective: To explore the link between altered thyroid profile and oxidative stress marker in females with unexplained infertility.

Methods: The cross-sectional case-control study was carried out at the Islamabad Clinic Serving Infertile Couples, Islamabad, Pakistan, from June 2016 to August 2017, and comprised women aged 18-40 years regardless of ethnic background who were divided into two groups; those with unexplained infertility were the cases, while fertile women acted as the controls. Serum was analysed for triiodothyronine, thyroxine and thyroid stimulating hormone as well as for oxidative stress markers including manganese superoxide dismutase, glutathione reductase and adrenaline using enzyme-linked immunosorbent assay. Data was analysed using SPSS 19.

Results: Of the 88 subjects, there were 44(50%) in each of the two groups. There was no significant difference in terms of thyroids markers except thyroxine and thyroid stimulating hormone ($p<0.05$). There were significant differences in terms of oxidative stress markers between the groups ($p<0.05$). A significant positive correlation of thyroid stimulating hormone was observed with manganese superoxide dismutase and adrenaline ($p<0.05$) with a weak non-significant association of glutathione reductase ($p>0.05$).

Conclusion: Increased thyroxine levels in females with unexplained infertility was associated with decrease in the serum levels of antioxidants.

Keywords: Oxidative stress, Thyroid profile, Glutathione reductase. (JPMA 70: 1345; 2020).

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Introduction

Infertility, as per definition of World Health Organisation (WHO), is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Approximately 25% of couples in developing countries are affected by infertility and 7-28% of such cases have female infertility as the main cause.[1] Causes of infertility could be narrowed down from hormonal to cellular changes in the body; one of which is the imbalance between pro- and anti-oxidants that results in oxidative stress (OS).

The oxidant status is critical in early embryonic development since it can modify key transcription factors that are important for implantation and fertilisation required for becoming fertile.[2] Enhanced reactive oxidative species (ROS) can disturb the normal physiological processes at different levels during embryogenesis, such as oocyte maturation, fertilisation, embryo development, pregnancy and even parturition that exacerbate the chances of infertility.[3] The ovum released from the ovary, the zygote or embryo and spermatozoa are all vulnerable to damage inflicted by OS.[3] Anti-oxidants, such as glutathione reductase (GR), react with ROS and convert them into benign compounds through reduction-oxidation (redox) system to control cellular structural damage and maintain homeostasis in oocytes.[4,5]

In addition to oxidants, there is also an increased demand of certain hormones during gestation and an imbalance could also be responsible for cellular damage. Research has shown the presence of thyroid hormones (TH) and thyroid stimulating hormone (TSH) receptors in the foeto-maternal unit (i.e. present in both the endometrium and

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the trophoblast), hence, there are potential paracrine and intracrine functions of TSH and TH during implantation.[6] At a cellular level, triiodothyronine (T3) works synergistically with follicular stimulating hormones (FSH) and epidermal growth factor to stimulate granulosa cell functions, facilitating FSH-mediated luteinizing hormone (LH)/human chorionic gonadotrophin (hCG) receptor induction, progesterone and human placental lactogen production (hPL).[6] This interplay explains synergy of FSH-mediated LH/HCG receptor to exert direct stimulatory effects on granulosa cell function e.g. progesterone production.[7]

TH alterations in both hyperthyroidism and hypothyroidism are found to be associated with OS, particularly peroxidation and anti-oxidant enzyme activities in animals and humans.[8] Both hyperthyroidism and hypothyroidism have been shown to be associated with OS and special cases are the autoimmune thyroiditis or the functional picture of low-T3 syndrome observed in acute and chronic non-thyroidal illness syndrome (NTIS).[9]

We are aware of the fact that excessive ROS production can lead to influx of macrophages during ovulation that yield in high concentrations of free radicals which may cause a number of reproductive disorders, including unexplained infertility.[10] The interplay between the hypothalamic-pituitary-ovarian (HPO) axis and the hypothalamic-pituitary-thyroid (HPT) axis is already documented.[7] Furthermore the role of altered TH status and debate on NTIS, which is manifested in decreased conversion of thyroxine (T4) to T3, led to the hypothesis that OS can cause a disturbance in TH profile or vice versa that may be the cause of unexplained fertility. The current study was planned to explore the link between altered thyroid profile and OS markers in females with unexplained infertility.

Subjects and Methods

The cross-sectional case-control study was conducted from June 2016 to August 2017 at the Islamabad Clinic Serving Infertile Couples (ICSI), Islamabad, Pakistan. After approval from the institutional ethics review committee, the sample size was calculated using Open-Epi version 3[11] and the formula n = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1) +p*(1-p)] in order to achieve a confidence interval of 80%, confidence limit of 5% and hypothesised frequency of outcome factor to be 17%.[12] The subjects were recruited from among those presenting to the clinic aged 18-40 years from all ethnic backgrounds. All infertile females with normal ovulation, body mass index (BMI) 18-25kg/m^2, regular menstrual cycle, and normal reproductive organs with normal serum levels of FSH <11 IU/L during the early follicular phase (day 2-5), prolactin <20 mg/L and TSH and TH levels in serum were recruited. Females with patent fallopian tubes were recognised by hysterosonosalpingography, and no detectable manifestation of endometriosis was determined upon clinical examination or ultrasonography. Their partners had total sperm count (TC) >39 million per ejaculate, total sperm motility (progressive and non-progressive) measured within 60 minutes of collection of more than 40%, and normal morphology of 4%, as defined by WHO criteria.[13] Recruitment was done using convenient random sampling.

Controls were healthy, parous women with normal ovulatory cycles, normal hormonal screen, and were matched for age and BMI. All women who had diagnosed infertility of their partner, known causes of infertility, polycystic ovarian syndrome (PCOS), uterine fibroids, endometriosis or tubal blockade menstrual irregularities with anovulatory cycles, more than 40 years of age, metabolic disorder like diabetes, obesity, hypertension, thyroid dysfunction, using oral contraceptive pills and/or having intrauterine device (IUD(placement were excluded.

After informed consent from each subject, blood was collected on the first visit. Serum was separated and kept at -70 degrees until further assay was performed. Enzyme-linked immunosorbent assay (ELISA) was used to monitor thyroid status. Levels of T3, T4 and TSH were detected using KITS (Diametra ELISA Kits Cat# DKO044, DKO045 and DKO013). At 95% confidence limit, detection limit for T3, T4 and TSH were reported as 5ng/dL, 0.4µg/dL and 0.01mIU/L respectively as per the manufacturer's protocol. OS markers assessed included manganese superoxide dismutase (MnSOD) (Cat# SG-10731, Sino Geneclon) and glutathione reductase manganese superoxide dismutase (MnSOD), GR (Cat# SG-00523, Sino Geneclon), adrenaline (Cat# SG-10545, Sino Geneclon) and cortisol (Cat# CO103S, Calbiotech) were evaluated using ELISA protocol of respective manufacturers.

Data was analysed using SPSS 19. Clinical characteristics were tabulated as frequencies and percentages for

qualitative variables (age group), and mean standard deviation (SD) for continuous/quantitative variables.

Table-1: Comparison of Thyroid Profile in Study Groups.

	Fertile Females (n=44)	Infertile Females (n=44)	p-value
Age (Years)	31.57±6.12	32.33±5.83	0.474
BMI (Kg/m^2)	23.67±1.66	28.59±4.58	<0.001
T3 (ng/dl)	131.67±24.04	135.57±32.55	0.494
T4 (ug/dl)	9.18 ±1.53	10.48± 1.89	0.001
TSH (mIU/L)	1.12 ± 0.54	1.49 ± 0.76	0.027

Values are mean ± Standard Deviation; Results compared by Mann Whitney test; BMI: Body mass index; T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid stimulating hormone.

Table-2: Comparison of Oxidative stress markers in Study Groups.

	Fertile Females (n=44)	Infertile Females (n=44)	p-value
Glutathione reductase (pg/ml)	854.5±335.4	150.9± 53.5	<0.001
MnSOD (ng/ml)	1.01 ± 0.42	6.06± 2.73	0.001
Adrenaline (pg/ml)	5.23 ± 2.06	40.13± 27.15	0.001

Values are mean ± Standard Deviation; Results compared by Mann Whitney test; MnSOD: Manganese superoxide dismutase.

Independent sample t-test was used for difference in the mean of two groups. Correlation of TH with markers of OS was done by Pearson correlation. P<0.05 was considered significant in all analysis.

Results

Of the 88 subjects, there were 44(50%) in each of the two groups. There was no significant difference in terms of thyroids markers except T4 and TSH (p<0.05) (Table 1). There were significant differences in terms of OS markers between the groups (Table 2).

There was a positively significant correlation of TSH with MnSOD and adrenaline, and a weak negative correlation with GR (Figure). There was a strong positive correlation of T4 with TSH (p=0.001), and a direct correlation of T4 with adrenaline (0.021), while T4 had a strong inverse relation with GR (p<0.001). Also, there was a positive correlation of T3 with T4 (p=0.017).

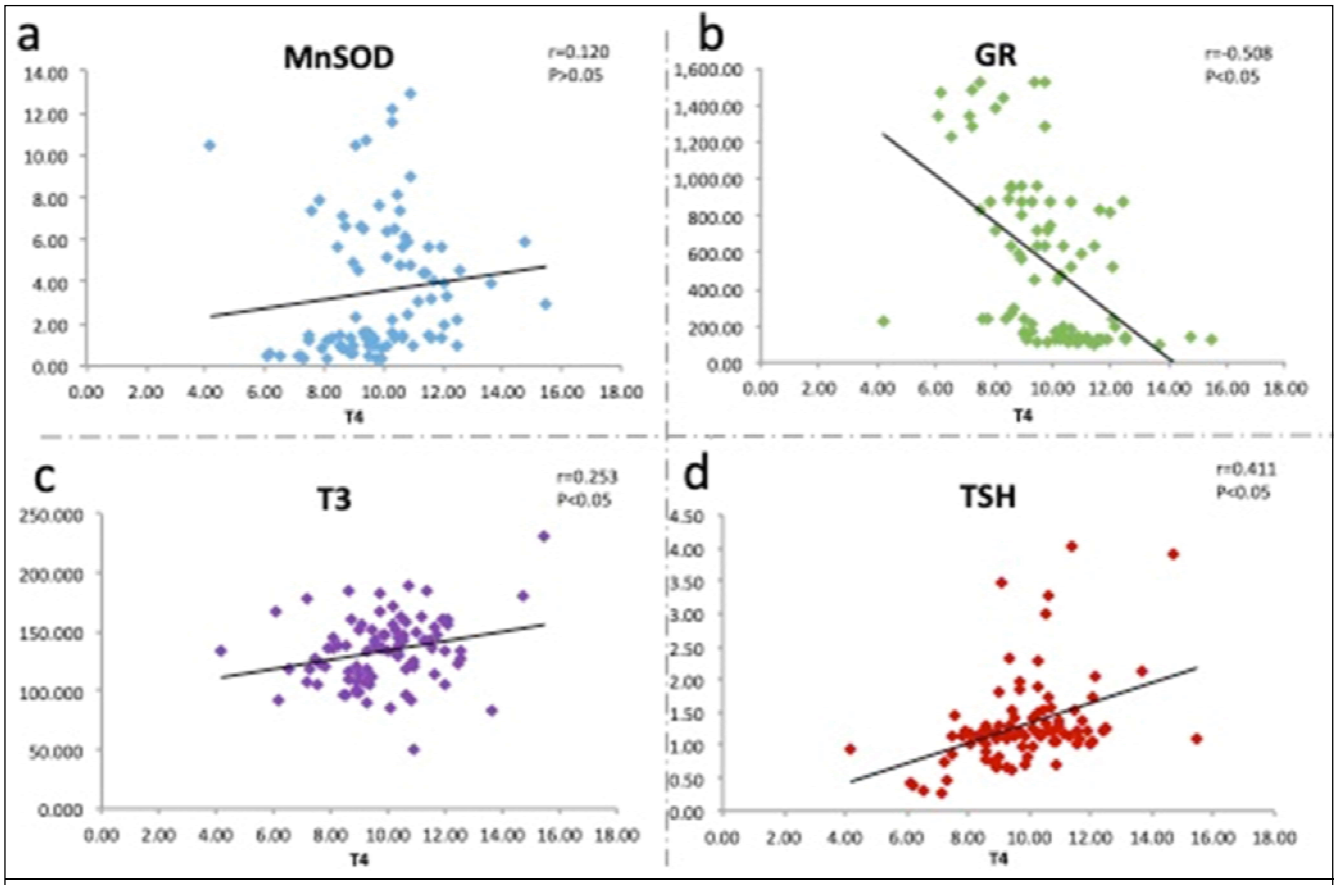

Figure-2: Association of thyroxine (T4) with thyroid hormones and Oxidative Stress Markers.
[a] insignificant correlation of T4 concentrations (ug /dl) with manganese superoxide dismutase (MnSOD) (ng/ml), [b] significant negative correlation of T4 concentrations (ug /dl) with glutathione reductase (GR) (pg/ml), [c] significant positive correlation of T4 concentrations (ug /dl) with T3 concentrations (mIU/L), [d] significant positive correlation of T4 concentrations (ug /dl) with thyroid stimulating hormone (TSH) (mIU/L).

Discussion

The diagnosis of unexplained infertility is challenging and mainly relies on tests that show normal semen parameter, ovulatory functions, tubal patency and a normal uterine cavity. The pathophysiology of unexplained infertility with low implantation rates still remains a scientific challenge with various unknown risk factors.[14] Elevated ROS levels in patients with unexplained infertility implies exhausted anti-oxidant defence, resulting in the inability to scavenge ROS and neutralise their toxic effects which we observed in our study by a significant decrease of GR in infertile females. A positive correlation of T4 and TSH in our study represents normal cascade of hypothalamo-pituitary thyroid hormone without the feedback regulation. Finding of a negative association of altered TH with GR, however, expresses impact of OS that could be one of the causative factors for unexplained infertility.

In the body, the anti-oxidant defence capability consists of enzymatic and non-enzymatic systems in which the latter is represented mainly by glutathione.[4] The body reproduces its own glutathione which can be altered by diet, pollution, toxins, medications, stress, trauma, aging, infections and radiation; and glutathione is normally recycled in the body. The basic function of glutathione in the reproductive system is related to its interactions with other systems, as a preventive mechanism against ROS.

GR plays a key role in protection against the detrimental effects of free radicals by providing detoxification of organic and inorganic peroxides.[15] Hence, the decreased endogenous anti-oxidant enzyme GR causes disruption of cell defence mechanism against mitochondrial ROS and affects the oocyte maturation, fertilisation, embryo development and pregnancy.[16] We observed reduced GR in infertile females, and Rocha et al. has also reported low GR activity in low responder females going through in vitro fertilization (IVF), hence low GR levels may decrease ovarian response.[17]

Thyroid hormones are considered to be "instrumental in reproductive physiology".[18] Association between female fertility and thyroid status is a well-established physiological phenomenon. For conception and progression of normal pregnancy, performance of TH should be optimal. Imbalance in levels of T3 T4 and TSH may result in inability to conceive or early miscarriages, especially in assisted reproduction.[19] In females, T3 is required for progesterone release during the luteal phase.[20] We observed no difference in T3 levels in fertile and infertile females, but T4 levels in infertile females was higher compared to fertile females which is similar to a study done by Al Fahham.[21]

TSH is known to be a very important parameter for conception, regulator of normal menstrual cycle and, thus, is strongly associated with female reproductive health.[22] We observed a high TSH concentration in infertile females which is similar to a study in which a high TSH was observed in females with all causes of infertility, like ovulatory dysfunction, tubal disorders and endometriosis.[22] High TSH levels are associated with reduced fertilisation potential of oocytes, which may lead to infertility. The correlation of TSH with markers of OS express interplay between the HPO and HPT axis.[7]

It has been observed that anti-oxidant capacity is poor in patients with hypothyroidism.[21] There is very little data available to describe the relationship between OS markers and TH levels in infertile females. In this study, TSH levels fell in the normal range <6.16 mIU/ml, but were raised in infertile females compared to fertile subjects. The association of TSH levels with OS marker GR observed in our study is also supported by Mancini A.[23]

In particular, THs play important roles in anti-oxidant modulation, as demonstrated in different in vitro and in vivo studies by generating OS in hyperthyroid states.[9] Reduced glutathione is an important co-factor of both anti-oxidant enzymes and de-iodinases, the enzymes responsible for the conversion of iodine to iodide and T4 to T3.[24] Increase in MnSOD with decrease in GR in hyperthyroid rats has been experimented upon, advocating the increased lipid peroxidation and perhaps organ damage due to OS. We also observed similar results with increased MnSOD and low levels of GR produced in infertile females, which justifies association with an increase in TSH. The role of THs on infertility was studied in animal model of rats[25] and demonstrated the susceptibility of THs with OS that may influence the lipid composition in rat hepatic tissues.

The current study is limited in terms of its small sample size. The sample size calculation demanded 93 subjects, but only 88 were recruited due to the limited funds for the ELISA kit. Despite the limitation, however, it is a unique research finding the association between TH

profile and unexplained infertility. Further studies are needed on the role of thyroid replacement medications or anti-oxidants to improve female fertility profile.

Conclusion

Disturbance in the oxidant/anti-oxidant levels due to raised T4 may have been the cause of unexplained infertility in females.

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Conflict of Interest: None.

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