THYROID DYSFUNCTION AND INFERTILITY TREATMENT

S. Lalani¹, I. Nizami¹, A.A. Hashmi¹, A. Saifuddin¹, R. Rehman^{1,*}¹Aga Khan University, Karachi, Pakistan**Abstract**

Objective. To find out the relationship of thyroid hormone profile of females with outcomes after intra cytoplasmic sperm injection (ICSI).

Method. It was a cross sectional study conducted in Islamabad Clinic Serving Infertile Couple from June 2013 till August 2015. T3 (triiodo thyronine), T4 (thyroxine) and TSH (thyroid stimulating hormone) of 168 consented females was estimated after they underwent the first step of treatment protocol (ovarian down regulation) for ICSI. Pregnant group had β hCG result more than 25 IU/mL while the rest were included in the non-pregnant group. Both groups were compared by using independent sample t-test. Pearson correlation coefficient was used to associate T3 and T4 with other pregnancy variables with their significance.

Results. Non pregnant women had significantly higher mean values for T3 and T4 as compared to pregnant women ($p < 0.05$, $p < 0.01$). Difference in mean TSH value between non-pregnant and pregnant women was not significant $p = 0.08$. It was found that T4 gave significant negative association with grading of embryo-I, blastocysts formed, thickness of endometrium and number of gestational sacs.

Conclusion. Disturbance in thyroid profile with raised T4 levels leads to alteration in endometrial thickness and quality of embryos required for implantation and hence conception.

Key words: Infertility, Intracytoplasmic sperm injection, Assisted reproductive technique, Thyroid profile, High levels of thyroxine.

INTRODUCTION

Infertility is a growing issue around the globe, estimating that 15% of population suffer from it (1). Reasons of infertility may range from poor reproductive health of either partner or deficiencies of crucial biochemical substances, which are usually overlooked or remain undiagnosed (1). Hypothyroidism falls in the latter category, where disturbance in thyroid hormones interferes with fertility. Since hypothyroidism

sometimes remains asymptomatic for a long time, therefore it cannot be easily caught as a contributing factor of infertility.

Many women may encounter hypothyroidism which can be primary, secondary or tertiary hypothyroidism depending upon the site of lesion. Irrespective of cause, alteration in thyroid hormone levels can influence conception and pregnancies. For instance, sub-clinical hypothyroidism (SCH), also called mild thyroid failure, is diagnosed when peripheral thyroid hormone levels are within normal reference laboratory range, but serum thyroid-stimulating hormone (TSH) levels are slightly elevated (≥ 4.25 mIU/L). Clinical hypothyroidism, on the other hand, has increased TSH and decreased T4 serum concentrations (2). Similarly, autoimmune thyroid disease causes antibodies production against thyroid gland and its products – Hashimoto's disease. In either case, it is the area of concern and major health burden globally.

Hypothyroidism has significant interference, in general, for couples who try to conceive and in particular for those who are undergoing infertility treatments. Developed countries have reported hypothyroidism to be 2-4% in fertile group (3). SCH has been recognized as major culprit. It is known that hypothyroidism causes increase in thyrotropin releasing hormone, which in turn, alters the level of prolactin and luteinizing hormone (LH) response – both are major contributor towards fertility. Therefore, any correction of hypothyroidism at initial level can be fruitful for the successful treatment of infertility (4).

On the contrary, the state of hyperthyroidism in females also poses hindrance in the way of conception due to poor reproductive state. Unlike hypothyroidism, it is a state in which TSH levels are suppressed with elevated T3 and T4. It may be caused by Plummer's disease, a state in which autonomous nodules are formed on the thyroid gland, which causes the condition of hyperthyroidism. Thyroiditis, a frequent

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thyroid disorder is yet another condition which results in hyperthyroidism. Another documented reason for hyperthyroidism is an autoimmune condition, Graves' disease, in which antibodies stimulate the thyroid gland, resulting in over production of thyroid hormones (5).

Intra cytoplasmic sperm injection (ICSI) is one of such treatments in which proper thyroxin levels are essential for the success of treatment. It is known from the literature that TSH is expressed by ovaries, while T3 along with thyroid hormone receptors are known to protect apoptosis of granulosa cells. Any fluctuation in TSH levels can influence the ICSI in a negative way (6). A decrease in thyroxin may cause early miscarriage which explains its role in implantation. On the other hand, literature suggests that over production of thyroid hormone does not impact the assisted reproduction significantly. However, fertile women with hyperthyroidism, autoimmune thyroid disorders in particular, are more prone to miscarriages, fetal abnormalities, etc. (5).

A number of studies have been conducted to detect ovulatory dysfunction or infertility with thyroid disorders (7). Although untreated thyroid disease can be a cause for infertility as well as sub-fertility, limited literature is available to explore the impact of thyroid dysfunction on infertility treatment procedures. To add further, a low success rate of ICSI (25-30%) requires the need to identify and elucidate all the treatable factors that could improve implantation after ICSI. (8) Since thyroid dysfunctions can be treated before starting the ICSI, therefore, an early detection and treatment of irregularities can contribute to successful ART. Our study was designed to relate thyroid hormone profile of females with outcome after ICSI to know the impact of thyroid profile on success after ICSI.

SUBJECTS & METHODS

The cross sectional study was carried out in "Islamabad Clinic for Serving Infertile Couples" from June 2013 till August 2015, after approval from the Ethical Review Board on the basis of convenience sampling. Participation was voluntary and ethical clearance was obtained from the Institutional review board. Respondents were given detailed information about the study and were assured of the confidentiality of the data. Informed consent was obtained before the start of the study.

The included females had age range from 23 till 41 years, with normal ovulatory cycles and uterine morphology. Although routine thyroid screening was

not done before recruitment of, yet women who had available prior reports reflecting abnormal thyroid profile (raised T3, T4 and reduced TSH proven by documented evidence) were excluded from our study. Moreover, women with other endocrine disorders, endometriosis and polycystic ovarian syndrome (PCOS) were also excluded. The GnRH receptors were down regulated with daily injection of Deca Peptyl (gonadotropin releasing hormone agonist) from day 21 of previous cycle. In order to assess the impact of thyroid profile on treatment procedure, blood samples for thyroid tests (T3, T4 and TSH) were taken after the participants were treated for ICSI and hence those who were identified to have abnormal thyroid levels after down regulation could not be excluded. This was done with the objective to see the effect of altered/undiagnosed thyroid status on ICSI results. Serum T3, T4 and TSH were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) subsequently by Diametra ELISA Kit with sensitivity reported as the lowest detectable concentration of T3, T4 and TSH that can be distinguished from the calibrator 0 is 5 ng/dL, 0.4 µg/dL and 0.01 mIU/L at the 95 % confidence limit, respectively.

After down regulation of ovaries, follicle stimulation was done by gonadotropin injections (Puregon) for fourteen days; the dose was titrated with respect to basal follicle stimulating hormone (FSH) and response to stimulation. Follicular monitoring by transvaginal ultrasound (TVS) was done three to four days after the commencement of the ovarian stimulation till maturity of maximum follicles to 20 mm. Human chorionic gonadotropin (β hCG) injection (10,000 IU) was given and procedure was done 3-6 hours after oocyte recovery on all morphologically intact ovocytes in Metaphase II stage as mentioned in our previous study (8). Fertilization was assessed by development of two pronuclei. Cleavage rate was assessed by counting the number of cells in the embryo on day three after 24 hours. Embryos that had six to eight cells on day three were considered to have cleaved at a normal rate, embryos with five cells or fewer were considered to have slow cleavage, and embryos with nine or more cells were considered to have accelerated cleavage. Embryos were evaluated on alternate days. All embryos were graded (Grades 1-5) as described by Aktan *et al.* (9).

Primary Outcome measure

Single serum β hCG was obtained in the respondents 14 days after ovocyte collection by

aseptic peripheral venipuncture method. TVS was performed at 8 weeks gestation on the basis of β hCG and TVS results were categorized into pregnant group comprised of females with β HCG more than 25 IU/mL and cardiac activity on TVS while the rest comprised of non-pregnant group.

Statistical analysis

The data feeding and analysis was done via SPSS (Statistical Packages of Social Sciences) version 15.0. Clinical characteristics were summarized in terms of frequencies and percentages for qualitative variables (age group), mean $\pm$ S.D or $\pm$ SEM for continuous/quantitative variables. Using independent sample t-test for quantitative variables performed statistical comparison. Pearson correlation was used to relate thyroid status with predictors of pregnancy. In all statistical analyses, only p-value <0.05 was considered significant.

RESULTS

In the study, 72/168 women conceived with a pregnancy rate of ~43%. Table 1 represents descriptive characteristics of study participants after down regulation for ICSI. The comparison of T3, T4 and TSH between pregnant and non-pregnant women using independent sample t-test, showed significantly higher mean for T3 and T4 in non-pregnant women as compared to pregnant women $p < 0.049$ and < 0.01 , respectively (Table 2). TSH did not give any significant differences in mean value between non-pregnant and pregnant women, $p = 0.08$.

Table 3 reports the Pearson correlation coefficient of T4 with other pregnancy parameters with their significance. It was found that T4 gave significant positive association with poor grading embryos (II, III). T4 gave significant negative association with grading of embryos I, blastocysts formed, thickness of endometrium, number of gestational sacs and ratio

Table 1. Descriptive statistics of study population after down regulation for ICSI

	Mean	Std. Deviation	Minimum	Maximum
Female age (years)	32.17	4.82	23.00	41.00
Duration of infertility (years)	6.54	3.51	2.00	18.00
BMI Kg/m ²	23.96	3.55	17.00	30.00
No. of oocytes/patient	7.75	1.63	5.00	12.00
No. of oocytes Metaphase II	7.39	1.66	2.00	11.00
No. of oocytes fertilized	6.14	1.28	2.00	9.00
No. of cleaved embryos	6.05	1.25	2.00	9.00
Grading of embryos I	2.19	1.54	00	6.00
Grading of embryos II	2.57	1.12	1.00	5.00
Grading of embryos III	1.29	0.50	00	2.00
Blastocyst formed	2.08	0.47	1.00	3.00
Grading of blastocyst I	1.55	0.81	00	4.00
Grading of blastocyst II	0.42	0.50	00	1.00
Grading of blastocyst II	0.15	0.45	00	2.00
Thickness of endometrium	8.80	3.37	3.00	14.00
No. of transferred embryos	1.69	0.60	1.00	3.00
Number of gestational sacs	0.63	0.80	00	2.00
T3 ng/dL	293.18	61.00	147.54	559.34
T4 μ g/dL	16.45	1.39	13.22	19.56
TSH mIU/L	2.41	1.60	1.18	14.86

T3 (triiodo thyronine), T4 (thyroxine) and TSH (thyroid stimulating hormone)

Table 2. Comparison of Mean Values for T3, T4 and TSH between Pregnant and Non Pregnant Women

	Groups	N	Mean	Standard Deviation	p-value
T3	Non-pregnant	96	304.2	62.41	0.049*
	Pregnant	72	278.4	56.59	
T4	Non-pregnant	96	17.4	0.82	$< 0.01^*$
	Pregnant	72	15.1	0.83	
TSH	Non-pregnant	96	2.1	0.76	0.08
	Pregnant	72	2.7	2.24	

* $p < 0.05$ considered significant using independent sample t-test

T3 (triiodo thyronine), T4 (thyroxine) and TSH (thyroid stimulating hormone)

Table 3. Correlation of T4 with Pregnancy Parameters

Correlation of T4 with Pregnancy Parameters	R	p-value
No. of oocytes/patient	-0.179	0.102
No. of oocytes Metaphase II	-0.156	0.156
No. of oocytes fertilized	-0.179	0.103
No. of cleaved embryos	-0.125	0.259
Grading of embryos I	-0.652	<0.01*
Grading of embryos II	0.54	<0.01*
Grading of embryos III	0.479	<0.01*
Blastocysts formed	-0.248	0.023*
Grading of blastocysts I	-0.603	<0.01*
Grading of blastocysts II	0.571	<0.01*
Grading of blastocysts III	0.188	0.086
Thickness of endometrium	-0.57	<0.01*
No. of transferred embryos	-0.045	0.685
Number of gestational sacs	-0.726	<0.01*
Number of sacs/ number of blastocysts * 100	-0.779	<0.01*

*P<0.05 considered significant for Correlation

value of number of sacs to number of blastocysts. There was no significant association found of T4 with number of oocytes, metaphase II, fertilized, cleaved embryos, grading of blastocysts II and number of transferred embryos.

DISCUSSION

Adequate levels of circulating thyroid hormones are of primary importance for normal reproductive function. A close relationship between the hypothalamic pituitary ovarian (HPO) and hypothalamic pituitary thyroid (HPT) axes can be explained by the presence of thyroid hormone receptors at the ovarian levels. The role of thyroid hormones on reproductive functions can be explained further by synergy with FSH on LH/hCG receptor to exert direct stimulatory effects on granulosa cell function, e.g. for production of progesterone required for endometrial receptivity and blastocyst implantation (10).

Evaluation of thyroid status in the infertile couple is not only important because it is significant and most common, but also its treatment is very simple and often has preventable effect on infertility (11). In the present study serum T3 and T4 levels were found to be significantly increased in infertile non pregnant females as compared to pregnant females.

According to the American Thyroid Association, the thyroid profile of a woman is different at different stages of pregnancy. The general trend is that TSH, T4 and T3 levels decrease as the pregnancy progresses. For example, TSH in normal, non-pregnant females ranges from 0.34 to 4.25 mIU/L and it decreases in pregnancy between 0.38 and 4.04 mIU/L by the third

trimester (12). Similarly, T4 levels range 5.4 to 11.7 µg/dL in normal, non-pregnant females while by the third trimester of pregnancy, they decrease to a range from 6.3 to 9.7 µg/dL (12). Total T3 levels, however, are less consistent with this trend: in normal, non-pregnant women the levels range from 60 to 181 ng/dL and 71 to 175 ng/dL and 104 to 182 ng/dL in the first and last trimester respectively (12).

In our study, the T3 and T4 levels were significantly higher in the non-conception group as compared to those with successful ICSI conceptions. In the non-pregnant, the average T3 level was 304.2 ng/dL, while in the pregnant women it was 278.4 ng/dL. Both of these levels are higher than normal according to the American Thyroid Association, but they were significantly higher than in the non-pregnant women. Similarly, the average T4 level was 17.4 µg/dL in the non-pregnant women while it was 15.1 µg/dL in pregnant women. Once again, it is demonstrated that T4 levels are higher than normal, and the T4 level in non-pregnant is significantly higher than that in pregnant women. The TSH levels, however, remained within normal limits but towards lower side despite increasing with pregnancy: 2.1 mIU/L in non-pregnant females and 2.7 mIU/L in pregnant females.

Disturbances in thyroid functions can lead to unsuccessful pregnancy. (11) Thus high T3, T4 and relatively lower TSH levels in non-pregnant females in our study explains disruption of HPT axis, and that may be the cause of treatment failures.

TSH levels reflect the integrity of hypothalamic pituitary thyroid (HPT) axis and are a "sensitive marker for thyroid dysfunction". During ART, increased TSH is attributed to stimulation by hCG and high E2 levels

which stimulate Thyroxin binding globulin to cause a decrease in free thyroid hormone and hence raised TSH (13). In our study, the levels were measured before ovarian stimulation which reflected a normal TSH (towards lower limit of range) with high T3 and T4 in non-pregnant females.

The number of mature oocytes, fertilized oocytes and cleaved embryos are all stages of normal embryogenesis. Blastocysts and their morphology are an important factor in the success of embryo transfer because this stage is well adapted to implant in the endometrium. There is a grading system in place that takes into account expansion of blastocoels, morphology of inner cell mass (ICM), cohesiveness of trophoectoderm (TE) and presence of excluded blastomeres or fragments from the formation of blastocysts, all of which are determinants of successful implantation (14). The negative correlation implies that higher T4 levels resulted in deterioration of embryo (blastocyst) quality. The low pregnancy with high T4 can be explained on the basis of poor quality of blastocyst which is associated with lesser chance of a successful implantation and conception (9). Keeping in mind the impact of thyroid dysfunction on blastocyst implantation and fetomaternal morbidities (7), increased awareness about detection of thyroid hormones in the infertility clinics should be reinforced. There is also a negative association of T4 levels with the thickness of endometrium and number of gestational sacs, both conditions that are essential for implantation of embryo. Endometrial thickness reflects the maturation of endometrium prepared to welcome the impending blastocyst (8). In our study decreased endometrial thickness due to higher T4 levels may be held responsible for the development of endocrine milieu, which synchronizes with the development and attachment of blastocysts. This again suggests that the high T4 levels are indicative of an inability to successfully conceive.

Our study has proved the importance of optimal T3 and T4 levels on the normal HPO axis with reference to oocyte and embryo parameters. In our study, thyroid profile was performed after down regulation of ovaries and indicates lack of complete correction of high T4 and T3 in terms of reduced TSH by HPT axis. This is manifested by presence of normal TSH with high T3 and T4 levels. It is a uni-centric study which has small sample size and we did not have their base line thyroid profiles and no immunological studies with anti-thyroperoxidase (TPO) and/or anti-thyroglobulin (Tg) antibodies were performed to establish a possible

correlation with autoimmune thyroiditis, a disorder that is believed to be the most common cause of thyroid dysfunction in recent years. Moreover, exact diagnosis of hyperthyroidism is based on increase in free T4 (FT4) and free T3 (FT3) which could not be made by our study. However, rise in total T4 and T3 gives us an avenue for complete thyroid screening of all infertile patients before they proceed for advanced infertility treatment procedures. Correction of thyroid function may help in improvement of results after infertility treatment procedures.

In conclusion, altered thyroid status in terms of high T3 and T4 in our patients was associated with failure of implantation and conception after ICSI. It has been observed that high T4 levels during the period of conception are associated with abnormal oocyte parameters (number, maturity and fertilization), embryo grading and endometrial thickness required for successful ICSI results. It is, therefore, recommended that thyroid hormone profile of all infertile females should be carried out and corrected before advanced infertility treatment procedures.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

The project was funded by The Aga Khan University – Research Module Funds of Department of Biological and Biomedical Sciences.

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