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Relationship between Thyroid Profile with Reproductive Hormones and Semen Quality

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Abstract

Semen quality is associated with various factors. The objective of the study was to investigate the impact of hypothyroidism and hyperthyroidism on human semen quality and reproductive hormones level if any. A total of 351 subjects were enrolled. They were subjected to assess the status of thyroid as well as reproductive hormones and semen quality. The subjects were grouped on the basis of thyroid hormone profile as, hypothyroid, hyperthyroid and normal thyroid hormone profile with respect to semen quality and reproductive hormones level. Semen volume, sperm count and viability was non-significant statistically in both hypothyroid and hyperthyroid subjects with respect to subjects with normal thyroid profile. Whereas, percent fast progressive Sperm was significantly lower in hypothyroid subjects, while non-motile sperm was significantly higher in hypothyroid subjects as compared to normal thyroid profile subjects. The data on reproductive hormones level indicated that FSH was higher and testosterone level was lower in both hypo and hyperthyroid subjects as compared to subjects with normal thyroid profile. Although the results were statistically non-significant. While LH level was also higher which was statistically significant in hypothyroid subjects with respect to subjects having normal thyroid profile. The data obtained suggest that impaired thyroid hormone has an impact on semen quality as well as reproductive hormone profile to some extent.

Keywords: Semen quality; Testosterone; LH; Hyperthyroid; Hypothyroid.

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1. Introduction

Reproduction is a basic drive in all living beings, including humans. Earlier studies have been concentrated on various factors of adverse effect on male reproductive system, such as decreasing quality of semen and increasing frequency of testicular cancer. Rate of fertility is decreasing during the last few decades is serious concern as defects passed on to the progeny are detrimental to the very existence of living beings. Further, it is necessary to consider the fact that reproduction is not just a proliferation of organism as it includes gamete's fusion and mix the genetic material from two different individual. So that, ancestral material should be passed unaffected to the next generation.

Facing infertility can be very difficult for both sexes and it causes emotional as well as social stress. About 13-18% of couple suffer from infertility regardless of race, ethnic group etc. Approximately one-half of them can be attributed to either partner [1]. In such cases men with no previous history associated with fertility problems were having normal findings on physical examination of sperm number, motility or morphology (or combination of these factors). These unexplained forms of male infertility may be associated with various exogenous and endogenous factors.

Endogenous hormones play important role in regulation of male reproductive function by maintaining spermatogenesis process [2]. The successful completion of male germ cell development depends upon the balanced endocrine function of hypothalamus, pituitary and testicular axis. Over and under secretion of endocrine glands leads to disorder of different organs and its function. They also mentioned that testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) has a major functional position in the male hypothalamopitutary-gonadal axis as well as in male reproductive system [2].

In recent years the role of thyroid hormones on germ cell development and process of spermatogenesis has been partially identified. Thyroid hormones might affect central (luteinizing hormone/follicle-stimulating hormone) and peripheral gonadal function by binding with sex hormone protein and ultimately affect semen quality [3]. The studies on impact of thyroid dysfunction on male reproductive function and semen quality are scanty and inconsistent. While the effect of hypothyroidism and hyperthyroidism in female's reproductive health, is well defined [4]. Owing to these, present study was carried out to explore the relationship if any between thyroid hormones with respect to male reproductive hormones and semen quality.

2. Materials and methods

***Ethical clearance*:** The paper is a part of major study for males. The ethical clearance was obtained from the Institutional Ethics Committee of National Institute of Occupational Health, Ahmedabad, India.

***Study subjects*:** The present study was conducted among the male partners of the couples attending for infertility. A total of 351 subjects were enrolled from the 20 to 45 years of age group.

***Inclusion and Exclusion criteria*:** Selected reproductive age group male attendees of the Out Patient Department (OPD) were enrolled. Subjects diagnosed with any sexually transmitted diseases, urogenital

problems or any other serious chronic diseases that might have an impact on reproductive system were not included. Subjects having history of taking drugs for specific disorder or supplementation of antioxidant that might affect semen quality were also excluded.

***Informed consent*:** A written consent was obtained from each subject to participate in the study. The information such as name, age, weight, height etc was recorded on pre-designed proforma for each subject.

***Collection of semen sample*:** Semen samples were obtained by masturbation within 3-6 days of sexual abstinence in a wide-mouth sterile container. Before sample collection, each patient was informed about the hygiene aspects and to avoid any spillage. They were explained that spillage would provide incorrect volume of semen, which in turn would affect the total count/ejaculate.

Collection of blood sample: Blood sample was collected from medial cubital vein using vacutainer syringe on the same day that the semen sample was collected. Blood sample was transferred into an anticoagulant free tube. After allowing for about 60 min for spontaneous blood clotting, the serum was separated by centrifugation at 3,000 rpm for 10 min at room temperature. Reproductive hormones such as testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid hormones such as triiodothyronine (T_3), thyroxin (T_4) and thyroid stimulating hormone (TSH) were measured in serum by EIA kit (Syntron Bioresearch, Inc., CA, USA).

Criteria for thyroid dysfunction

Thyroid function is considered as normal when subject having normal level of T_3, T_4 and TSH (Normal range of TSH is 0.50 to 4.10 μIU/m, T_4 is 4.58 to 11.88 UG/dL, T_3 is 0.56 to 1.88 ng/ml) provided in kit. Further, thyroid function was categorized as hyperthyroid [increase in T_3 (>1.88 ng/ml), increased T_4 (>11.88 UG/dL) and decrease in TSH (<0.50 μIU/m) level], Hypothyroidism [decrease in T_3 (<0.56 ng/ml), decrease T_4 (<4.58 UG/dL) and increase TSH (>4.10 μIU/m) level].

Physical examination of the semen:

***Volume*:** The volume of the semen sample was measured by determining the weight of the container before and after collection of the sample as per WHO (World health organization) criteria [5]. The difference in weight of the two provide the volume (ml) of the semen sample, assuming the density of semen to be 1g/ml.

***Observation of semen sample microscopically*:**

***Sperm motility*:** Sperm motility was assessed as per WHO criteria [6]. After liquefaction, about 10μl of well-mixed semen sample was taken on a clean glass microslide and covered with a cover slip.

The preparation was allowed to settle, and the slide was observed under the light microscope *(Zeneval, Carl Zeiss, Germany)* using 400x magnifications. At least 200 sperms were observed. During the assessment, all the rapid progressive sperms were counted first, followed by slow progressive sperms. After counting the

progressive sperms, non-progressive sperm than immotile sperms were counted in the same field. Percentage of sperms in each category was calculated.

***Sperm viability*:** Sperm viability was evaluated by eosin-nigrosin method. A drop of undiluted, well-mixed liquefied semen was mixed with two drops of eosin Y solution (1% aqueous eosin Y solution). After 30 seconds, three drops of nigrosin solution (10% aqueous nigrosin solution) was added and mixed.

A smear was prepared and air-dried. The viable sperms remained unstained while the dead sperms took the colour of the dye. The result is the proportion of vital (live) spermatozoa expressed as an integer percentage.

***Sperm count*:** The concentration of spermatozoa was determined as described in WHO guidelines [7]. Dilution of the semen sample was carried out in the proportion of 1:20 using semen diluent (5% of $NaHCO_3$, 35% v/v neutral formalin). The sperm counting was carried out with the help of Neubauer's chamber. Two separate aliquots were loaded in the Neubauer's chamber. Two separate readings were taken and the mean of the two was considered.

***Sperm morphology*:** Sperm morphology was assessed in stained samples using papanicolaou staining method [7]. Papanicolaou stain (Haematoxylin, Orange G and EA 50) gives a clear difference between the basophilic and acidophilic cell constituents and thereby enables the evaluation of sperm morphology.

***Morphological classification*:**

The morphological assessment was carried out at 100x under oil immersion using light microscope (*Zeneval, Carl Zeiss, Germany*). A minimum of two hundred spermatozoa were counted for each subject and categorized as below:

***Normal category*:**

Spermatozoa having an oval head, distinct midpiece region, normal tail and clear well defined acrosomal area (~40-70% of the head area) [7].

***Abnormal category*:** Morphological defects were categorized into head, midpiece, tail and multiple defects.

Statistical analysis Statistical analyses were conducted using SPSS 16.0 for windows. Descriptive statistics are reported as Mean ± SE. All other qualitative characteristics have been presented as count and percentage. To compare mean values among groups, for all reproductive hormone levels and semen quality variables, analysis of variance (ANOVA) was applied followed by Dunnett procedure in multiple comparisons.

All statistical tests performed using two-sided tests at a 5% significance level unless otherwise specified. A significant P value of less than 0.05 will be considered statistically significant.

3. Results

On the basis of thyroid hormones level subjects were divided into normal, hypothyroid and hyperthyroid groups. In that 45 (12.82%) subjects were hypothyroid, 39 (11.11%) were hyperthyroid and rest 267 (76.07%) had normal thyroid profile.

The data shows that mean age of the study population having normal thyroid profile was 31.95±0.36 years. Marginal elevation in age was observed in the subjects having imbalanced thyroid profile with respect to subjects with normal thyroid profile. No significant alteration was found in BMI between all three groups. Semen volume was decreased in both hypothyroid and hyperthyroid subjects as compared to subjects having normal thyroid profile. Variation in sperm viability was marginally lower in subjects with impaired thyroid profile as compared to group with normal thyroid profile, which was statistically non-significant (Table 1).

Semen quality parameters showed that mean sperm count was lower non-significantly in hyperthyroid and hypothyroid subjects as compared to subjects with normal thyroid level. Whereas, fast and total progressive motility percentage was significantly decreased and non-motile sperms were significantly higher in hypothyroid and hyperthyroid subjects as compared to subjects having normal thyroid profile (Table 2).

Sperm morphology with respect to thyroid profile is depicted in table 3. Normal sperm morphology percentage was slightly higher in subjects having normal thyroid profile as compared to hypothyroid and hyperthyroid subjects. Although the changes were statistically non-significant.Non-significant decrement in the level of testosterone was also observed in hypothyroid and hyperthyroid subjects as compared to subjects with normal thyroid profile. Whereas FSH level was non-significantly higher in both hypothyroid and hyperthyroid subjects as compared to normal subjects. However, LH level was increased in hyperthyroid and hyperthyroid subjects as compared to subjects having normal thyroid profile, Which was significant in hypothyroid subjects as compared to normal thyroid profile group (Table 4). Further, mean data of T_3, T_4 and TSH were analyzed with respect to impaired thyroid profile indicated that variation in T_3, T_4 and TSH were significant in hypothyroid and hyperthyroid subjects as compared to subjects with normal thyroid profile (Table 5).

Table 1: Age, BMI, semen volume and sperm viability with respect to thyroid profile

Parameters	**Normal**	**Hypothyroid**	**Hyperthyroid**
Age (yrs)	31.95 ± 0.36	33.22 ± 0.90	33.33 ± 1.00
BMI (kg/m^2)	22.15 ± 0.23	21.84 ± 0.60	22.99 ± 0.76
Semen volume (ml)	3.12 ± 0.09	2.60 ± 0.18	3.06 ± 0.25
Sperm viability (%)	41.25 ± 0.86	40.37 ± 2.70	37.33 ± 2.17

Values are expressed as Mean ± S.E

Table 2: Sperm count and sperm motility with respect to thyroid profile

Parameters	Normal Thyroid	Hypothyroid	Hyperthyroid
Sperm count (millions/ml)	42.69 ± 1.68	38.62 ± 5.42	33.56 ± 4.59
Fast progressive motility (%)	25.59 ± 0.62	21.79 ± 2.16†	22.04 ± 1.87
Slow progressive motility (%)	25.27 ± 0.36	23.21 ± 1.37	24.62 ± 0.72
Non-progressive motility (%)	18.91 ± 0.31	18.27 ± 1.11	19.53 ± 0.98
Non-motile (%)	30.38 ± 0.84	36.71 ± 3.25†	34.03 ± 2.38
Total progressive motility (%)	50.86 ± 0.76	45.00 ± 2.81†	46.66 ± 2.16

Values are expressed as Mean ± S.E, † p<0.05

Table 3: Sperm morphology with respect to thyroid profile

Parameters	Normal Thyroid	Hypothyroid	Hyperthyroid
Normal Morphology (%)	16.85 ± 0.41	15.35 ± 1.07	15.55 ± 1.22
Total head abnormality (%)	53.52 ± 0.51	53.64 ± 1.27	55.36 ± 1.52
Total mid piece abnormality (%)	20.12 ± 0.34	21.69 ± 0.90	19.83 ± 0.73
Total tail abnormality (%)	7.62 ± 0.25	7.44 ± 0.64	7.60 ± 0.79

Values are expressed as Mean ± S.E

4. Discussion

It is known that endocrine dysfunction leads to impairments in various organs including reproductive system. It is slowly recognizing the fact that thyroid play an important role in reproduction and associated functions. Decades back it was believed that male fertility could not be affected by thyroid hormones. The interest is

growing to understand the role of thyroid in male reproduction are now being growing interest because of the identification of thyroid hormone receptors (TRs) on nursing Sertoli cells of testis. As Sertoli cells play crucial role in process of spermatogenesis, thyroid hormones must play identical role in sperm cell development and function [8]. Earlier studies also reported the effect of thyroid hormone on Sertoli and Leydig cell proliferation and functional status of these cells, which might affect semen quality [9].

Table 4: Level of Reproductive hormones with respect to thyroid profile

Parameters	Normal	Hypothyroid	Hyperthyroid
Testosterone (ng/ml)	3.67 ± 0.13	3.06 ± 0.27	3.26 ± 0.36
FSH (mIU/ml)	7.58 ± 0.56	10.37 ± 1.53	9.16 ± 1.82
LH (mIU/ml))	5.86 ± 0.30	7.27 ± 0.65†	7.67 ± 1.07

Values are expressed as Mean ± S.E, $^{\dagger}p<0.05$

Table 5: Level of T_3, T_4 and TSH with respect to thyroid profile

Parameters	Normal	Hypothyroid	Hyperthyroid
T_3 * (ng/ml)	1.03 ± 0.01	0.33 ± 0.01	3.40 ± 0.75
T_4 * (ug/dl)	7.34 ±0.11	3.49 ± 0.09	14.20 ± 0.28
TSH * (uIU/ml)	2.18 ± 0.05	5.94 ± 0.24	0.28 ± 0.01

*Values are expressed as Mean ± S.E, * p<0.05*

They also mentioned that the testis was unresponsive organ to the thyroid hormones but studies of recent years suggested that thyroid hormones dysfunction is not only associated with functional status of testis but it also affect fertility and sexual activity in men. In the present study, the data with respect to thyroid profile showed that 12.82% subjects were hypothyroid, 11.12% subjects were hyperthyroid and remaining 76.06% subjects having normal thyroid profile based upon the level of T_3, T_4 and TSH in the study population. The data also showed that imbalanced thyroid profile declined sperm count non-significantly, whereas non-motile sperms were significantly higher in hypothyroid subjects as compared to normal thyroid subjects. Earlier Poppe and his colleagues [10] also found that low sperm density, low motility and decreased sperm count was observed in imbalanced thyroid profile patient with respect to euthyroid patients. Later Krassas and his colleagues [11] concluded that semen quality specifically sperm progressive motility and semen volume was negatively affected in hypothyroid conditions. The present study also corroborates these findings.

It has been reported that human and rat testes contain thyroid hormone receptor (TRs) from neonatal to adult life

[12, 13]. T_3 binds directly to the TRs which are present in Sertoli cells of seminiferous tubules and in this way thyroid hormones might play role in growth and development of male testis. Further, testicular maturation may also be affected because of changes in thyroid hormones level during early testis development, which might impact on reproduction in later life [14]. Semen quality parameters were more deteriorated in subjects with hypothyroidism as compared to subjects with normal thyroid profile. The study also suggests that thyroid may also have role in spermatogenesis of adulthood also in addition, its role during testicular development.

In the present study percent normal morphology was higher non-significantly in normal thyroid profile subjects as compared to altered thyroid profile subjects. Krassas and his colleagues investigated twenty-five hypothyroid men and fifteen normal individuals and they found that sperm morphology was affected significantly among hypothyroid subjects [15].

The level of testosterone was marginally decreased in both hypothyroid and hyperthyroid subjects as compared to normal thyroid profile group which was statistically non-significant. Earlier Kumar and his colleagues found normal testosterone response of eight hypothyroid patients after achieving euthyroid condition [16]. This also showed the role of thyroid in reproduction. LH level was significantly increased in hypothyroid subjects as compared to normal thyroid subjects. FSH level was non significantly increase in hyperthyroid and hypothyroid subjects with respect to normal group. Wajner and his colleagues found that prepubertal males who has primary hypothyroidism shown elevated level of FSH and LH frequently [9]. Recently Kumar and his colleagues also reported that male reproduction is governed by the classical hypothalamohypophyseal testicular axis: Hypothalamic gonadotropin releasing hormone (GnRH), pituitary leteininzing hormne (LH) and follicle stimulating hormone (FSH) and the gonadal steroid, principally, testosterone [16]. They also reported that thyroid hormones have a moderately influence on this axis and which in turn sexual and spermatogenic functions. Earlier Abalorich and his colleagues also reported that hyperthyroidism causes marked alteration of the gonadotropic and PRL axis and dramatically affect apermatic function [17]. These observations lend supports the findings of deterioration of semen quality with respect to thyroid profile in the present study.

5. Conclusion and limitations

The present data suggests that thyroid hormones have some role in the male reproduction, as semen quality parameters were impaired among subjects with thyroid hormones dysfunction. The reason behind impaired semen quality parameters among imbalanced thyroid profile subjects can be ascertained by performing more studies regarding molecular mechanisms by which thyroid hormones act on Sertoli and Leydig cell.

The study has a limitation of one time collection of semen and blood sample from the subjects who were attending OPD at civil hospital and IKD institute for their fertility problems. Hence, it was difficult to convince them to provide the blood and semen sample for further study.

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