GONADAL FUNCTION IN SUBCLINICAL HYPOTHYROIDISM
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ABSTRACT

BACKGROUND
We wanted to assess the basal gonadotropin and estradiol levels in women of reproductive age group with subclinical hypothyroidism and evaluate the response of pituitary ovarian axis to leuprolide.

METHODS
Both in cases and controls, we measured basal follicular stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) on 3rd or 4th day of menstrual cycle. Leuprolide subcutaneous injection 20 mcg/Kg was given on the same day. 1 hour after injection, stimulated LH levels and E2 measured after 24 hours. Basal values and leuprolide stimulated values were compared between cases and controls.

RESULTS
Mean basal E2 was 44.16 vs. 55.37 in cases and controls respectively. Mean basal LH in cases was 6.06 ± 2.01 m IU/L vs. 6.63 ± 2.38 m IU/L in controls with a significant p value of 0.01 (p < 0.05). Mean basal FSH is 8.39 ± 3.26 mIU/Ls 7.76 ± 2.90 m IU/L in cases and controls respectively with no significant difference. Mean peak LH in cases was 20.37 ± 10.32 m IU/ml and in controls it was 20.80 ± 8.67 mIU/ml after leuprolide stimulation. Mean peak E2 in cases and controls was 56.12 and 65.54 pg/ml respectively with no significant difference.

CONCLUSIONS
Basal LH was lower in cases than controls which is statistically significant. After stimulation with leuprolide, there was no significant difference in LH and E2 levels between cases and controls. It can be concluded that in mild thyroidal failure, the response of pituitary gonadotrophs to leuprolide is normal.

KEYWORDS
Subclinical Hypothyroidism, Pituitary Ovarian Axis, LH, Estradiol


BACKGROUND
Normal thyroid function is important to maintain normal reproduction, via its interaction in several pathways.1 Subclinical hypothyroidism (SCH) is a condition of mild thyroid failure defined biochemically by normal levels of thyroid hormones with mildly elevated thyroid stimulating hormone (TSH) concentrations.2

Prevalence of SCH has been reported to be between 4-10% of general population according to two large population based studies, The Whickham Survey,3 in and National Health And Nutrition Examination Survey III.4 India has estimated 42 million people suffering from thyroid diseases, with prevalence of SCH as high as 9.4% with female dominance of 11.4 vs. 6.2% in men.5 According to 20 yr. follow up of Whickham survey, the annual rate of progression to overt hypothyroidism was 4.3% in women with both raised serum TSH and anti-thyroid antibodies, 3% if only serum TSH was raised, and 2% if only antithyroid antibodies were present. So, SCH can be transient or permanent. Progression from mild to overt hypothyroidism may be related to thyroid peroxidase (TPO) antibody positivity, higher basal TSH value, old age and female sex.6,7 A diagnosis of persistent SCH can be verified by reevaluating TSH concentration after 6 or 12 months. TSH may normalize within 2 years, this is more commonly seen in individuals with negative antithyroid antibodies and serum TSH levels of <10 µIU/L. Whether to treat subclinical hypothyroidism is a dilemma. Most clinicians treat Subclinical Hypothyroidism with TSH >10 m IU/L. Opinions differ regarding management when TSH is between 4.5 and 10 m IU/L. The three societies American association of clinical endocrinology, American thyroid society and endocrine
society recommends routine treatment of subclinical hypothyroidism with TSH of 4.5-10 m IU/L if subject has high cardiovascular risk, goiter, anti TPO positivity, infertility, pregnancy, or symptoms of hypothyroidism. Both primary hyperthyroidism and hypothyroidism in males and females have been well documented to produce variable degrees of gonadal dysfunction.

Hypothyroid women have decreased rates of metabolic clearance of androstenedione and estrone and exhibit an increase in peripheral aromatization. Plasma binding activity of sex hormone binding globulin (SHBG) is decreased, which results in decreased plasma concentrations of both total testosterone and estradiol (E2), but their unbound fractions are increased. Gonadotropins levels are usually normal. However, blunted or delayed LH response to GnRH has been reported in some hypothyroid women. Alterations in steroid metabolism disappear when a euthyroid state is restored. In women of fertile age, hypothyroidism results in changes in cycle length and amount of bleeding (i.e., oligomenorrhea and amenorrhea, polymenorrhea and menorrhagia). Goldsmith et al. found that eight of 10 patients with primary hypothyroidism had menstrual disturbances. Scott and Mussey observed that 56% of hypothyroid women presented menstrual irregularities, mainly metrorrhagia or menorrhagia alone or combined. Joshi et.al. found that 68% of hypothyroid women had menstrual irregularities, compared with only 12% in controls. Taken together, these findings indicate that the frequency of menstrual disturbances in hypothyroidism is approximately three times greater than in the normal population. Overall, thyroid dysfunction shows many effects on reproductive health ranging from menstrual disturbances, ovulatory dysfunction, infertility, miscarriage and pregnancy related complications.

METHODS
A total of 40 women of reproductive age group were included in our observational, case control study who attended outpatient department of endocrinology, King George Hospital, Visakhapatnam, Andhra Pradesh, between March 2014 and September 2015.

Inclusion Criteria
- Newly detected subclinical hypothyroid subjects.
- Females in the age group 18-35.
- Cycle interval ranging from 21-35 days with variability less than 3 days.

Exclusion Criteria
- Pregnancy
- Lactation
- Exposure to chemotherapy or radiotherapy.
- Subjects with any chronic systemic illness (diabetes mellitus, chronic kidney disease, chronic liver disease, chronic infections, systemic inflammatory disorders, malignancies, congestive heart failure etc.)
- Cycle length <21 or >35 days.
- Subjects on drugs affecting the thyroid hormone and gonadal hormone metabolism.
- TSH > 10 µ IU/ml with normal T4 so that effects of even mild thyroidal failure can be assessed.

Total T4, TSH were measured by chemiluminescence immunoassay (CLIA). T4 normal range- 4-12 µg/dl. TSH 4.5-10 µ IU/ml were considered as cases with subclinical hypothyroidism. TSH 0.5-4.5 µ IU/ml were considered as euthyroid controls. Both in cases and controls, basal FSH, LH, E2 on 3rd or 4th day of menstrual cycle at 8 AM on fasting is measured by CLIA. Leuprolide subcutaneous injection 20 mcg/Kg is given on the same day. 1 hour after injection, stimulated LH and E2 are measured after 24 hours. Basal values and leuprolide stimulated values are compared between cases and controls.

RESULTS
Presenting complaints in our subclinical hypothyroidism (SCH) cases were shown as in table 1. In this study over all, most of the subjects presented with non-specific symptoms. Most common symptom pertaining to thyroid is goiter.

<table>
<thead>
<tr>
<th>Complaint</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysphagia</td>
<td>1</td>
</tr>
<tr>
<td>Goiter</td>
<td>8</td>
</tr>
<tr>
<td>Infertility</td>
<td>2</td>
</tr>
<tr>
<td>Joint Pains</td>
<td>1</td>
</tr>
<tr>
<td>Lethargy</td>
<td>1</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>1</td>
</tr>
<tr>
<td>Threat Pain</td>
<td>1</td>
</tr>
<tr>
<td>Wt. Gain</td>
<td>2</td>
</tr>
<tr>
<td>Nil</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
</tbody>
</table>

Graph 1

Graph 2
1. Mean age of subjects with SCH is 27.05 years and mean age of euthyroid controls is 29 years. No significant difference in age between cases and controls with 'p' value >0.05

2. Mean levels of total T4 in SCH subjects is 7.96 ± 1.44 µg/dl vs controls it is 8.72 ± 1.15 µg/dl. No significant difference between cases and controls. P value = 0.94

3. Mean TSH in SCH subjects it is 8.01±1.144 µIU/ml and in controls it is 2.70 ± 1.106 µIU/ml

4. In SCH cases mean value of basal FSH is 8.395 ± 3.26 m IU/ml vs controls in whom it is 7.786 ± 2.90 m IU/ml. When cases and controls are compared, no difference in basal FSH values with a P value of 1.04 which is non-significant.

5. Basal LH in cases is 6.06 ± 2.10 m IU and in controls it is 6.63 ± 2.38 m IU/m. Basal LH is significantly more in controls when compared to SCH cases with a P Value 0.01(<0.05).

6. In SCH cases mean basal estradiol is 44.16 ± 24.8 pg/ml vs. euthyroid controls it is 55.37 ± 17.3 pg/ml. There is no significant difference of basal estradiol levels between cases and controls.

7. Peak LH- 1 hr, After Leuprolide Stimulation: Mean levels of stimulated LH in cases is 20.37 ± 10.32 m IU/ml and in controls it is 20.802 ± 8.67 m IU/ml. We observed no significant difference in leuprolide stimulated LH levels between cases and controls.

8. Mean Peak Estradiol Levels: Mean estradiol levels 24 hrs after leuprolide stimulation in controls is 65.54 pg/ml and in cases it is 56.12 pg/ml. There is no significant difference in peak estradiol levels between cases and controls.

9. Mean Increment in LH After Stimulation: Mean increment of LH in cases is 13.73 mIU/ml and in controls it is 14.74 m IU/ml When increments in LH are compared, no significant difference is there between cases and controls.

**DISCUSSION**

Thyroid dysfunction causes menstrual disturbances and anovulation. Menstrual disturbances ranges from oligomenorrhea, amenorrhea, polymenorrhea, menorrhagia. Hypothyroidism is associated with increase in TRH (thyrotropin releasing hormone) which increases both TSH (thyroid stimulating hormone) and prolactin. Prolactin in turn inhibits the secretion of GnRH (gonadotropin releasing hormone) thereby decreasing the secretion of gonadotropins. The decreased gonadotropins leads to oligomenorrhea and amenorrhea, common menstrual disturbances seen in hypothyroidism. These classical hormonal changes seen in patients with overt hypothyroidism. However in mild thyroid failure these hormonal changes may not be evident. Prolactin also acts directly on ovary and inhibits process of ovulation. Hypothyroidism is associated with various forms of infertility ranging from disorder in ovulation to corpus luteal phase defects.

Follicular fluid composition might be important factor for developing oocyte. Both T3 and T4 are found in follicular...
fluid of humans and positive correlation between serum T4 and follicular fluid T4 levels. Thyroid hormone receptors are located on human oocyte. So, thyroid hormone directly act on oocyte. Serum levels of follicular stimulating hormone (FSH) and luteinizing hormone (LH) are significantly low in overt hypothyroid women in a study by Neemaacharya et al. Serum estradiol (E2) was also reduced significantly in hypothyroidism. In our study, basal LH levels were significantly lower in cases compared to controls. However, the basal FSH levels and estradiol levels were normal in both the groups. Ours study was done in subclinical hypothyroidism which might have resulted in the difference in the result.

Subclinical hypothyroidism has been associated with infertility. The prevalence of subclinical hypothyroidism in infertile women range from 1 to 4% and most cases associated with ovulatory dysfunction. Subclinical hypothyroidism associated with luteal phase defects, abortions, stillbirths, premature and gestational hypertension. The basal estradiol levels were normal in both cases and controls in our study. This suggests that estradiol levels were not affected in mild degree of thyroidal failure. Study by Sanjaysaran et al., although serum E2 was less in cases than controls respectively. These differences were statistically significant with a P <0.05. This study was done in overt hypothyroidism which would potentially affect the reproductive axis as compared to euthyroid controls. In the present study only SCH subjects were taken. So the difference might not be obtained.

According to Sanjaysaran et al., although serum FSH and LH were lower in cases as compared to controls respectively, but these differences were statistically insignificant P = 0.09 & P = 0.45, respectively. In another study by Neemaacharya et al., in the subclinical hypothyroid group serum FSH & LH levels were decreased in females who had menorrhagia and infertility. The conflicting results among these quoted studies and the present study can be explained by different categories of hypothyroidism, presence of autoimmunity, and also variability in presence and severity of symptoms. This is the first study in subclinical hypothyroidism done to assess the hypothalamo-pituitary-ovarian axis to leuprolide stimulation. One of them by Velazquez et al. concluded that there is defective LH response to GnRH in hypothyroid men. But this is not observed in subjects with mild hypothyroidism in the present study, suggesting that this mild degree of thyroid dysfunction will not affect pituitary gonadotrophs responsiveness to GnRH. In our study, the basal levels of LH are significantly more in controls compared to cases, with no difference in basal FSH and E2. On leuprolide stimulation there is no difference in peak LH and E2 between cases and controls.

A study by T. Veeresh, D. Moulali et al. the result of this study indicates that there is significant increased basal levels of serum LH as compared to FSH. Thus the ratio of LH: FSH altered from 1:1 to 6:1, and also there is significant increase in serum prolactin levels in hypothyroidism. They concluded that the alteration in menstrual cycle and decreased reproductive performance of women are similar to polycystic ovarian disease and can be explained on the basis of altered hormone profile of LH, FSH and prolactin. Increased levels of prolactin and LH with normal FSH in hypothyroid cases was seen, indicating their susceptibility for the development of polycystic ovarian syndrome. All the above referred studies were done in subjects with overt hypothyroidism except one study by Neemaacharya et al. Here, we evaluated basal levels of gonadotropins as well as assess the response of gonadotropins to leuprolide. So, this is the first study to assess the response of gonadotropins to leuprolide in subclinical hypothyroidism.

Finally, mean TSH in the present study in cases was 8.01±1.14 µIU/L. In the study by Vaishalideshmukh et al. mean TSH was 9.82±7.86 µIU/L which is higher than the present study because it was a prevalence study of subclinical hypothyroidism and included all age groups and all TSH values with normal T4 levels, whereas we excluded patients with TSH value >10 µIU/L.

**Limitations**

- Sample size is small.
- Aetiology of subclinical hypothyroidism is not assessed (autoimmunity).
- Prolactin levels are not measured which can be affected in hypothyroidism and prolactin can affect the reproductive system.

**CONCLUSIONS**

In mild thyroidal failure, the response of pituitary gonadotrophs to leuprolide is normal, in contrast to overt hypothyroidism where the response is sluggish. This is the first study to be done in subclinical hypothyroid subjects to assess both basal and stimulated gonadotropin levels. Further studies with large samples are required to confirm these findings.

**REFERENCES**


