

Frequency of Subclinical Hypothyroidism in Subfertile Females and the Effect of Thyroxine Replacement

Shehla Arif¹, Farah Deeba Nasrullah¹, Riffat Jaleel¹, Farah Shabi UI Hasnain¹ and Aman Ullah Abbasi²

¹Department of Obstetrics and Gynaecology, Dr. Ruth K.M. Pfau Civil Hospital, Karachi, Pakistan

²Department of Medicine, Dr. Ruth K.M. Pfau Civil Hospital, Karachi, Pakistan

ABSTRACT

Objective: To determine the frequency of subclinical hypothyroidism (SCH) and autoimmune thyroid disease (ATD), assess dominant follicular formation and conception rates after thyroxine replacement, and evaluate the association between SCH and ATD with conception among subfertile females.

Study Design: Quasi-experimental study.

Place and Duration of the Study: Department of Obstetrics and Gynaecology, Dr. Ruth K. M. Pfau Civil Hospital, Karachi, Pakistan, from August 2022 to August 2023.

Methodology: The study was carried out on 67 women with primary or secondary subfertility diagnosed with SCH, with or without thyroid autoimmunity. History, examination, subfertility workup, thyroid function tests, and anti-TPO antibody testing were performed and recorded on a predesigned proforma. The participants were administered 50 microgram thyroxine (LT4) supplementation and later given three cycles of ovulation induction with clomiphene citrate. The dominant follicular formation and conception rates were noted. Descriptive statistics were calculated as frequencies, percentages, means, and standard deviations. Univariate logistic regression was applied to predict pregnancy using thyroid function tests and anti-TPO results. The p-value of ≤ 0.05 was considered significant.

Results: The frequency of subfertility was 25.8%, SCH 9.9%, and TAI 1.9%. The mean age was 28.88 ± 3.6 years, and most participants had secondary subfertility (48, 71.6%). As a result of LT4 supplementation, a dominant ovarian follicle formed in 24 (35.8%) participants, and conception was achieved in 12 (17.9%) participants. No significant association was found between thyroid function tests, ATD, and conception.

Conclusion: SCH is not uncommon in subfertile females of reproductive age. However, a significant relationship could not be established between SCH and subfertility in the studied patients, and conception rates were low after thyroxine supplementation.

Key Words: Subclinical hypothyroidism, Female subfertility, Thyroid autoimmunity, LT4 supplementation.

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INTRODUCTION

Thyroid disorders are ranked as the second most common endocrine problems affecting child-bearing women.¹ Thyroid hormones are essential for the optimum function of every organ system, and its association with reproductive function and pregnancy is well-known. Dysfunction is related to anovulation, oligomenorrhoea, cycle irregularities, and subfertility.² These women are at higher risk of miscarriages and adverse foetomaternal outcomes.³ Subclinical hypothyroidism (SCH) is defined as biochemical evidence of thyroid hormone deficiency without any symptoms. It is diagnosed when an elevated thyroid-stimulating hormone (TSH) concentration is associated with a normal concentration of free thyroxine (FT4).⁴

SCH occurs in 3-8% of women of childbearing age and is more prevalent among those with unexplained subfertility.⁵ The probable causes of subfertility in these patients include anovulatory cycles, defective ovarian follicular development and maturation, elevated prolactin levels, and luteal phase or sex hormone abnormalities. Autoimmune thyroid disease (ATD) is present in 30-40% of SCH cases and is associated with the presence of thyroid autoantibodies.⁶ It is detected by measurement of anti-thyroid peroxidase (anti-TPO) antibodies and thyroglobulin (Tg) antibodies. The American Society of Reproductive Medicine (ASRM) recommends thyroid function tests and treatment with levothyroxine if levels are raised in subfertile females.⁷ There was some confusion in the past regarding the upper cut-off limit of serum TSH, which has been addressed by the American Thyroid Association (ATA) in their 2017 guidelines, recommending 4.0 mU/l when considering SCH.⁸ Levothyroxine replacement therapy should be carefully initiated and monitored to maintain thyroid hormone levels within the recommended range, to help conception and prevent adverse foetomaternal outcomes during pregnancy. While dealing with women with unexplained subfertility and SCH, clinicians should be aware of the complex nature of this disease and the limitations of available evidence.

Correspondence to: Dr. Shehla Arif, Department of Obstetrics and Gynaecology, Dr. Ruth K. M. Pfau Civil Hospital, Karachi, Pakistan
E-mail: mrsashar01@gmail.com

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This study aimed to determine the frequency of SCH and ATD, assess the effects on follicle formation and conception after thyroxine replacement, and evaluate the association between SCH and ATD with conception among subfertile females.

METHODOLOGY

This study was conducted in the Outpatient Subfertility Clinic of the Department of Obstetrics and Gynaecology, Dr. Ruth K. M. Pfau Civil Hospital and Dow University of Health Sciences, Karachi, Pakistan. It was a quasi-experimental study conducted from August 2022 to August 2023. The sample size was calculated using OpenEpi Software. The estimated sample size was 64 with 95% confidence level and 10% confidence limit on the basis of prevalence of 20.8%. It was collected by a non-probability consecutive method. The couples attending the Subfertility Clinic underwent a workup, individualised to patient needs, which included hormone analysis (FSH, TSH, and Prolactin), transvaginal ultrasound, semen analysis, and hysterosalpingogram to determine the probable causes for subfertility, following the recommendations of the NICE guidelines for infertility. Females aged 21-35 years with a history of primary or secondary subfertility for one year or more, elevated TSH ≥ 4.0 mIU/ml (repeated after four weeks), normal FT4, and no other cause for subfertility were included after obtaining written informed consent. The anti-TPO levels were further carried out to diagnose ATD. Patients with endometriosis, male-factor subfertility, active pelvic inflammatory disease, tubal disease, any organic pelvic lesion, primary hyperprolactinaemia, overt hypothyroidism (already on thyroxine), a history of thyroidectomy or radiotherapy, FSH >8 IU/ml, polycystic ovarian syndrome or other pre-existing medical conditions were excluded from the study. The sample for thyroid function tests was obtained through peripheral venous blood and analysed by electrochemiluminescence immunoassay (ECLIA) on a Cobase 601 analyser in the hospital laboratory. The patients were given levothyroxine therapy (50 micrograms) in consultation with an endocrinologist and were followed up. Ovulation induction (OI) was given on the 2nd or 3rd day of the next menstrual cycle using 50 mg clomiphene citrate (CC) for five days. Ovarian response was checked by the number and size of dominant follicles (1.8-2.5 cm) on the 12th day of the menstrual cycle through transvaginal ultrasound and by measuring day-21 serum progesterone levels. Pregnancy was confirmed by a positive pregnancy test and serum beta hCG when the next period was missed. Ovulation induction was continued for three consecutive cycles in incremental doses, i.e., 50, 100, and 150 mg, and the patients were followed up. If conception did not occur, the patient was counselled regarding other treatment options. All patient-related information and investigations were recorded on a pre-designed proforma by the investigators and postgraduate students of the ward.

Data were stored and analysed using the SPSS version 26.0. For all categorical variables, including residence and occupation, frequencies and percentages were calculated. Continuous data, such as age and TSH levels, were measured using mean

and standard deviation (SD). Univariate logistic regression was applied to predict pregnancy by using thyroid function tests and anti-TPO. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

A total of 2,615 women attended the gynaecological Outpatient Clinic with 675 (25.8%) subfertility cases during the study period. The frequency of SCH was 9.93% (n = 67), and of ATD was 19.4% (n = 13). About half of the participants belonged to the age range of 26 to 30 years, and most were of normal height and build. The majority of participants were housewives and urban residents (46, 67.21% and 44, 65.7%, respectively). History of recurrent miscarriage was present in 10 participants (15%). Table I displays the demographic and other basic characteristics of the participants.

The rates of dominant ovarian follicle formation and conception as a result of thyroxine replacement are given in Table II.

Univariate logistic regression analysis showed an odds ratio (OR) of 1.7 for TSH; however, the result was not statistically significant. No association was observed between conception and thyroid function tests or thyroid autoimmunity (TPO antibody status), as shown in Table III.

None of the patients reported any side effects of levothyroxine therapy during the study period.

Table I: Characteristics of the study participants.

Characteristics	Mean $\pm$ SD	n (%)
Age (years)	28.88 $\pm$ 3.658*	
21-25		12 (17.9)
26-30		35 (52.2)
31-35		20 (29.9)
Parity (number)	1.52 $\pm$ 1.307*	
BMI (kg/m ²)	25.20 $\pm$ 3.896*	
History of miscarriages		
None		42 (62.7)
<3		15 (22.4)
≥ 3		10 (14.9)
Type of subfertility		
Primary		19 (28.4)
Secondary		48 (71.6)
Duration of subfertility (years)	3.097 $\pm$ 1.108*	
Thyroid peroxidase antibody (TPO Ab)		
Negative (<35 IU/ml)		54 (80.6)
Positive (≥ 35 IU/ml)		13 (19.4)
Systolic BP (mmHg)	118.73 $\pm$ 12.80*	
Diastolic BP (mmHg)	76.72 $\pm$ 9.43*	
TSH (0.4-4 mIU/L)	5.05 $\pm$ 0.64*	
Free T3 (1.8-4.2 mcg/dL)	2.82 $\pm$ 0.71*	
Free T4 (0.8-1.9 ng/dL)	1.17 $\pm$ 0.29*	

*Data are presented in the mean $\pm$ SD. BMI: Body mass index; SD: Standard deviation.

Table II: Reproductive outcomes after levothyroxine supplementation.

Outcomes	n	%
Dominant ovarian follicle formation (1.8-2.5 cm)		
Formed	24	35.8
Not formed	43	64.2
Conception (pregnancy test)		
Positive	12	17.9
Negative	55	82.1

Table III: Results of the univariate logistic regression analysis of conception by age, parity, subfertility duration, thyroid function, and TPO antibody status.

Conception	OR	95% CI for OR		p-values
		Lower	Upper	
Age	0.989	0.833	1.175	0.900
Parity	1.322	0.780	2.239	0.299
Duration of subfertility	0.933	0.534	1.631	0.809
TSH (0.4 - 4 normal)*	1.700	0.598	4.833	0.319
Free T3 (1.8 - 4.2 normal)*	0.933	0.389	2.236	0.876
Free T4 (0.8 - 1.9)*	0.325	0.042	2.524	0.282
History of miscarriages				
None	Reference			
<3	2.800	0.315	24.895	0.356
≥3	0.300	0.067	1.349	0.116
Thyroid peroxidase antibody				
Negative, <35	Reference			
Positive, ≥35	0.391	0.097	1.582	0.188

*Data are presented in the mean ± SD; Ref: Reference category; Binary logistic regression was used to assess the p-value. OR: Odd ratio; CI: Confidence interval.

DISCUSSION

Subfertility is an emerging health concern, with grave psychosocial consequences, an increased risk of divorce, and multiple marriages trends in developing countries such as Pakistan. The incidence of subfertility has increased worldwide, including both high-, middle- and low-income countries. The overall prevalence is around 22% in Pakistan, indicating that a significant proportion of couples are unable to conceive spontaneously. In the current study, the rate has doubled compared to that reported in a national study conducted twenty years ago.⁹ Another study from Nigeria has also supported this increasing prevalence in developing countries.¹⁰ The frequency of SCH observed in the present study is comparable to other studies reported worldwide,^{4,11} except for a systematic review from India, which reported a higher value (25-40%).¹² The predominant type of subfertility found was secondary, which is consistent with other studies from the developing world;¹⁰ however, this contrasts with developed countries, where primary subfertility is more common. This trend is probably due to an increased risk of infection after miscarriage or delivery, as well as sexually transmitted infections that give rise to tubal and peritubal adhesions and tubal blockage, leading to secondary subfertility in these countries. The mean duration of subfertility before seeking medical advice was shorter in the study, as women sought subfertility treatment early. This finding is in agreement to other studies from the developing world.^{10,13}

Many studies have shown that a substantial percentage of both pregnant and non-pregnant females in Pakistan are iodine-deficient, leading to SCH or overt hypothyroidism. This condition can result in conception failure, recurrent pregnancy loss, poor foetomaternal outcomes, and developmental issues in infants and children. Hypothyroidism interferes with ovum development and ovulation and is a well-known cause of anovulatory infertility. It is not only important for ovum formation and maturation, but also essential for foetal growth, brain and other organ system maturation, and neonatal survival. A good number of patients in the

present study demonstrated dominant follicular development as a result of thyroxine replacement; however, a considerable improvement in conception rates was not found, possibly due to factors working at the cellular or molecular level — such as abnormal immune mechanisms, autoimmunity, concurrent undiagnosed pelvic endometriosis, or vitamins and micronutrient deficiency, which remain unidentified — that may interfere with implantation and conception. The role of thyroid autoimmunity in SCH, regardless of thyroid hormonal status, has been associated with pregnancy losses, including miscarriage and preterm labour.^{14,15} Lower pregnancy rates were found in anti-TPO-positive women compared to those without antibodies, although the difference was not statistically significant.

The main concern and rationale for identifying the subset of patients with ATD among reproductive-age females was to start thyroxine treatment early in the preconception period, allowing these women to enter pregnancy with optimised thyroid hormones. This approach may help reduce pregnancy losses and other complications related to hypothyroidism. Another important reason is the increased annual risk of progression to overt hypothyroidism when it is associated with thyroid autoimmunity.^{16,17} The available literature is limited to a few population-based studies demonstrating the association of SCH and ATD with subfertility, and the evidence remains inconclusive.¹⁸ The findings of the current study are in agreement of other studies that also failed to prove a definitive relationship between borderline-high TSH levels and poor reproductive outcomes.^{19,20}

Not all these females in reproductive age with SCH should receive levothyroxine supplementation, as advised by the ATA. This may be because these females exhibit different clinical and demographic characteristics compared to those in Western population, necessitating the need to develop local, population-specific guidelines.

The greatest strength of this study lies in its quantitative design. Another strength was the definitive inclusion of patients after four weeks of repeated TSH testing, rather

than relying on a single measurement, as TSH level varies over time, and the rise may be transient. However, this was a single-centre tertiary care study with a limited sample size. Secondly, participants could not be divided into cases and controls, as it was not ethically justifiable to deprive the Control group from some essential treatment. Thirdly, other thyroid antibodies, such as anti-thyroglobulin, could not be assessed due to limited resources.

CONCLUSION

SCH is common among local subfertile women. However, a significant relationship between SCH and subfertility could not be established. No remarkable improvement in conception rates was noted, and the overall conception rate remained low after LT4 supplementation. Larger-scale, population-based studies with bigger sample sizes are recommended to further validate this relationship.

ETHICAL APPROVAL:

Ethical approval was obtained from the Institutional Review Board of Dow University of Health Sciences, Karachi, Pakistan (IRB-2601/DUHS/Approval/2022/985).

PATIENTS' CONSENT:

Informed consent was obtained from all the participants.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

SA: Concept, design, and manuscript writing.

FDN: Data analysis and writing of the manuscript.

RJ: Critical review and manuscript approval.

FSUH: Data acquisition, literature search, and referencing.

AUA: Writing, editing, and proofreading.

All authors approved the final version of the manuscript to be published.

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