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ASSESSMENT OF ENDOMETRIAL THICKNESS AND VASCULARITY BY TRANSVAGINAL DOPPLER ULTRASOUND AND THEIR RELATIONSHIP WITH ENDOMETRIAL MICROBIAL INFECTION IN REPRODUCTIVE-AGED WOMEN WITH SUBCLINICAL HYPOTHYROIDISM: A COMPARATIVE STUDY

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ABSTRACT

Background: Subclinical hypothyroidism is a mild thyroid disorder that involves having a high level of TSH and a normal level of free T4. It is not always clinically obvious but can influence reproductive function, menstruation, uterine development and blood flow. A further disruption of endometrial receptivity and fertility may be caused by endometrial microbial infection. TV-Doppler ultrasound is a non-invasive technique for evaluating vascularity and thickness of the endometrium.
Objective: To assess endometrial thickness and vascularity by transvaginal Doppler ultrasound and determine their relationship with endometrial microbial infection in reproductive-aged women with subclinical hypothyroidism.
Methods: This comparative study was carried out at Muhammad College of Medicine Peshawar, between January 2025 and June 2025. There were 73 women of reproductive age included and divided into subclinical hypothyroidism and euthyroid control groups. Demographic, clinical, thyroid profile, transvaginal ultrasound, Doppler vascularity and microbiological results were collected. The following parameters were compared between the groups: endometrial thickness, Doppler indices of uterine arteries, vascularity grade and microbial culture results. Data were analysed statistically with SPSS version 26 and p value ≤ 0.05 was taken as statistically significant.
Results: Women with subclinical hypothyroidism had significantly higher mean endometrial thickness than controls. Thickened endometrium, heterogeneous endometrial pattern, intrauterine fluid, and increased vascularity were more frequent in the subclinical hypothyroidism group. Uterine artery pulsatility index and resistance index were higher, while peak systolic velocity was lower among women with subclinical hypothyroidism. Endometrial microbial infection was detected more frequently in the subclinical hypothyroidism group than in controls. Infection was significantly associated with greater endometrial thickness, heterogeneous endometrium, intrauterine fluid, increased vascularity, and raised uterine artery resistance. TSH showed a positive correlation with endometrial thickness, pulsatility index, and resistance index.
Conclusion: Subclinical hypothyroidism was associated with increased endometrial thickness, altered Doppler vascularity, and higher frequency of endometrial microbial infection. Transvaginal Doppler ultrasound may be useful for identifying endometrial abnormalities in reproductive-aged women with subclinical hypothyroidism, particularly when symptoms of infection or infertility are present.

Keywords: Subclinical hypothyroidism, endometrial thickness, transvaginal ultrasound, Doppler vascularity, endometrial infection, reproductive-aged women.

INTRODUCTION

Subclinical hypothyroidism is characterized by a high thyroid stimulating antibody (TSH) and a normal level of free thyroxine (T4) in the blood stream. It is seen in reproductive age women, and may be unsuspected due to non-specific symptoms or low clinical suspicion. Thyroid dysfunction can affect reproductive physiology, even though it is presented in a subtle way, via the effects on the ovarian function, cyclicity of the menstrual period, sex hormone metabolism, implantation and endometrial receptivity. A slight thyroid disturbance can thus be a cause of menstruation disturbance, infertility, early pregnancy loss and abnormal response of the endometrium [1-3].

Endometrium is a tissue that changes in structure and vascularity in a cyclic manner and is responsive to hormonal stimulation during the reproductive years. Estrogen, progesterone, thyroid hormones, immune mediators and local vascular factors are all critical to normal endometrial development and must interact to maintain it. Endometrial thickness is a popular ultrasound measure of endometrial growth and receptivity. An abnormally thin, thick or heterogeneous endometrium may be a sign of hormonal imbalance, inflammation and infection or other gynecological pathology. Subclinical hypothyroidism may cause abnormal endometrial morphology due to the altered endocrine function in women with this disease [4-6].

The transvaginal ultrasound is an important imaging technique used in the evaluation of the uterus and endometrium. It can measure the thickness of the endometrium and evaluate the pattern, echogenicity and intrauterine fluid. Doppler ultrasound also provides the functional data by assessing uterine and endometrial blood flow. Uterine artery pulsatility index, uterine artery resistance index, peak systolic velocity and endometrial vascularity grade can provide a valuable assessment of uterine perfusion and vascular resistance. These parameters can specifically be valuable in women of reproductive age who are infertile, have irregular periods, or a suspected pathological endometrium [7-9].

Another factor that could have a profound impact on reproductive health is endometrial microbial infection. Microbial invasion or chronic endometrial inflammation may affect the structure, vascularity, immunity and implantation ability of the endometrium. May be clinically silent or manifest with symptoms of pelvic pain, abnormal vaginal discharge, menstrual irregularity, recurrent pregnancy loss or infertility. Ultrasonographic findings of endometrial inflammation from infection can include heterogeneous echotexture, thickening of the endometrium, intrauterine fluid, and increased vascularity on Doppler imaging. Thus, use of ultrasound along with microbiological evaluation might enhance the probability of detecting clinically relevant endometrial abnormalities [10, 11].

The association of subclinical hypothyroidism, endometrial vascularity and microbial infection remains poorly studied. Thyroid Dysfunction can affect the regulation of the immune system, tone of the blood vessels, and response of the endometrium, making you more susceptible to infection or making inflammatory changes in the endometrium worse. In the same way, a bacterial infection can also independently cause thickened endometrium and Doppler vascularity. This is of significance because untreated thyroid disorders and/or uterine infections can adversely affect fertility and reproductive outcome [12] .
The present study was conducted to assess endometrial thickness and vascularity using transvaginal Doppler ultrasound and to determine their relationship with endometrial microbial infection in reproductive-aged women with subclinical hypothyroidism. The study aimed to compare ultrasound and Doppler findings between women with subclinical hypothyroidism and euthyroid controls and to evaluate whether microbial infection was associated with abnormal endometrial morphology and vascularity.

METHODOLOGY

This comparative study was conducted at Muhammad College of Medicine, Peshawar, from January 2025 to June 2025. The study was carried out to assess endometrial thickness and vascularity using transvaginal Doppler ultrasound and to determine their relationship with endometrial microbial infection among reproductive-aged women with subclinical hypothyroidism. A total of 73 women were included in the study after fulfilling the selection criteria. The participants were divided into two groups: women diagnosed with subclinical hypothyroidism and euthyroid women who served as controls.
The women seeking care at the gynecology outpatient department were screened for reproductive age.Reproductive aged women were identified for screening. Women between the ages of 18-45 were included. In the subclinical hypothyroidism group, women had elevated levels of TSH and normal free T4 levels. The control group consisted of women of reproductive age who had normal thyroid function test results. The exclusion of women with overt hypothyroidism, hyperthyroidism, pregnancy, lactation, known uterine malignancy, diagnosed endometriosis,

fibroid uterus distorting the uterine cavity, recent use of hormonal therapy, intrauterine contraceptive device use, recent antibiotic therapy, or previous uterine surgery was to avoid confounding effects on endometrial morphology, vascularity, and microbial findings.

Demographic and clinical data were collected on a proforma after informed consent was obtained. The data were age, BMI, place of residence, marital status, parity, menstrual pattern, pelvic pain, vaginal discharge, history of infertility, previous miscarriage, and history of pelvic infection. Menstrual and gynecological histories were taken from the participants in detail. Clinical assessment was carried out as necessary and appropriate laboratory investigations reviewed prior to recruitment.

Blood samples were taken to measure thyroid profile. Thyroid-stimulating hormone, free thyroxine and free triiodothyronine were determined by conventional laboratory techniques. In addition, anti-thyroid peroxidase antibody status was recorded, if available. Subclinical hypothyroidism was defined as an elevated serum TSH level with a normal free T4 level. Normal TSH and normal free T4 levels were used to classify the women as a control group.
All participants underwent transvaginal ultrasound with a high-frequency probe by a highly trained sonologist. The thickness of the endometrium was measured from the deepest point from one endometrial-myometrial interface to the other in the midsagittal plane. All measurements were taken in millimeters. Endometrial pattern was observed as: Trilaminar, Homogeneous or Heterogeneous. Other intra-pelvic changes and presence of intrauterine fluid, as well as abnormal echogenicity were noted. In women with regular cycles, the ultrasound assessment would preferably take place during the peri-ovulatory or middle cycle phase, and if the cycle was irregular, the timing of the scan was recorded.

Uterine and endometrial blood flow was then evaluated by Doppler ultrasound. Pulsatility index of uterine artery, resistance index of uterine artery, peak systolic velocity, and the endometrial or subendometrial vascularity were recorded. The extent of the visual Doppler flow signals in and around the endometrium were classified as poor, moderate or increased, and used to grade the vascularity of the endometrium. To assure consistency in the vascular assessment all the participants had Doppler settings as close as possible to each other.
Endometrial microbial infection was tested by taking a sample of the endometrial tissue under sterility procedure. Samples were taken and sent to the microbiology laboratory to perform Gram staining and culture. The growth of the microorganisms was either positive or negative. The organisms were identified using the standard microbiological procedures and classified as bacterial, fungal or mixed infection. Chronic endometritis was confirmed in cases in which the diagnosis could be supported by clinical, microbiological, and/or histological diagnosis. The organisms tested were E. coli, S. aureus, S. species, Gardnerella vaginalis, and Candida species.

The main outcome was measuring endometrial thickness and Doppler vascularity parameters in women with subclinical hypothyroidism and euthyroid controls. The secondary outcome was the relationship between endometrial thickness and vascularity to the presence of an infection of the uterus. To evaluate the correlation of TSH level with endometrial thickness, uterine artery Doppler indices and infective status, additional analysis was conducted.

Data were entered and analysed with SPSS 26. The quantitative variables (age, Bmi, TSH, free T4, endometrial thickness, uterine artery pulsatility index, resistance index, peak systolic velocity) were presented as mean ± standard deviation. For qualitative variables like menstrual irregularity, vaginal discharge, vascularity grade, thickened endometrium, culture positivity and type of microbial infection, frequencies and percentages were used. A two-tailed independent sample t test was used to compare quantitative variables between the two groups and chi-square test or Fisher's exact test was used for categorical variables when applicable. Pearson correlation was used to evaluate the correlation between TSH level and ultrasound/Doppler parameters. The level of p value < 0.05 was considered to be statistically significant.

RESULTS

The total number of reproductive aged women in the study was 73. Of these, 37 women were determined to have subclinical hypothyroidism, and 36 women were found to have normal thyroid function and were used as controls. The average age of the participants was 30.8 ± 5.7 years. The majority of the participants were married and the two groups did not differ significantly in age, parity, body mass index and place of residence. Women with subclinical

hypothyroidism, however, had more frequently menstrual irregularity, pelvic discomfort, vaginal discharge and history of infertility.

Table 1. Baseline demographic and clinical characteristics of study participants

Variable	Subclinical hypothyroidism n=37	Control n=36	p-value
Mean age, years	31.2 ± 5.9	30.4 ± 5.5	0.551
BMI, kg/m²	26.4 ± 3.8	25.7 ± 3.5	0.414
Married	34 (91.9%)	33 (91.7%)	0.972
Nulliparous	14 (37.8%)	11 (30.6%)	0.512
Rural residence	16 (43.2%)	14 (38.9%)	0.705
Menstrual irregularity	21 (56.8%)	10 (27.8%)	0.012
Pelvic pain/discomfort	18 (48.6%)	9 (25.0%)	0.037
Vaginal discharge	20 (54.1%)	11 (30.6%)	0.043
History of infertility	15 (40.5%)	7 (19.4%)	0.049

The mean TSH level was significantly higher in subclinical hypothyroidism women than in the control group in the thyroid profile. Both groups had levels of free T4 in the normal range, establishing the diagnosis of subclinical hypothyroidism. The subclinical hypothyroidism group also had a higher prevalence of positivity for anti-TPO antibodies.

Table 2. Thyroid profile of study participants

Thyroid variable	Subclinical hypothyroidism n=37	Control n=36	p-value
Mean TSH, mIU/L	7.18 ± 1.64	2.31 ± 0.74	<0.001
Free T4, ng/dL	1.08 ± 0.19	1.15 ± 0.21	0.139
Free T3, pg/mL	2.91 ± 0.43	3.02 ± 0.47	0.301
Anti-TPO positive	13 (35.1%)	4 (11.1%)	0.016

Women with subclinical hypothyroidism had a significantly thicker mean endometrial thickness than the controls, when assessed by transvaginal ultrasound. In addition, the subclinical hypothyroidism group had more heterogeneous endometrial appearance and intrauterine fluid. In women with subclinical hypothyroidism, some had a thickened endometrium (37.8%) compared with 13.9% of healthy controls.

Table 3. Comparison of transvaginal ultrasound findings between groups

Ultrasound variable	Subclinical hypothyroidism n=37	Control n=36	p-value
Mean endometrial thickness, mm	10.9 ± 2.6	8.7 ± 2.1	<0.001
Thin endometrium	4 (10.8%)	6 (16.7%)	0.462
Normal endometrium	19 (51.4%)	25 (69.4%)	0.117
Thickened endometrium	14 (37.8%)	5 (13.9%)	0.020
Trilaminar pattern	15 (40.5%)	21 (58.3%)	0.129
Heterogeneous endometrium	16 (43.2%)	7 (19.4%)	0.028
Intrauterine fluid	8 (21.6%)	2 (5.6%)	0.047

Women with subclinical hypothyroidism exhibited changes in vascular parameters as noted in Doppler assessment. Increased vascularity of the endometrium was more common in this population. The mean value of the uterine artery pulsatility index and resistance index were significantly elevated in the subclinical hypothyroidism group and the peak systolic velocity was significantly lower, indicating lower uterine perfusion despite increased local inflammatory vascularity.

Table 4. Doppler vascularity parameters among study participants

Doppler variable	Subclinical hypothyroidism n=37	Control n=36	p-value
Uterine artery PI	2.18 ± 0.46	1.82 ± 0.39	0.001
Uterine artery RI	0.78 ± 0.08	0.70 ± 0.07	<0.001
Peak systolic velocity, cm/s	24.6 ± 5.8	28.1 ± 6.2	0.015

Poor vascularity	9 (24.3%)	17 (47.2%)	0.041
Moderate vascularity	14 (37.8%)	15 (41.7%)	0.735
Increased vascularity	14 (37.8%)	4 (11.1%)	0.008

The incidence of endometrial microbial infection was 28.8% in the entire study population. Subclinical hypothyroidism was associated with a significantly increased risk of infection. Escherichia coli, Staphylococcus aureus, Streptococcus spp, Gardnerella vaginalis and Candida spp were the most frequently cultured organisms. A significant higher proportion of mixed microbial growth occurred in women with subclinical hypothyroidism.

Table 5. Frequency and pattern of endometrial microbial infection

Microbial variable	Subclinical hypothyroidism n=37	Control n=36	p-value
Any microbial infection	16 (43.2%)	5 (13.9%)	0.006
Bacterial infection	12 (32.4%)	4 (11.1%)	0.027
Fungal infection	2 (5.4%)	1 (2.8%)	0.573
Mixed infection	2 (5.4%)	0 (0.0%)	0.159
Chronic endometritis	11 (29.7%)	3 (8.3%)	0.021

Table 6. Microorganisms isolated from endometrial samples

Organism isolated	Frequency n=21	Percentage
Escherichia coli	6	28.6%
Staphylococcus aureus	4	19.0%
Streptococcus species	3	14.3%
Gardnerella vaginalis	3	14.3%
Candida species	3	14.3%
Mixed bacterial growth	2	9.5%

Endometrial thickness was significantly higher in the case of women with microbial infection. More infected participants also had increased vascularity. The Doppler indices were higher in infected women, suggesting that microbial infection may play a role in abnormal vascular and endometrial changes.

Table 7. Association of endometrial findings with microbial infection

Variable	Infection present n=21	Infection absent n=52	p-value
Mean endometrial thickness, mm	11.6 ± 2.4	8.9 ± 2.2	<0.001
Thickened endometrium	12 (57.1%)	7 (13.5%)	<0.001
Heterogeneous endometrium	13 (61.9%)	10 (19.2%)	<0.001
Intrauterine fluid	6 (28.6%)	4 (7.7%)	0.018
Increased vascularity	11 (52.4%)	7 (13.5%)	<0.001
Uterine artery PI	2.26 ± 0.44	1.88 ± 0.43	0.001
Uterine artery RI	0.80 ± 0.07	0.71 ± 0.08	<0.001

There was a correlation with positive relationship between TSH level and endometrial thickness. TSH also had a positive correlation with uterine artery PI and RI, and a negative correlation with the peak systolic velocity. Microbial infection was found to be strongly associated with endometrial thickness, indicating that microbial infection and thyroid dysfunction might affect endometrial morphology.

Table 8. Correlation of TSH with ultrasound and Doppler parameters

Correlation variable	r-value	p-value
TSH with endometrial thickness	0.46	<0.001
TSH with uterine artery PI	0.39	0.001
TSH with uterine artery RI	0.42	<0.001
TSH with peak systolic velocity	-0.31	0.008
Endometrial thickness with infection status	0.51	<0.001

Vascularity grade with infection status	0.44	<0.001

In summary, women with subclinical hypothyroidism had significantly thicker endometrium, Doppler vascular indices that were different from the euthyroid controls, and a higher incidence of endometrial infection with microorganisms. Thick and heterogeneous endometrium, increased vascularity and higher resistance of the uterine arteries were associated with microbial infection. The results of these studies indicate that subclinical hypothyroidism may be associated with adverse endometrial changes and risk of microbial infection among reproductive-aged women.

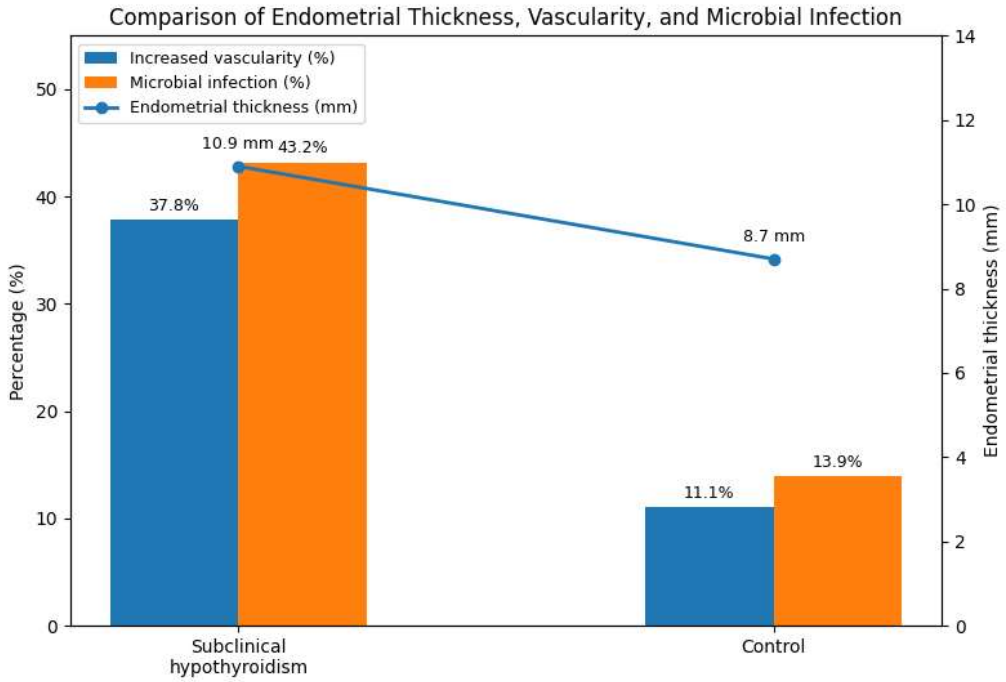

Figure 1. Comparison of mean endometrial thickness, increased endometrial vascularity, and microbial infection frequency between women with subclinical hypothyroidism and euthyroid controls.

DISCUSSION

The present study assessed endometrial thickness and vascularity by transvaginal Doppler ultrasound and evaluated their relationship with endometrial microbial infection among reproductive-aged women with subclinical hypothyroidism. The findings showed that women with subclinical hypothyroidism had significantly greater mean endometrial thickness compared with euthyroid controls. Thickened and heterogeneous endometrium was also more commonly observed in the subclinical hypothyroid group. These findings suggest that even mild thyroid dysfunction may influence endometrial morphology. Thyroid hormones play an important role in reproductive physiology by affecting ovarian function, menstrual cyclicity, sex hormone metabolism, and endometrial receptivity. Raised TSH, even with normal free T4, may disturb the hormonal environment and contribute to altered endometrial development [13, 14].

The Doppler ultrasound showed a significant vascular difference between the two groups in this study. Uterine artery PI and RI were increased and PSV was decreased in women with subclinical hypothyroidism compared with controls. These findings show increased vascular resistance and relatively decreased uterine perfusion. Concurrently, subclinical hypothyroid group had more increased endometrial vascularity. However, this may be accounted for by local inflammatory/receptive changes in the endometrium, whereas the overall flow resistance in the uterine arteries is higher. For this reason Doppler assessment could be beneficial and not just measurement of the endometrial thickness [15, 16].

In this study, a difference was observed between the two groups in the amount of vascularity detected by Doppler ultrasound. Uterine artery PI and RI were increased and PSV was decreased in women with subclinical hypothyroidism compared with controls. The results indicate that the vascular resistance is increased and uterine perfusion is relatively decreased. At the same time, subclinical hypothyroid group exhibited more increased

endothelial growth of the endometrium. This could be explained by local inflammatory/receptive changes of the endometrium, whereas the overall flow resistance in the uterine arteries is increased. For this reason Doppler assessment could be beneficial and not just measurement of the endometrial thickness [17, 18].

There was a positive correlation between the presence of microorganisms and abnormal endometrial findings. The thickness of the endometrium was significantly larger in women with infection, as was the presence of heterogeneous endometrium, intrauterine fluid, and Doppler resistance indices, when compared with non-infected women. These findings suggest that inflammation in the endometrium can occur due to microbial infection and may lead to thickening, changes in the echotexture and increased vascularity of the local tissues. The Doppler higher vascular signals could be due to inflammatory angiogenesis or congestion in the endometrial tissue. As a result, if a thickened endometrium is encountered in a woman of reproductive age with abnormal Doppler vascularity, it should be suspected that there is underlying infection of the endometrium, particularly if there are symptoms of vaginal discharge, pelvic pain, or infertility [19].

The correlation analysis also confirmed the association between thyroid dysfunction and endometrial changes. TSH was positively correlated with endometrial thickness, uterine artery pulsatility index, and resistance index while there was a negative correlation between TSH and peak systolic velocity. Infection status was also significantly related to endometrial thickness and vascularity grade. The results indicate that there may be a combined effect of subclinical hypothyroidism with microbial infection affecting the health of the endometrium. Subclinical hypothyroidism may establish a micro-environment of the endometrium that promotes abnormalities that would lead to an abnormal response, and microbial infection may further enhance inflammatory and structural abnormalities. This dual action can be clinically significant in women who are experiencing irregular periods, fertility issues, recurrent miscarriage, abdominal discomfort, or vaginal discharge [20].

The findings of this study have important clinical implications. Transvaginal Doppler ultrasound is a non-invasive and valuable method to assess endometrial morphology and blood flow. Doppler evaluation may be useful in women with subclinical hypothyroidism for the detection of abnormal endometrial vascularity, particularly for women with gynecologic symptoms, and for additional evaluation of their infection. Prompt diagnosis of endometrial infection is crucial as untreated infection can impact fertility, implantation, and reproductive outcomes. The research also underscores the need for thyroid screening on reproductive-age females who have abnormal findings on endometrial biopsy or unexplained infertility.

There were several limitations in this study. First, it was a small sample size and the study was performed at a single center, which could affect generalizability. Second, the cross sectional comparative study design may demonstrate association but will not be able to demonstrate causality of subclinical hypothyroidism, endometrial changes, and microbial infection. Third, endometrial thickness and vascularity may vary depending on the different stages of the menstrual cycle; an attempt was made to either document or standardise the time of the scan wherever possible. Fourth, there are some bacteria that are not detected by culture-based microbial assessment, especially fastidious or anaerobic bacteria. A future multicenter study with larger number of patients, molecular microbiological techniques, hormonal profile and follow-up after thyroid and/or antimicrobial therapy is recommended to elucidate the causal relationship and the clinical outcome.

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