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"RESEARCH METHODOLOGY IN BIOMEDICINE, BIOSTATISTICS AND CLINICAL BIOINFORMATICS"

Reproductive factors in Subclinical Hypothyroidism: a case-control study

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Abstract

Purpose: The prevalence of subclinical hypothyroidism (SH) is more frequent in females than males. Therefore, the aim of this thesis was to evaluate for the first time the association of reproductive factors, particularly age at menarche, with SH risk.

Methods: In a retrospective case-control study, reproductive factors, such as age at menarche, age at menopause and at first birth, lactation, parity, full-term pregnancies, reproductive years, use of oral contraceptives and hormonal replacement therapy, somatometric data, insulin resistance and lipid parameters were recorded in 72 consecutive female patients with SH and 72 healthy female controls matched on age (± 5 years) and date of diagnosis (± 1 month).

Results: SH cases exhibited significantly younger age at menarche than controls ($p<0.001$). Cases exhibited later age at first pregnancy with a lower number of full-term pregnancies ($p=0.04$). Other reproductive factors were similar between cases and controls ($p>0.05$). Early age at menarche was independently associated with SH risk, above and beyond age, date of diagnosis, BMI, thyroid autoimmunity, hip circumference, HOMA-IR and alcohol consumption ($p<0.001$, OR: 0.22, 95% CI: 0.11 – 0.44).

Conclusion: It seems that an interplay of early exposure to estrogens, as expressed by early menarche, and induction of thyroid autoimmunity are associated with SH risk. More prospective studies shedding light on the role of estrogens in SH are required to confirm these findings.

Key words: autoimmunity; estrogen; hypothyroidism; menarche; reproductive; subclinical; thyroid;

«Αναπαραγωγικοί Παράγοντες και Υποκλινικός Υποθυρεοειδισμός: Μελέτη Ασθενών-Μαρτύρων»

ΠΕΡΙΛΗΨΗ

Εισαγωγή: Ο επιπολασμός του υποκλινικού υποθυρεοειδισμού είναι συχνότερος στις γυναίκες από ότι στους άντρες. Ως εκ τούτου, ο στόχος αυτής της διπλωματικής εργασίας ήταν να αξιολογηθεί, για πρώτη φορά, η συσχέτιση των αναπαραγωγικών παραγόντων, ιδιαίτερα της ηλικίας εμμηναρχής, με τον κίνδυνο ανάπτυξης υποκλινικού υποθυρεοειδισμού.

Υλικό και μέθοδοι: Στο πλαίσιο μιας αναδρομικής μελέτης τύπου ασθενών-μαρτύρων μελετήθηκαν οι αναπαραγωγικοί παράγοντες όπως: ηλικία εμμηναρχής, ηλικία εμμηνόπαυσης, ηλικία κατά τον πρώτο τοκετό, γαλουχία, τεκνοποίηση, αριθμός τελειόμηνων κυήσεων, αναπαραγωγικά έτη, χρήση αντισυλληπτικών και θεραπεία ορμονικής υποκατάστασης, σωματομετρικά δεδομένα, ινσουλινοαντίσταση και λιπιδαιμικοί παράγοντες σε 72 διαδοχικές γυναίκες ασθενείς με υποκλινικό υποθυρεοειδισμό και 72 υγιείς γυναίκες μάρτυρες εξομοιωμένες ως προς την ηλικία (± 5 χρόνια) και την ημερομηνία διάγνωσης (± 1 μήνας) με τις ασθενείς.

Αποτελέσματα: Οι ασθενείς εμφάνισαν σημαντικά μικρότερη ηλικία εμμηναρχής από τις μάρτυρες ($p<0.001$). Επίσης οι ασθενείς εμφάνισαν μεγαλύτερη ηλικία κατά το πρώτο τοκετό με μικρότερο αριθμό τελειόμηνων κυήσεων ($p=0.04$). Οι υπόλοιποι αναπαραγωγικοί παράγοντες ήταν παρόμοιοι μεταξύ ασθενών και μαρτύρων. Η πρώιμη ηλικία εμμηναρχής συσχετίστηκε ανεξάρτητα με τον υποκλινικό υποθυρεοειδισμό μετά από αμοιβαίο έλεγχο για τους παράγοντες εξομοίωσης (ηλικία και ημερομηνία διάγνωσης), τον δείκτη μάζας σώματος, την παρουσία

θυρεοειδικής αυτοανοσίας, την περιφέρεια ισχίων, τον δείκτη HOMA-IR και την κατανάλωση αλκοόλ ($p<0.001$, OR: 0.22, 95% CI: 0.11 – 0.44).

Συμπεράσματα: Ενδέχεται η αλληλεπίδραση της πρώιμης έκθεσης στα οιστρογόνα, όπως εκφράζεται από την πρώιμη εμμηναρχή, και της θυρεοειδικής αυτοανοσίας να συσχετίζεται με τον κίνδυνο ανάπτυξης υποκλινικού υποθυρεοειδισμού. Απαιτούνται περισσότερες προοπτικές μελέτες, οι οποίες να αποσαφηνίζουν τον ρόλο των οιστρογόνων στον υποκλινικό υποθυρεοειδισμό, για να επιβεβαιώσουν τα παραπάνω ευρήματα.

Λέξεις κλειδιά: Αναπαραγωγή, Αυτοανοσία, Εμμηναρχή, Θυρεοειδής Αδένας, Οιστρογόνα, Υποκλινικός, Υποθυρεοειδισμός

List of abbreviations:

anti-TG: anti-thyroglobulin antibodies; anti-TPO: anti-peroxidase antibodies; BMI: Body Mass Index; BP: blood pressure; CI; Confidence Interval; CVD: Cardiovascular Disease; DBP: Diastolic Blood Pressure; DM: Diabetes Mellitus; HDL-C: High-density lipoprotein cholesterol; HOMA: Homeostasis model assessment score of insulin resistance; HT: Hashimoto's thyroiditis; HC: hip circumference; HRT: Hormone Replacement Therapy; LDL-C: Low-density lipoprotein cholesterol; OR: Odds Ratio; SBP: Systolic Blood Pressure; SD: standard deviation; SH: Subclinical Hypothyroidism; SLE: Systemic Lupus Erythematosus; T3: Triiodothyronine; T4: Thyroxine; TG: Thyroglobulin; TPO: Thyroid peroxidase; TSH: Thyroid stimulating hormone; US: ultrasonography; WHR: Waist-to-hip ratio; WC: waist circumference;

Introduction

Subclinical hypothyroidism (SH) is considered a mild thyroid dysfunction which can be diagnosed from elevated thyrotropin levels (TSH) in the presence of normal free T3 and T4 levels [1]. A significant percentage of patients are asymptomatic while others present with weight gain, muscle weakness, fatigue, depression, cold intolerance and constipation. SH has also been linked to cardiovascular disease, particularly when TSH levels are above 10mIU per liter, metabolic syndrome, atherosclerosis, dyslipidemia, diabetes mellitus (DM) type 2 and hypertension [2-5]. Prevalence of SH is more common in women in midlife with a tendency to increase over the age of 50 years. Hashimoto's thyroiditis (HT) is considered the most frequent determinant of progressive thyroid dysfunction and SH in adults. In such cases, when thyroperoxidase or thyroglobulin antibodies are not easily detected, thyroid ultrasonography is performed in order to evaluate thyroid damage [3].

There are few established risk factors for SH, with thyroid autoimmunity being the most important [6]. Because SH is more frequent in women, with a female to male ratio of approximately 5 to 1 in the general population, reproductive, menstrual and hormonal parameters, particularly age at menarche, may be of paramount importance [7,14]. The aim of the present thesis was to explore the potential role of hormonal and reproductive factors, particularly age at menarche, in SH risk among women living in the Metropolitan region of Attica.

Materials and Methods

The study enrolled 72 women suffering from SH and 72 women as healthy control participants matched on age and date of diagnosis with cases, between February 2012 and December 2016 inclusive. All women were of Greek nationality and same residency area (Metropolitan area of Athens & Attica region, Greece). This area is

considered iodine-sufficient. Medical records were retrieved from the medical records of Theodora Stratigou, MD, PhD, consultant Endocrinologist at the Endocrinology Department of Evangelismos Hospital. All medical records were reviewed and interviews were performed by the same physician (TS), in order to obtain information on demographic characteristics, medical history, consumption of coffee and alcoholic beverages, tobacco smoking and physical exercise per week. Determination of weight, height, waist circumference (WC), hip circumference (HC), and blood pressure (BP) were performed by the same physician for all patients. Waist-to-hip ratio (WHR) was calculated and body mass index (BMI) was determined based on the equation: body weight (kg)/height^2 (m^2). Body fat percentage was calculated based on the Deurenberg equation: BF% = 1.20 * BMI + 0.23 * age - 10.8 * sex - 5.4. Each woman participant was submitted to two BP measurements, with the same instrument, 5 min apart after 10 min of rest. BP was calculated by taking into account the mean of two measurements. Interviews and measurements were accomplished under similar circumstances and at the same time in the morning (8 –9.30 am) and. The study protocol followed the ethical guidelines of the Declaration of Helsinki. All participants gave an informed consent before enrollment in the study.

Selection of cases

Seventy-two women suffering from SH (TSH level higher than 5 μIU/L measured at least twice in independent determinations) and who were not receiving L-T4 treatment before the baseline visit. Both patients and controls were enrolled by Dr T. Stratigou. Hashimoto's thyroiditis (HT) was diagnosed based on elevated levels of anti-thyroglobulin (anti-TG) and/or anti-thyroid peroxidase (anti-TPO) in serum and/or the presence of heterogeneous echogenicity as observed by thyroid ultrasonography (US).

Selection of control participants

The control group was randomly selected among women who were healthy subjects and came for an annual check-up examination. For every eligible patient with SH, an attempt was made to randomly identify a control as closely as possible in time (±1 month) and matched to patients on age (± 5 years). In total, 72 women control participants without any known disease involving any thyroid disease were included in the study. Circulating TSH, fT4, fT3, anti-TG and anti-TPO were measured and HT was excluded also by thyroid US.

Exclusion criteria

The exclusion criteria comprised age less than 18 and older than 75 years old; any medications that could cause thyroid dysfunction; previous thyroidectomy; hypertension, CVD, renal or hepatic disease; autoimmune or endocrine disorders; muscular, neurologic or psychiatric disorders; cancer; pregnancy and post-partum period; anti-lipidemic drugs; arduous physical activity or sports activity; and infection at the chronic period of blood drawn. All subjects were on an unrestricted diet.

Laboratory determinations

All blood draws were performed early in the morning after 12 hours of fasting. Serum thyroid parameters (TSH, fT3, fT4, T3, T4, anti-TG and anti-TPO) and insulin were performed using electro-chemiluminescent immunoassays (Cobas, Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Glucose and lipid parameters (total cholesterol, LDL-C, HDL-C, triglycerides) were determined using an automated analyzer (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Homeostasis model assessment score of insulin resistance (HOMA-IR) was derived from the formula: fasting serum insulin (μU/ml) x fasting serum glucose (mmol/l)/22.5.

Statistical analysis

The statistical analysis of the data was performed using IBM-SPSS® version 24 for Windows. In the beginning, data were assessed through simple cross-tabulations and by using χ^2 test for categorical variables, t-test for normally distributed variables, and Mann-Whitney U for not normally distributed variables. Normality hypothesis was tested by using the Shapiro-Wilk test. Spearman correlation coefficients were used as measurements of correlation for continuous variables. Multiple binary logistic regression analysis was employed to identify whether age of menarche, expressed as quartiles, is independently associated with risk of SH (dependent variable), adjusting for matching factors and significant clinical parameters found in univariate analyses. For logistic regression analysis, transformation in quartiles was performed for continuous variables not normally distributed. A two-sided *P* value of less than 0.05 was considered significant. Bases on a previous clinical study [4], it was calculated that a total sample size of at least 140 women is needed to achieve 98% power at the 0.05 level of significance in order to detect a 5-6% difference in age at menarche between groups.

Results

Clinical and laboratory biomarkers in SH cases and controls

Descriptive categorical features and continuous clinical parameters including patient history information, somatometric, metabolic, thyroid, and blood pressure and lipid factors of all participants are presented in Tables 1 and 2. In comparison to controls, women with SH presented similar educational level, smoking history and physical exercise. (Table 1, $p>0.05$). However, cases tended to present a more frequent diagnosis of thyroid autoimmunity ($p<0.001$). Moreover, alcohol and coffee

consumption were significantly higher in patients than controls (p=0.03 and p=0.01 respectively).

As expected, women with SH had significantly higher levels of TSH, anti-TG and anti-TPO antibodies as seen in figures 1,2,3 and almost similar levels of total T3, free T3, free T4 (Table 2). Only total T4 was significantly lower in patients than controls (p<0.001). Regarding somatometric variables, women with SH presented lower hip circumference than controls (Fig.4, p<0.05), and BMI was slightly different between cases and controls (Fig.5, p=0.08). BP parameters were similar for both groups. Comparison of metabolic profiles revealed that women with SH showed significantly higher insulin levels and HOMA-IR score than controls (Fig.6, p<0.001). Also, they presented significantly higher concentrations of total cholesterol, LDL-C and triglycerides (p<0.05) but not HDL-C (p=0.35).

Comparison of reproductive factors between SH cases and controls

26.4% of women had their menarche until the age of 12 years and 56.9% were postmenopausal. Specifically, 38 women with SH and 44 controls were at menopause (Fig.7). Women with SH presented an earlier age at menarche than controls as seen in figure 8 (p<0.001). Women with SH became menopausal later than controls, but the difference was not significant (p=0.55, Fig.9). Interestingly, SH cases exhibited a significantly older age at first pregnancy (p=0.04, Fig.10) and significantly smaller number of full-time pregnancies (p=0.04) than controls. The duration of reproductive years, as far as it concerns the menopausal group, showed a nearly significant difference between cases and controls (p=0.07, Fig.11). Compared to controls, women with SH did not differ in terms of menses and period duration, parity (Fig.12) and lactation (Fig.13), (Table 3, p>0.05). The use of Hormone Replacement Therapy (HRT) was the same for both cases and controls (Table 3, Fig.14, p=1.00). Regarding

the use of oral contraceptives (OC) or combined use of OC and HRT, there weren't any statistically significant differences (Table 3, Fig.15, 16, $p>0.05$).

Correlations of age at menarche with somatometric, metabolic, thyroid cardiovascular parameters and reproductive factors

As showed in Table 4, in all study women, age at menarche was positively correlated with weight, BMI, WC, WHR, fat mass, free fat mass, fat percentage, DBP, total T4, glucose and HDL-C. Age at menarche presented negative correlations with TSH, anti-TPO and reproductive years. In particular, cases with SH exhibited a similar pattern of correlations but with the exception of WC, WHR, free fat mass, DBP, TSH, total T4, anti-TPO and HDL-C ($p>0.05$). Additionally, there were positive correlations with anti-TG, HOMA-IR, triglycerides and total cholesterol. In controls, there were positive correlations of age at menarche with weight, BMI, waist circumference, WHR, fat mass, free fat mass, fat percentage, anti-TG, HOMA-IR, glucose, triglycerides, HDL-C. Moreover, controls presented negative associations with reproductive years, number of full-term pregnancies, HRT and a positive one with age at first pregnancy ($p<0.05$).

Age at menarche is independently associated with SH

Table 5 portrays multiple logistic regression-derived adjusted odds ratios (OR) and 95% confidence intervals (CI) for associations of SH in relation to age, date of diagnosis (matching factors), age at menarche, BMI, hip circumference, thyroid autoimmunity, HOMA-IR and alcohol consumption. There was statistically significant evidence that earlier age at menarche expressed by quartiles was associated with SH, before and after adjusting for the abovementioned parameters (OR=0.22, 95% CI 0.11 – 0.44, $p<0.001$). Regarding BMI, an individual with 1kg/m^2 more had about 1.21 times more likely the risk to develop SH than someone with

normal BMI (OR=1.21, 95% C.I. 1.02 – 1.43, p=0.03). Regarding hip circumference, a woman with 1 quartile more in HC presented with lower risk of SH (OR=0.41, 95% C.I. 0.26 – 0.65, p<0.001). There is also strong and statistically significant evidence that thyroid autoimmunity, specifically Hashimoto's thyroiditis, is associated with increased risk of SH. Interestingly, controlling for other parameters, an individual who consumes more alcohol had about 2.12 times more likely the risk to develop SH (OR=2.12, 95% C.I. 1.37 – 3.47, p<0.001). Finally, HOMA-IR score was not associated with SH risk (p=0.12).

Discussion

To the best of our knowledge, this is the first study showing that early age at menarche was independently associated with SH risk. This thesis shows that women with delayed menarche present a decreased risk of SH. This thesis also confirms the strong association between thyroid autoimmunity and higher BMI with SH risk, as observed in many studies [3, 5]. However, we did not find any associations between other reproductive factors, such as lactation, parity, age at menopause and at first birth, full-term pregnancies, menopausal status, reproductive years, menses and period duration, use of oral contraceptives, HRT and exogenous use of hormones with SH risk.

It is well known that early age at menarche has been linked to several other disease states, such as metabolic syndrome, CVD, diabetes mellitus (DM) type 2, decreased bone mineral density, pre-eclampsia and breast cancer [7-9]. Nowadays, the age of menarche is decreasing due to many parameters, such as style of living, diet, socio-economic and environmental factors, and educational level [10]. Women with higher socio-economic profiles presented lower age at menarche in contrast to those who were settled in low-income or rural areas with lower educational level [11]. Puberty is

a significant hormonal milestone in a woman's life that is characterized by alterations in growth hormone, hypothalamus-pituitary-gonadal (HPG) axis, gonadotropins, luteinising hormone, follicle-stimulating hormone, estrogens and insulin growth factor- I [12,13].

Whilst it is unlikely that age at menarche *per se* in a causal determinant for SH, it could be a marker of an underlying pathway that confers an elevated risk. It is possible that early menarche and early exposure to estrogens trigger thyroid autoimmunity in general. It is well-known that autoimmune diseases, including thyroid autoimmunity, are more frequent in women than men (Female to Male Ratio 5:1) [14]. Indeed, early age at menarche (age 12 or younger) was strongly associated with a risk of Systemic Lupus Erythematosus (SLE) in big cohorts like NHS and NHSII [15]. Estrogen receptors are expressed in the majority of immune cells; nevertheless, their effects depend on receptor type and could be complicated. Estrogen may influence antibody-mediated immunity, including the suppression of B-cell maturation [16]. Estrogens play a critical role in B-cell maturation and may have an aftereffect on the breakdown of immune tolerance observed in autoimmune disorders [15].

However, the association between early menarche and SH was independent from thyroid autoimmunity; therefore, there might be other more complex factors playing a role. The role of estrogens and estrogen receptors in thyroid autoimmunity and progression to SH, may be explained also by early menarche. Estrogen receptors and earlier exposure to estrogens may influence the initiation and progression of thyroid autoimmunity. In vitro studies have shown that estrogen significantly increased proliferation, inflammation and invasive properties of thyroid cancer cell lines [5, 17-19]. Finally, another mechanism may comprise the fact that early menarche may be

associated with body fatness, insulin resistance and cardio-metabolic risk factors in the majority of Western studies [20, 21]. The abovementioned factors are also associated with SH risk [5, 22-25].

This thesis presents many methodologic strengths. A properly powered study was implemented which was sufficient to replicate prior established associations of the study parameters with SH risk and to produce new statistically significant associations with age at menarche. Finally, to minimize the chances of potential uncontrolled exposures, this thesis included controls who were carefully matched to cases and drawn from the same study population.

However, this thesis presents some limitations to take into consideration. First of all, it was impossible to collect any somatometric data at childhood, as well as follow-up data, that may affect menarcheal age and negative health outcome of later life. Secondly, the cross-sectional nature of this thesis impedes conclusions as per the existence of a causal relationship, despite the fact that menarcheal age precedes SH. Third, menarcheal age was reported by recall; nevertheless, menarche is an important event in a woman's life, therefore, it is reliable throughout many years [26]. The case-control design cannot demonstrate causality but may raise reliable hypotheses to be bolstered and extended in future prospective and longitudinal studies. Although confounding variables were properly controlled for by employing standard statistical procedures, residual confounding by other undetermined variables and hormones is always a possibility that may be explored in future larger studies. Finally, another limitation of the thesis is the moderate sample size, which eventually was sufficient to replicate previous associations of risk factors with SH risk.

In conclusion, this thesis showed an independent association of early menarche with an increased risk of SH. Although it is still unclear how hormonal and reproductive

factors are related to SH, this thesis raises reliable hypothesis regarding the interplay of early exposure to estrogens with SH risk. Further larger prospective studies and genetic research of the association of reproductive factors with SH are needed. More investigation is warranted to explain the greater prevalence of SH amid women.

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Table 1 Descriptive Categorical Features of subclinical hypothyroidism cases (n=72) and control participants (n=72).

Categorical Variables	**Cases (n=72)** N (%)	**Controls (n=72)** N (%)	**p-value**
Education (years)			0.74
6	8 (11.1)	4 (5.6)	
9	10 (13.9)	8 (11.1)	
12	32 (44.4)	36 (50.0)	
>12	22 (30.6)	24 (33.4)	
Smoking history			0.50
Non-Smoker	40 (55.6)	36 (50.0)	
Ex-Smoker	14 (19.4)	20 (27.8)	
Current Smoker	18 (25.0)	16 (22.2)	
Alcohol consumption			**0.03**
Never	8 (11.1)	8 (11.1)	
Rarely (1-2 glasses per month)	12 (16.7)	24 (33.3)	
Weekly (1-2 glasses per week)	24 (33.3)	28 (38.9)	
Weekly (3-4 glasses per week)	20 (27.8)	8 (11.1)	
Coffee consumption			**0.01**
Never	4 (5.6)	0 (0.0)	
Few times per month	10 (13.9)	8 (11.1)	
Few times per week	6 (8.3)	20 (27.8)	
Daily	52 (72.2)	44 (61.1)	
Physical activity (≥ 2 hours/week)	10 (13.9)	16 (22.2)	0.19
Presence of thyroid autoimmunity (yes, %)	21 (29.2)	4 (5.6)	**<0.001**

Table 2 Baseline Continuous Parameters of subclinical hypothyroidism cases (n=72) and control participants (n=72).

Continuous variables	**Cases (n=72)**	**Controls (n=72)**	**p-value**	**Reference**
	Mean (SD)	Mean (SD)		**range**
Age, years	48.6 (15.6)	49.9 (14.3)	0.74	-
Weight, kg	67.8 (11.4)	64.6 (8.2)	0.05	-
Height, m	1.64 (0.04)	1.64 (0.04)	0.87	-
BMI, kg/m^2	25.2 (4.0)	24.0 (3.1)	0.08	-
Waist circumference, cm	80.9 (11.6)	83.6 (10.1)	0.15	-
Hip circumference, cm	91.1 (10.5)	95.6 (7.3)	**0.01**	-
WHR	0.9 (0.08)	0.9 (0.06)	0.09	-
Fat %	35.9 (5.9)	34.9 (4.9)	0.22	-
Fat mass, kg	24.8 (7.5)	22.8 (5.6)	0.06	-
Free fat Mass, kg	42.9 (5.2)	41.8 (3.8)	0.20	-
SBP, mmHg	128.4 (18.8)	125.8 (11.4)	0.27	-
DBP, mmHg	80.1 (12.1)	79.7 (6.8)	0.84	-
Mean arterial BP^{∂}, mmHg	96.2 (13.9)	95.1 (8.1)	0.47	-
TSH, μIU/mL	8.5 (1.8)	2.7 (0.7)	**<0.001**	0.27 – 4.2
free T3, pg/mL	3.1 (0.8)	3.1 (0.6)	0.94	2.0 – 4.4
free T4, ng/mL	1.4 (0.4)	1.5 (0.3)	0.09	0.8 – 2.0
Total T3, ng/mL	1.3 (0.3)	1.3 (0.3)	0.45	0.8 – 2.0
Total T4, μg/dL	8.0 (1.8)	11.1 (1.3)	**<0.001**	5.1 – 14.1
Anti-TG Ab, IU/mL	56.5 (37.7)	34.5 (29.8)	**<0.001**	0 - 115
Anti-TPO Ab, IU/mL	26.4 (23.7)	13.1 (7.5)	**<0.001**	0 - 34
Glucose, mg/dL	83.5 (11.2)	80.3 (10.9)	0.09	74 - 106
Insulin, μU/mL	22.2 (5.1)	19.3 (3.1)	**<0.001**	2.6 – 24.9
HOMA-IR score	4.7 (1.6)	3.9 (0.9)	**<0.001**	-
Total cholesterol, mg/dL	209.7 (14.7)	196.8 (14.9)	**<0.001**	140 - 220
Triglycerides, mg/dL	138.0 (51.2)	86.2 (24.5)	**<0.001**	<200

HDL-C, mg/dL	56.7 (6.7)	58.0 (6.8)	0.35	♀: 45 - 65
LDL-C, mg/dL	137.1 (11.5)	129.2 (12.4)	**0.001**	<159

[d]Mean Arterial BP= (2*DBP+ SBP)/3

Reproductive years = Age at menarche – Age at menopause

Ab: antibody; BP: blood pressure; BMI: Body Mass Index; DBP: Diastolic Blood Pressure; HDL-C: High-density lipoprotein cholesterol; HOMA: Homeostasis model assessment score of insulin resistance; LDL-C: Low-density lipoprotein cholesterol; SBP: Systolic Blood Pressure; SD: standard deviation; T3: Triiodothyronine; T4: Thyroxine; TG: Thyroglobulin; TPO: Thyroid peroxidase; TSH: Thyroid stimulating hormone; WHR: Waist-to-hip ratio

Table 3 Reproductive factors of subclinical hypothyroidism cases (n=72) and control participants (n=72).

Reproductive factors	**Cases (n=72)**	**Controls (n=72)**	**p-value**
Continuous variables	***Mean (SD)***	***Mean (SD)***	
Age at menarche, years	12.6 (1.2)	13.3 (0.8)	**<0.001**
Age at menopause, years	***(n=38)***	***(n=44)***	0.55
	45.0 (2.7)	44.3 (3.1)	
Reproductive years	***(n=38)***	***(n=44)***	0.07
	32.2 (3.0)	31.0 (3.3)	
Menses duration, days	29.1 (1.9)	28.6 (1.7)	0.14
Period duration, days	5.07 (0.51)	5.08 (0.44)	0.84
Age at first pregnancy, years	29.6 (5.22)	28.27 (4.71)	**0.04**
Full-term pregnancies	1.75 (0.49)	2.02 (0.63)	**0.04**
Categorical Variables	***N (%)***	***N (%)***	
Use of oral contraceptives yes, %)	7 (9.7)	6 (8.3)	0.77
Hormone Replacement Therapy (yes, %)	3 (4.2)	3 (4.2)	1.00
Exogenous use of hormones* (yes, %)	10 (13.9)	9 (12.5)	0.81
Parity (yes, %)	40 (55.6)	44 (61.1)	0.50
Lactation (yes, %)	23 (31.95)	28 (38.89)	0.66
Menopausal (yes, %)	38 (52.8)	44 (61.1)	0.31

*Exogenous use of hormones: Hormone Replacement Therapy & Oral Contraceptives

Table 4 Correlations of study variables with age at menarche

Variables	Whole Population n=144		Cases with SH n=72		Controls n=72	
	r	p	r	p	r	p
Age	0.09	0.30	0.14	0.25	-0.06	0.60
Height	0.09	0.26	0.14	0.24	0.05	0.65
Weight	0.37	**<0.001**	0.30	**0.01**	0.67	**<0.001**
Body Mass Index	0.34	**<0.001**	0.30	**0.01**	0.60	**<0.001**
Waist circumference	0.29	**<0.001**	0.17	0.16	0.40	**0.001**
Hip circumference	0.08	0.35	0.002	0.99	0.05	0.66
WHR	0.27	**0.001**	0.18	0.14	0.51	**<0.001**
Fat %	0.33	**<0.001**	0.29	**0.02**	0.49	**<0.001**
Fat mass	0.35	**<0.001**	0.31	**0.008**	0.58	**<0.001**
Free fat Mass	0.27	**0.001**	0.20	0.09	0.45	**<0.001**
SBP	0.07	0.44	0.17	0.15	-0.06	0.59
DBP	0.23	**0.001**	0.22	0.06	0.23	0.06
Mean arterial BP	0.12	0.15	0.18	0.14	0.05	0.68
TSH	-0.25	**0.003**	0.08	0.52	-0.05	0.68
free T_3	0.01	0.92	0.08	0.53	-0.06	0.63
free T_4	0.16	0.05	0.14	0.23	0.22	0.85
Total T3	0.03	0.75	0.03	0.82	-0.003	0.98
Total T4	0.21	**0.01**	-0.15	0.22	0.01	0.95
Anti-TG Ab	0.15	0.07	0.29	**0.01**	0.26	**0.03**
Anti-TPO Ab	-0.20	**0.02**	-0.18	0.13	0.03	0.79
HOMA-IR	0.15	0.08	0.24	**0.04**	0.25	**0.03**

Glucose	0.21	**0.01**	0.31	**0.01**	0.25	**0.04**
Insulin	0.08	0.32	0.15	0.21	0.21	0.08
Triglycerides	0.03	0.72	0.26	**0.02**	0.32	**0.007**
Total Cholesterol	0.13	0.12	0.31	**0.008**	0.21	0.07
LDL-C	-0.04	0.14	0.12	0.32	-0.06	0.59
HDL-C	0.24	**0.004**	0.16	0.19	0.35	**0.003**
Age at menopause	-0.18	0.10	-0.09	0.60	-0.28	0.07
Reproductive years	-0.49	<**0.001**	-0.51	<**0.001**	-0.35	**0.02**
Menses duration	-0.10	0.22	-0.16	0.17	0.07	0.54
Period duration	-0.05	0.55	-0.09	0.47	-0.05	0.66
Age at first pregnancy	-0.02	0.89	-0.19	0.22	0.40	**0.01**
Full-term pregnancies	-0.13	0.25	-0.06	0.71	-0.41	**0.01**

Ab: antibody; DBP: Diastolic Blood Pressure; HDL-C: High-density lipoprotein cholesterol; HOMA: Homeostasis model assessment score of insulin resistance; LDL-C: Low-density lipoprotein cholesterol; SBP: Systolic Blood Pressure; SD: standard deviation; SH: Subclinical Hypothyroidism; T3: Triiodothyronine; T4: Thyroxine; TG: Thyroglobulin; TPO: Thyroid peroxidase; TSH: Thyroid stimulating hormone; WHR: Waist-to-hip ratio

Table 5 Association of age at menarche, autoimmune thyroid disease, BMI, Hip Circumference and HOMA-IR with risk of subclinical hypothyroidism in 72 cases with subclinical hypothyroidism and in 72 controls matched on age and date of diagnosis; multiple logistic regression-derived, adjusted odds ratios (OR‡) and 95% confidence intervals (95% CI)

Variables	**Category or Increment**	**p-value**	**OR**	**95% CI**
Age at menarche	1 quartile more	**<0.001**	0.22	0.11 – 0.44
BMI	1 kg/ m^2 more	**0.03**	1.21	1.02 – 1.43
Hip Circumference	1 quartile more	**<0.001**	0.41	0.26 – 0.65
Thyroid Autoimmunity	Yes versus No	**<0.001**	45.27	5.84 – 350.79
HOMA – IR	1 SD more	0.12	1.66	0.89 – 3.11
Alcohol consumption	1 category of alcohol consumption more	**0.001**	2.12	1.37 – 3.47

‡ adjusted for age and date of diagnosis and blood draw (matching factors)

SD: standard deviation

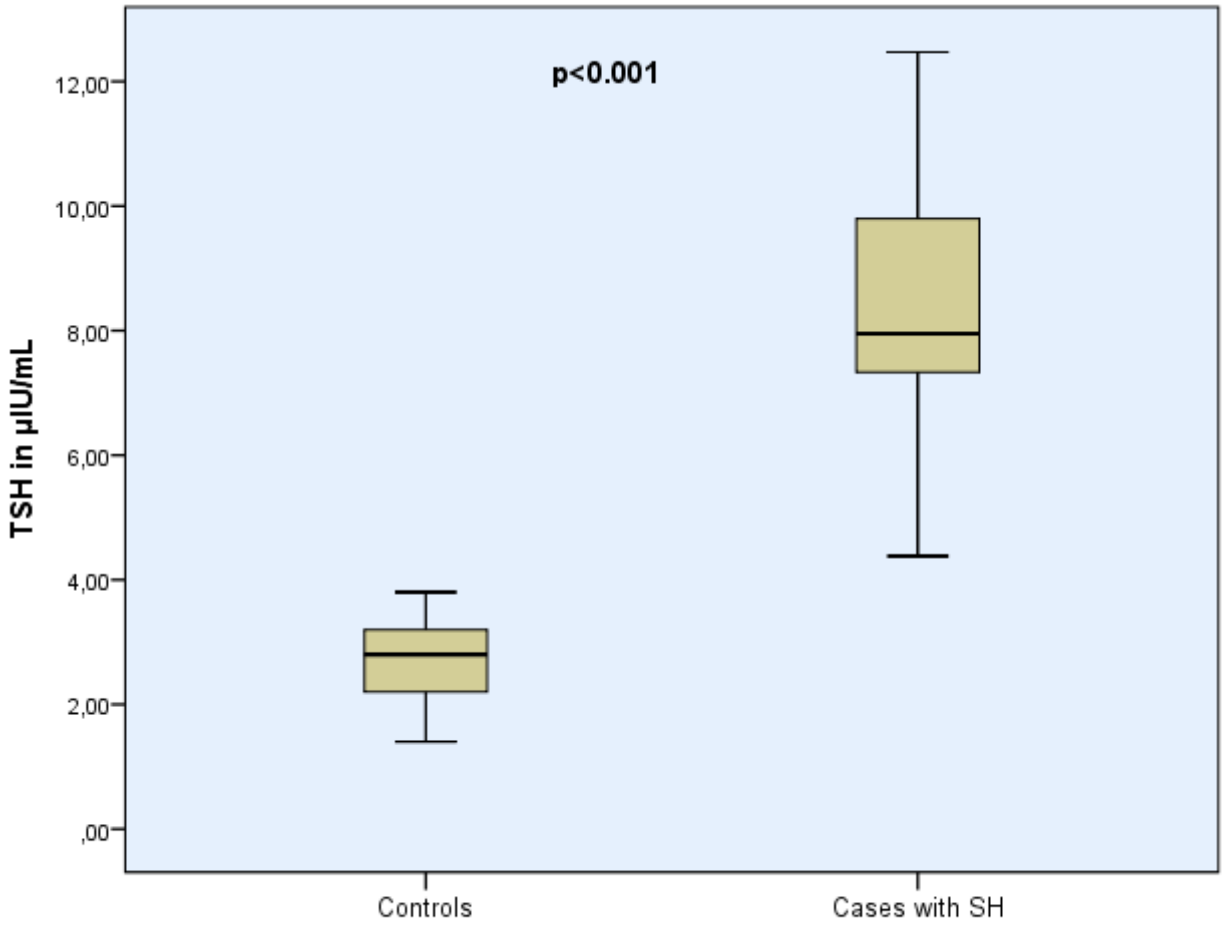

Fig.1 Box plots of TSH in 72 cases with subclinical hypothyroidism (8.5±1.8) and 72 controls (2.7±0.7, p<0.001).

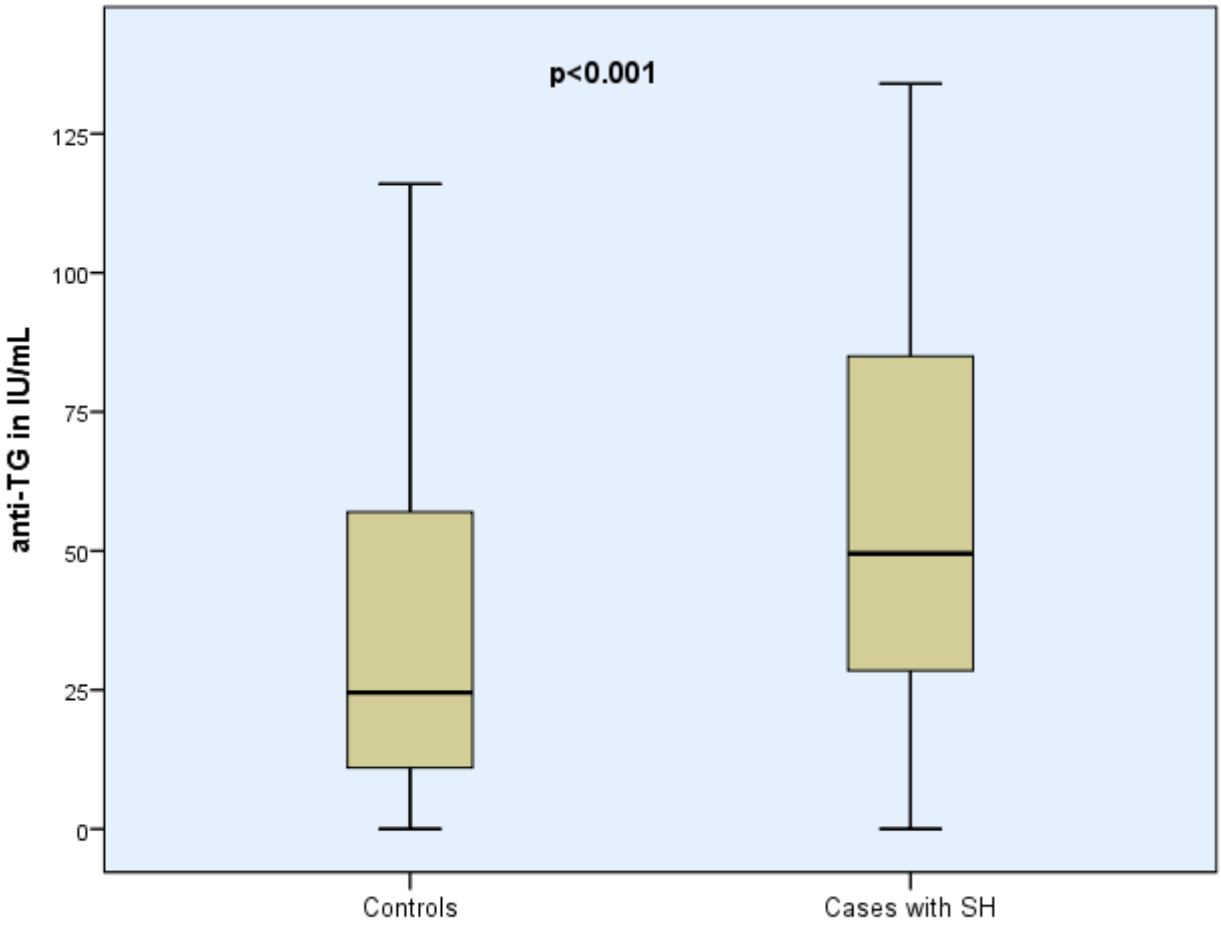

Fig.2 Box plots of anti-TG antibodies in 72 cases with subclinical hypothyroidism (56.5±37.7) and 72 controls (34.5±29.8, p<0.001).

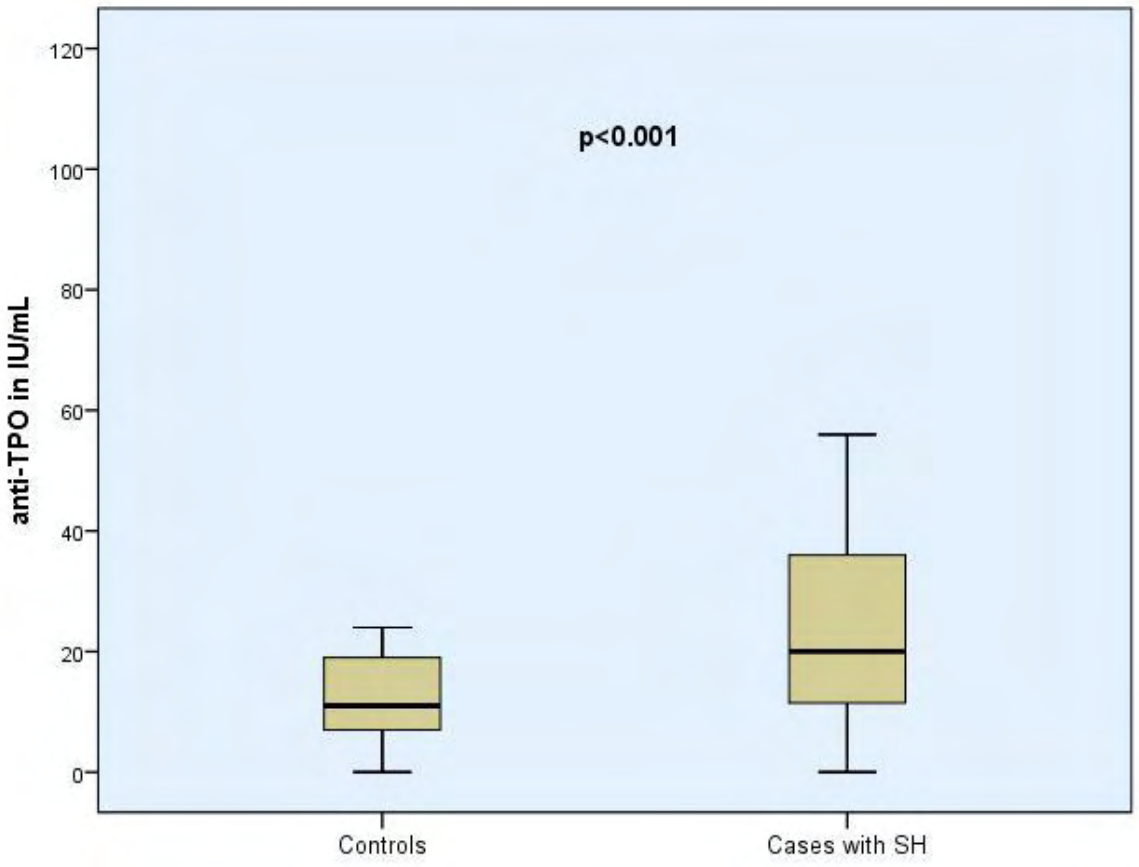

Fig.3 Box plots of anti-TPO antibodies in 72 cases with subclinical hypothyroidism (26.4±23.7) and 72 controls (13.1±7.5, p<0.001).

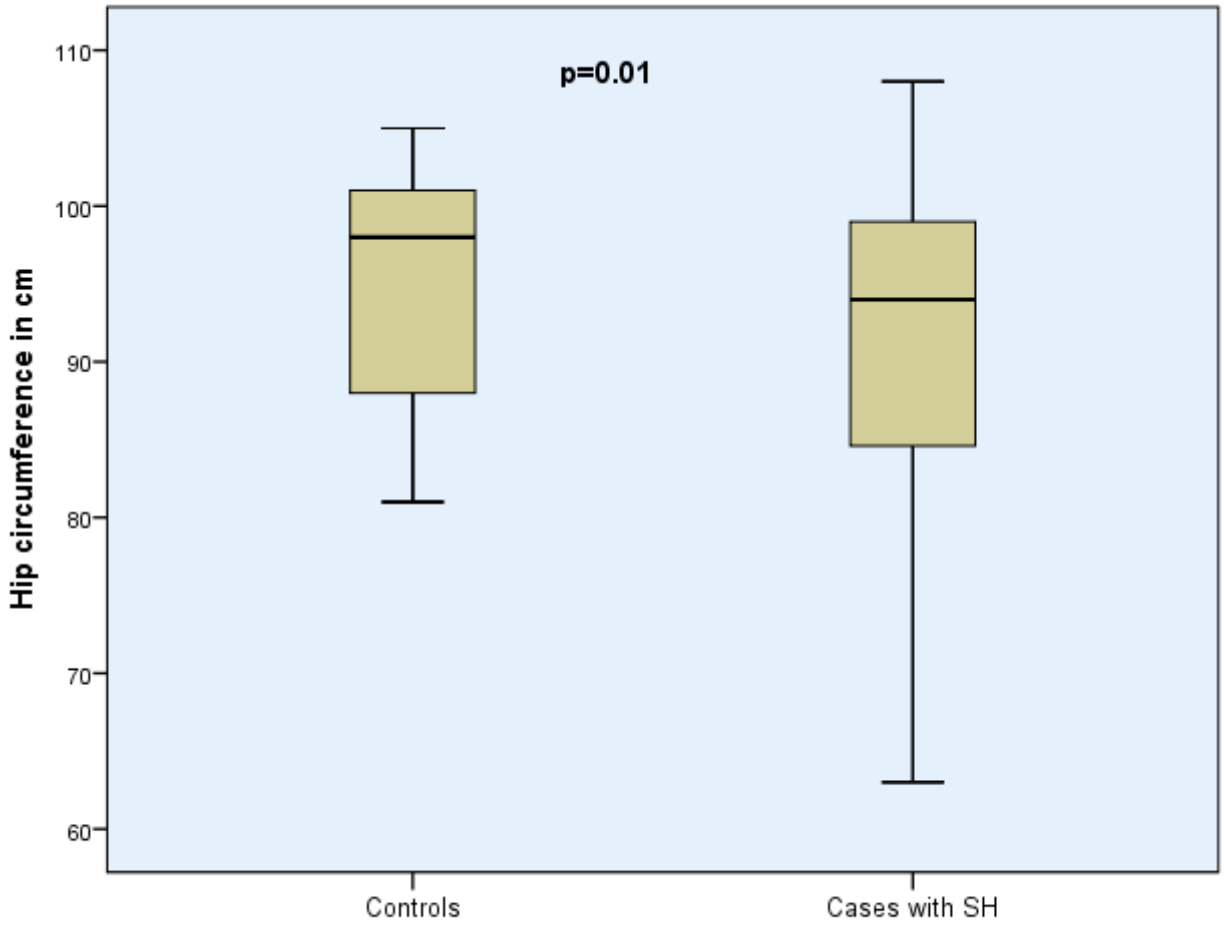

Fig.4 Box plots of hip circumference in 72 cases with subclinical hypothyroidism (91.1±10.5) and 72 controls (95.6±7.3, p=0.01).

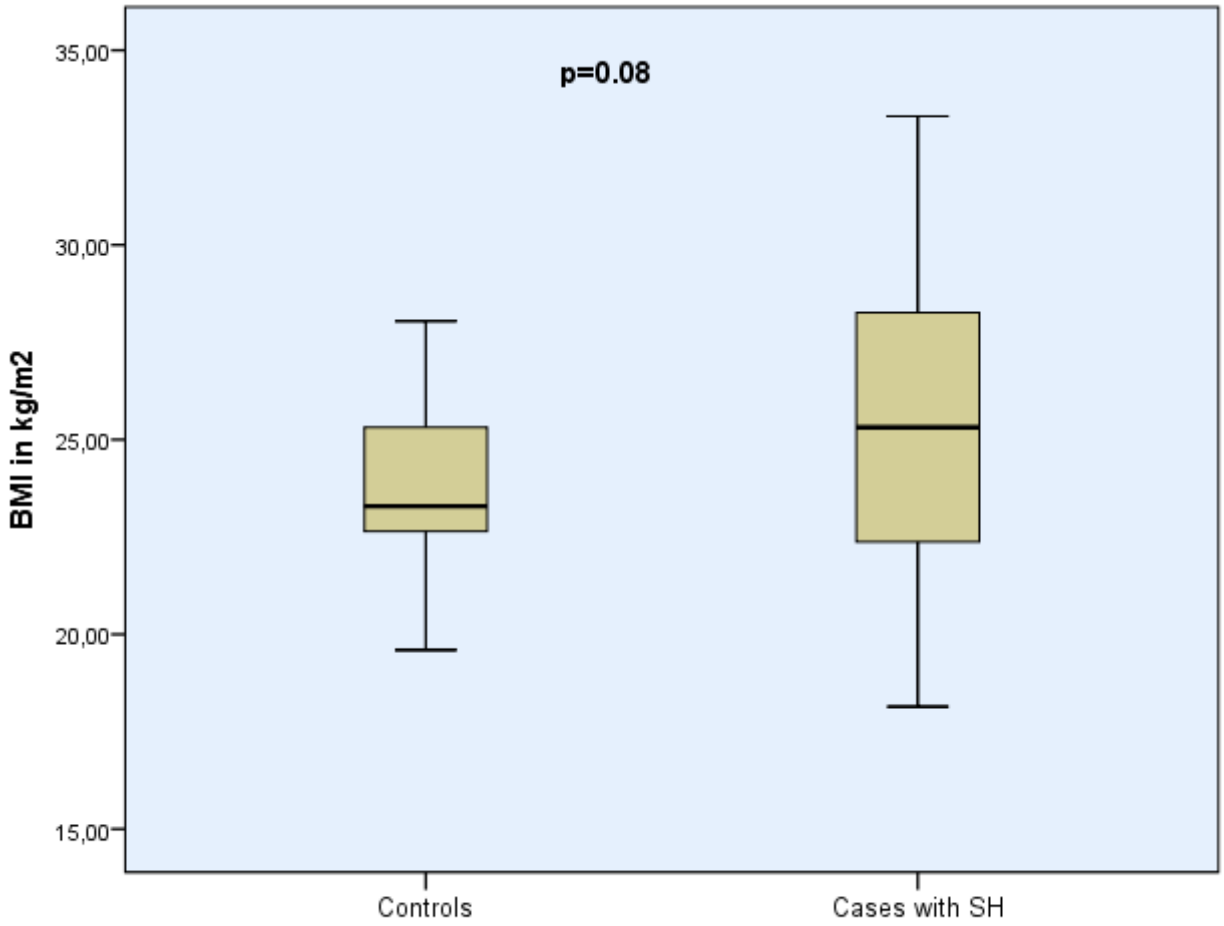

Fig.5 Box plots of BMI in 72 cases with subclinical hypothyroidism (25.2±4.0) and 72 controls (24.0±3.1, p=0.08).

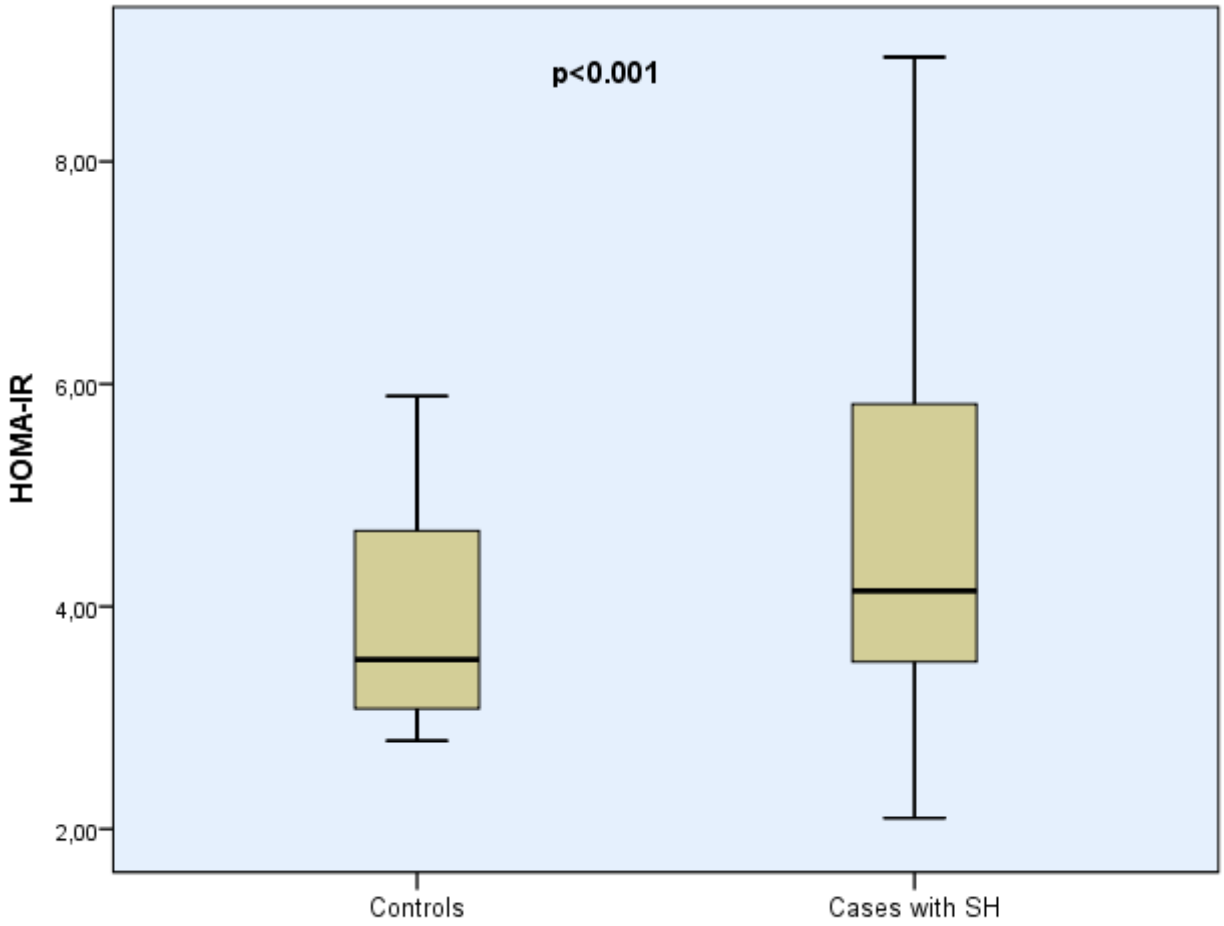

Fig.6 Box plots of HOMA-IR in 72 cases with subclinical hypothyroidism (4.7±1.6) and 72 controls (3.9±0.9, p<0.001).

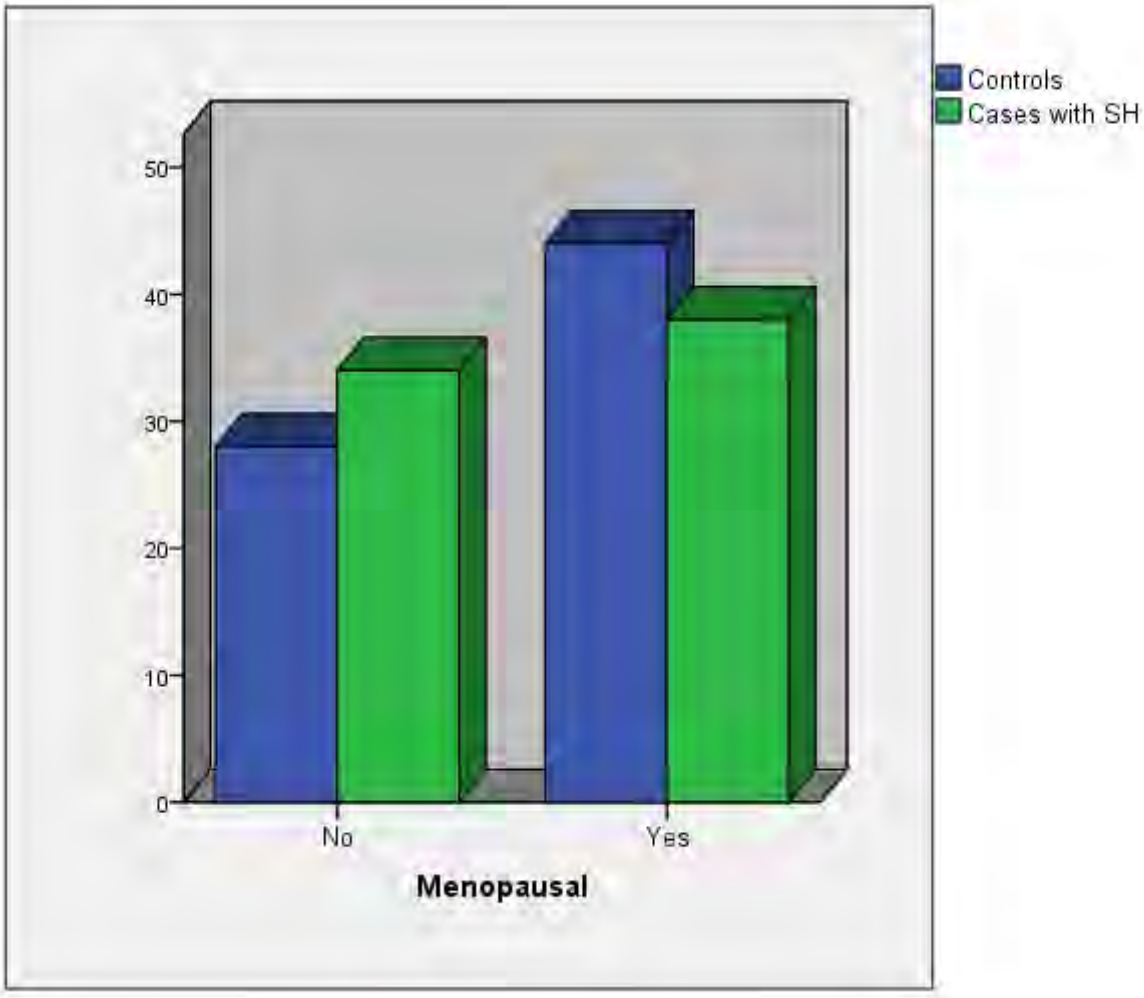

Fig.7 Cases with subclinical hypothyroidism did not differ in terms of menopausal status compared to controls (52.8% vs. 61.1%. p=0.31).

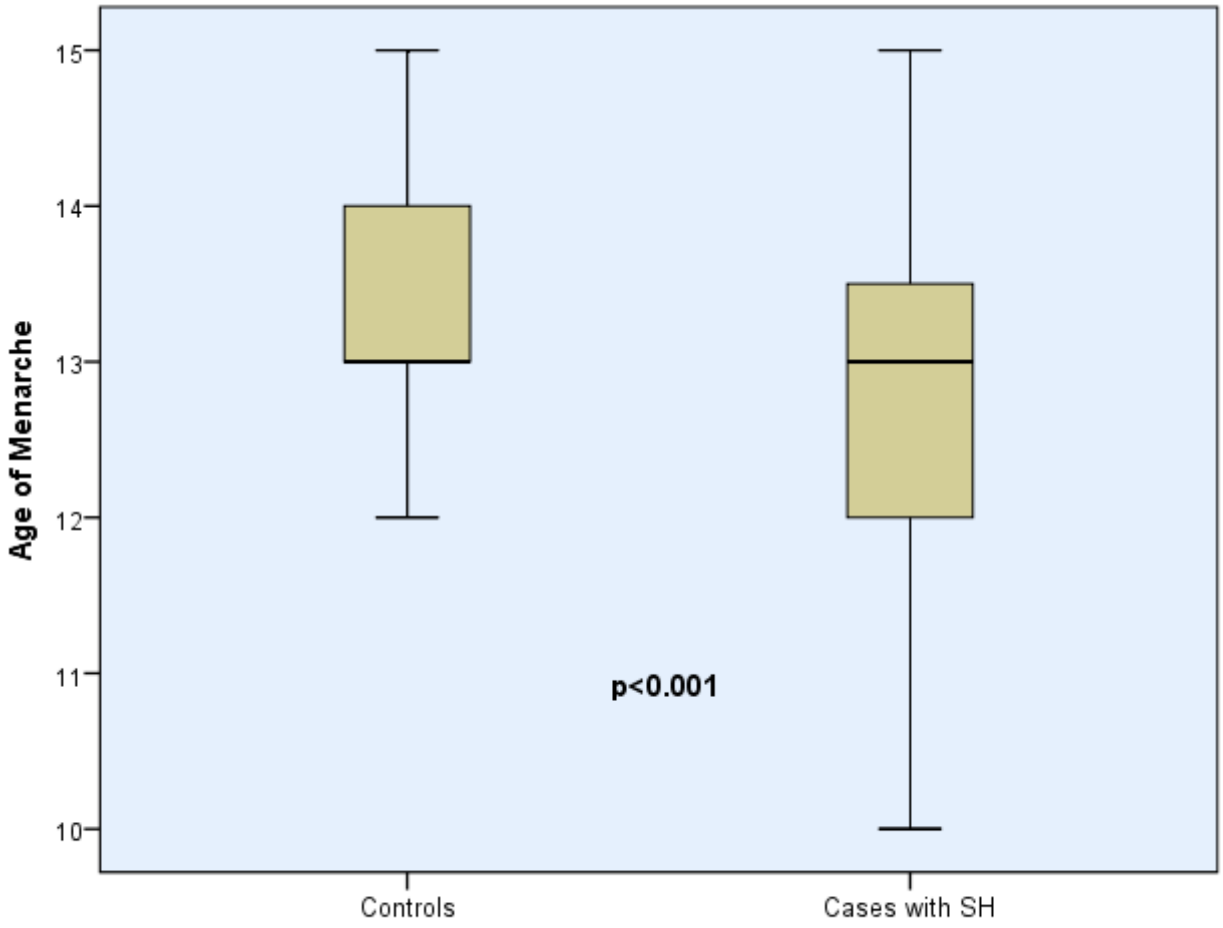

Fig. 8 Box plots of age at menarche in 72 cases with subclinical hypothyroidism (12.6±1.2) and 72 controls (13.3±0.8, p<0.001).

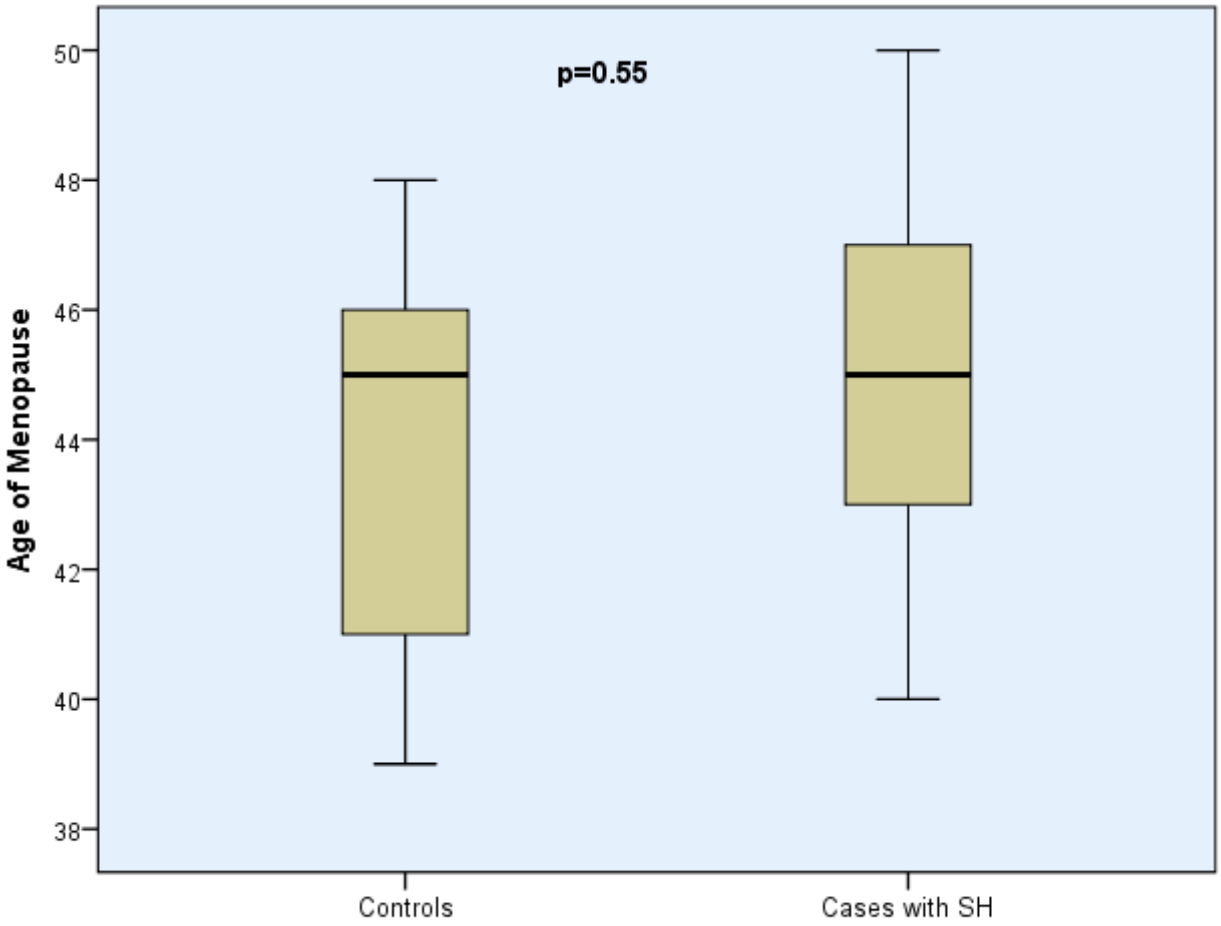

Fig. 9 Box plots of age at menopause in 38 cases with subclinical hypothyroidism (45.0±2.7) and 44 controls (44.3±3.1, p=0.55).

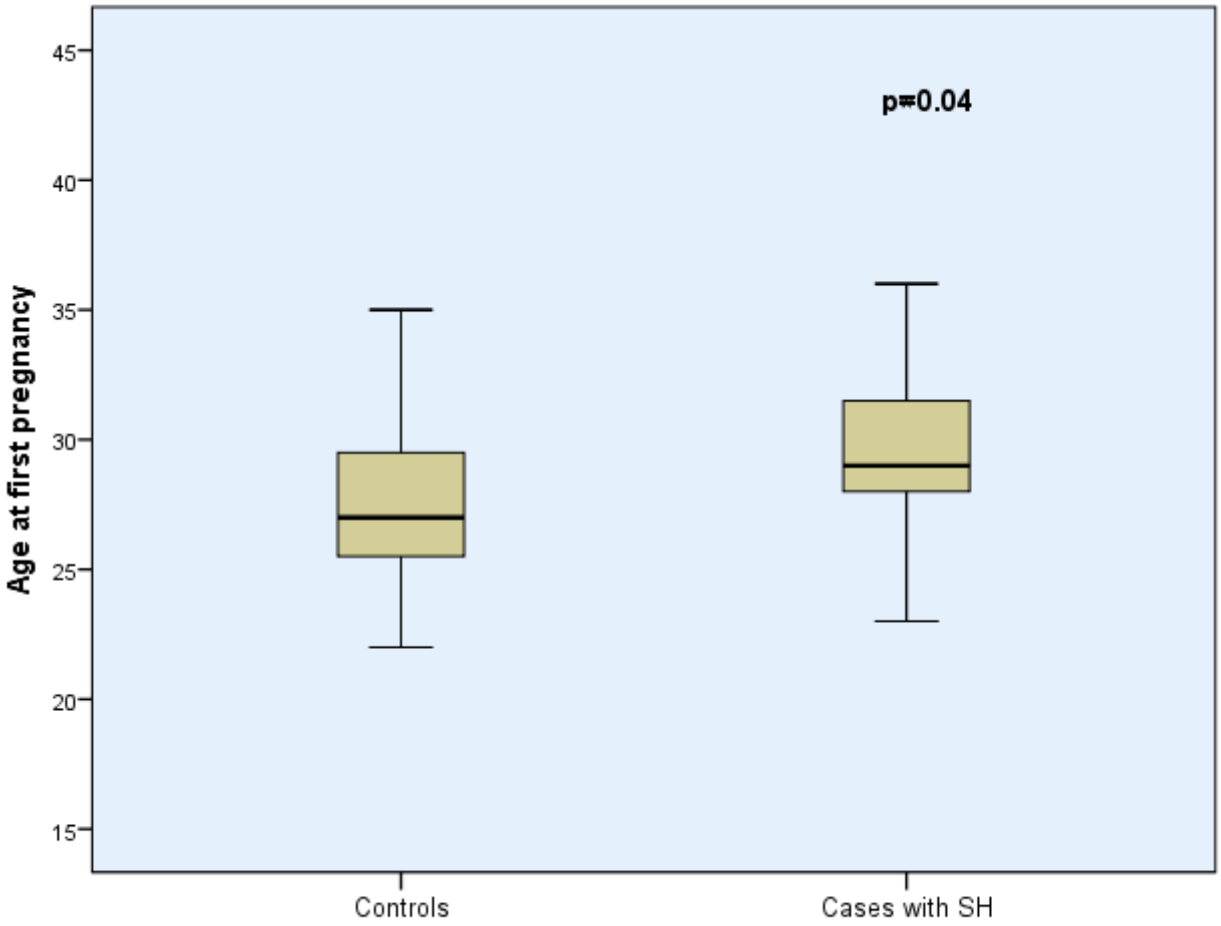

Fig.10 Box plots of age at first pregnancy in 40 cases with subclinical hypothyroidism (29.6±5.22) and 44 controls (28.3±4.71, p=0.04).

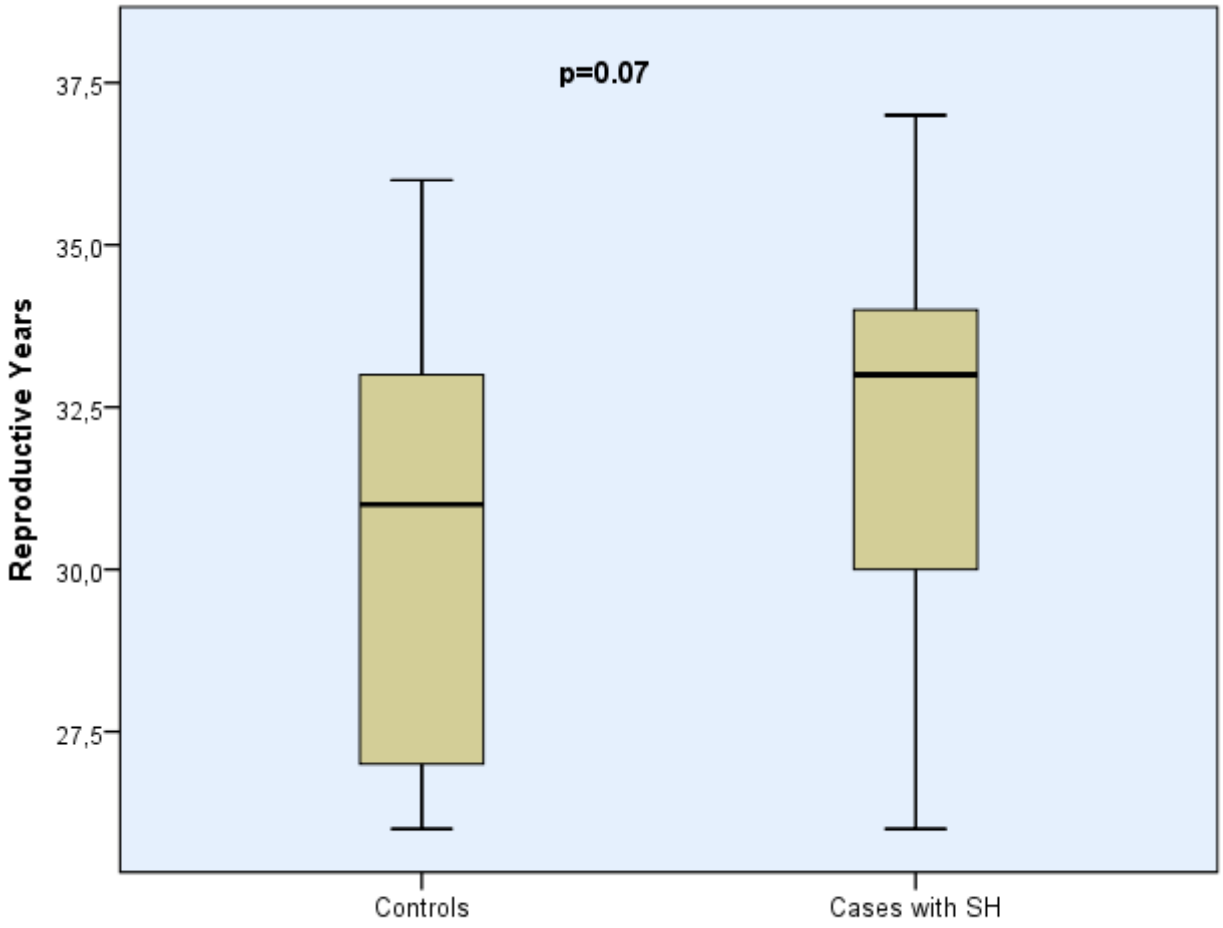

Fig.11 Box plots of reproductive years in 38 cases with subclinical hypothyroidism (32.2±3.0) and 44 controls (31.0±3.3, p=0.07).

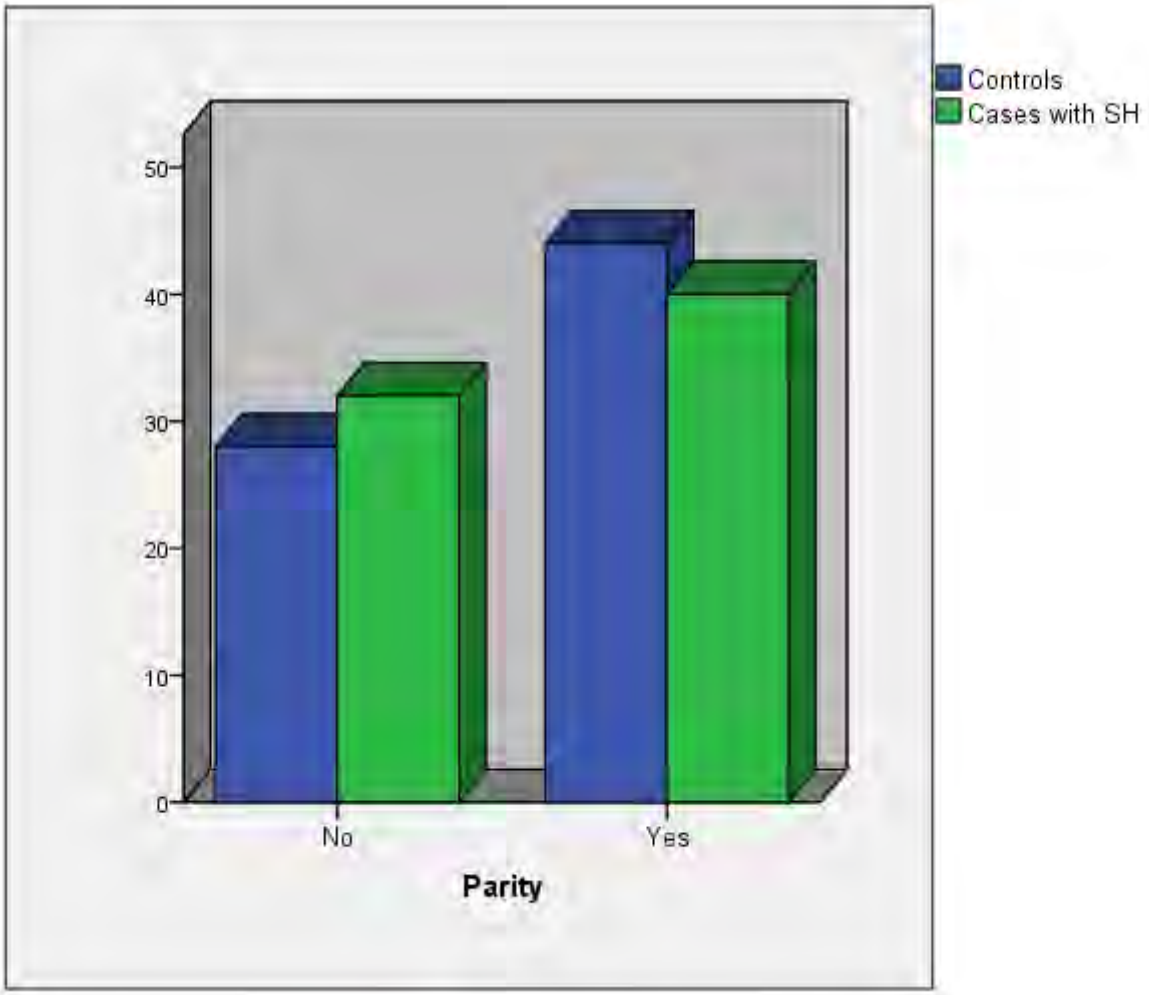

Fig.12 Cases with subclinical hypothyroidism did not differ in terms of parity compared to controls (55.6% vs. 61.1%. p=0.50).

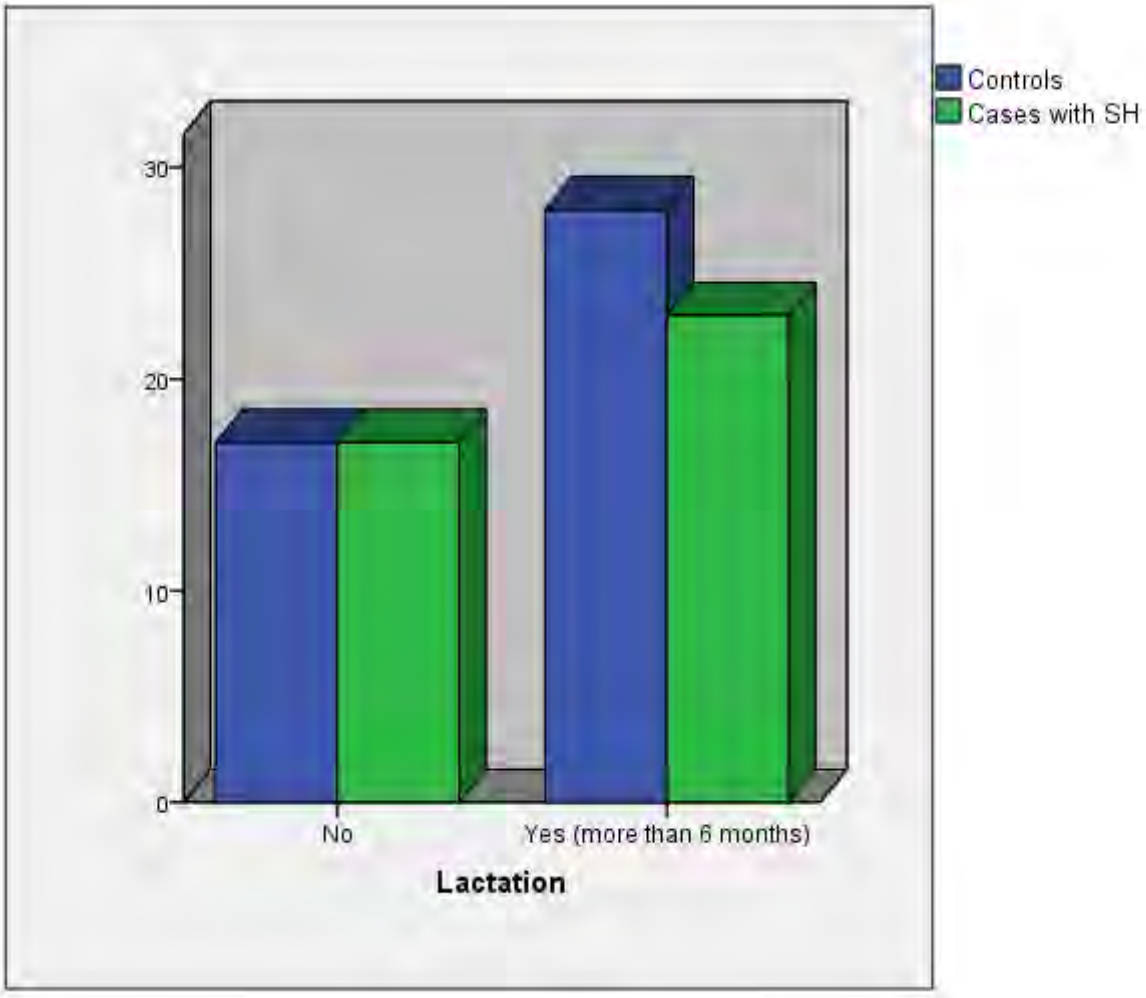

Fig.13 Cases with subclinical hypothyroidism did not differ in terms of lactation compared to controls (31.95% vs. 38.89%. p=0.66).

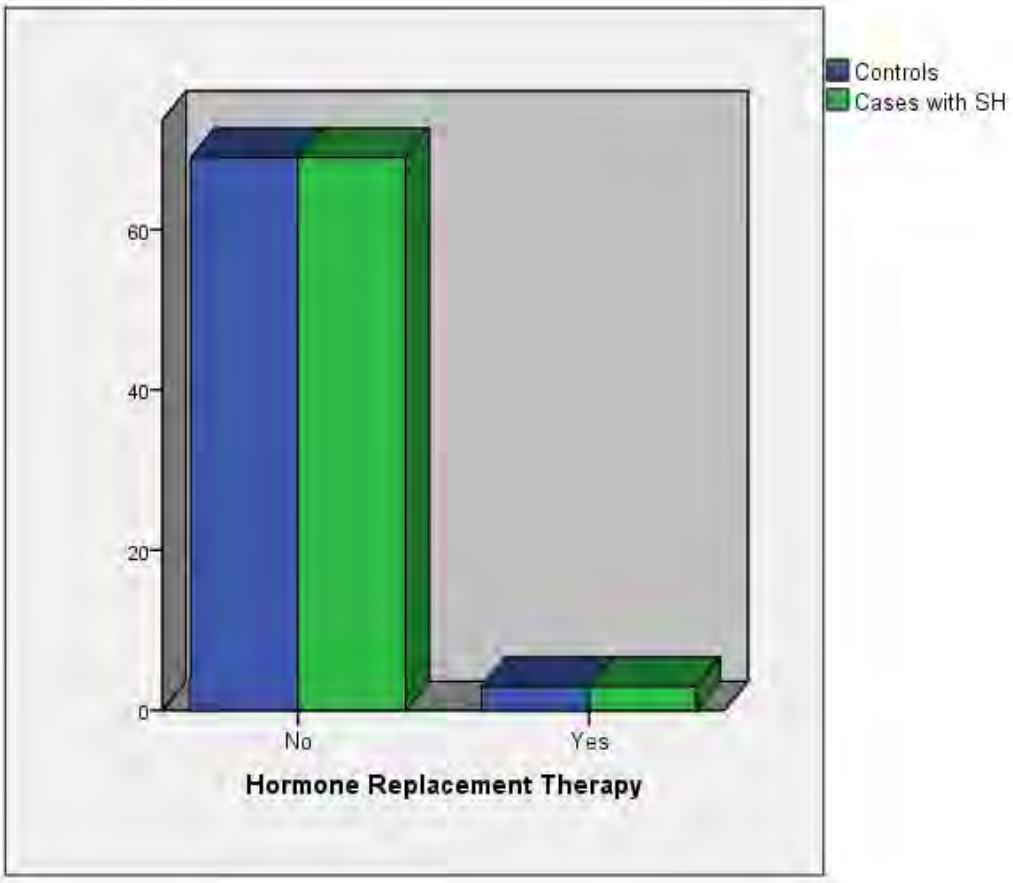

Fig.14 Cases with subclinical hypothyroidism did not differ in terms of use of Hormone Replacement Therapy compared to controls (4.2% vs. 4.2%. p=1.00).

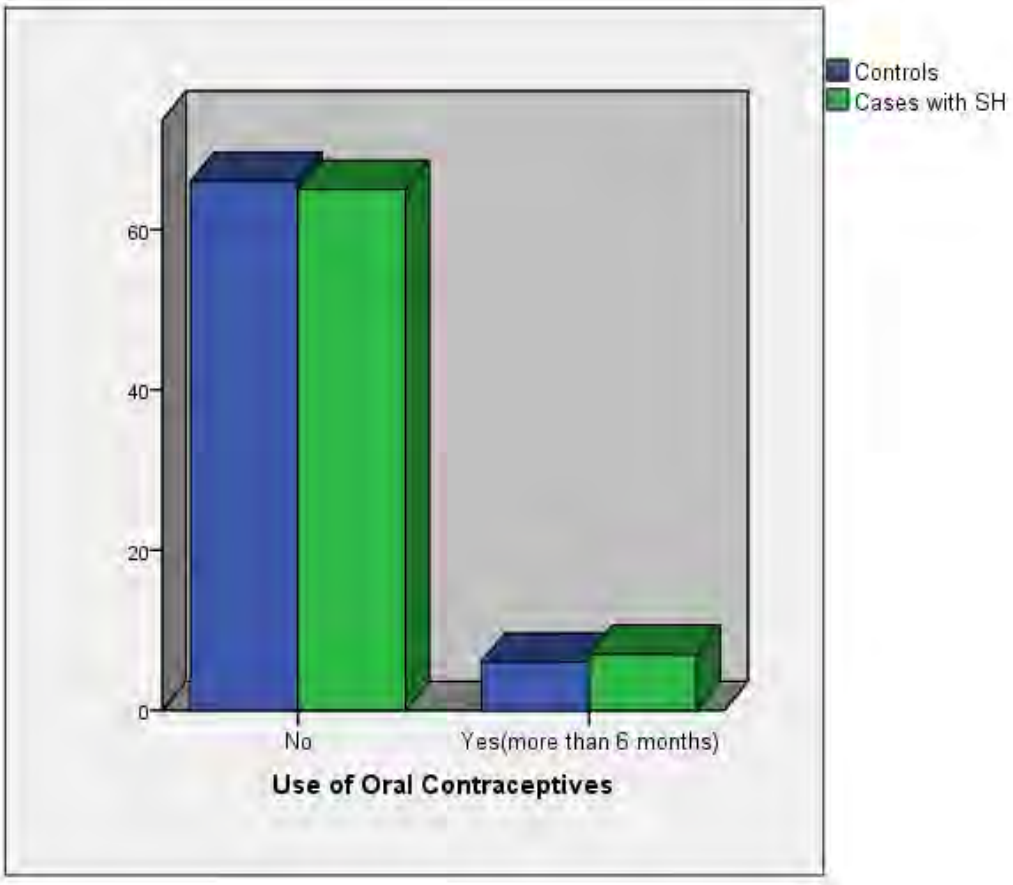

Fig.15 Cases with subclinical hypothyroidism did not differ in terms of use of oral contraceptives compared to controls (9.7% vs. 8.3%. p=0.77).

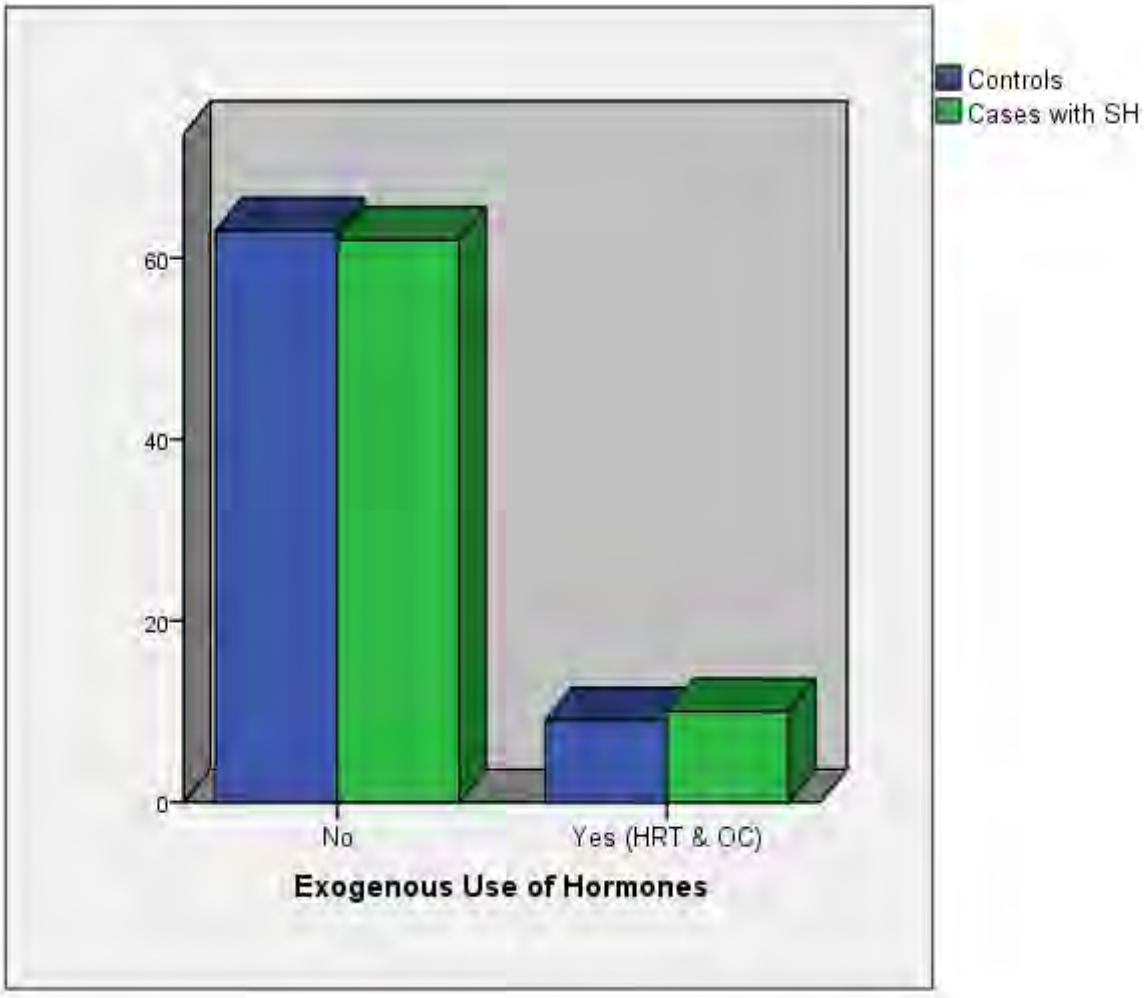

Fig.16 Cases with subclinical hypothyroidism did not differ in terms of Exogenous Use of Hormones compared to controls (13.9% vs. 12.5%. p=0.81).