Primary Women Infertility and Thyroid Disorders

Madha Mohammed Saleh[1], Ola Abbas Khdhair[2], Amina Salman Ali[3]

[1]Assistant Professor in Immunology, College of Health and Medical Technology, Baghdad, Iraq, [2]M.Sc. in Immunology, Assistant Lecturer, Department of Medical Laboratory Techniques, Al-Mustaqbal University College, Babylon, Iraq, [3]M.Sc., Researcher in Microbiology, Babylon, Iraq

Abstract

The study was conducted to explore the prevalence of thyroid autoimmune disorders among primary infertile women. Hormonal and immunological serum markers were tested including: TSH, T3, T4, TPO Ab, prolactin and Interleukin-6. Thyroid abnormalities were observed in 38.5% of the primary infertile women. Thyroid autoimmunity was more prevalent among primary infertile women (9.6%) than pregnant (4.0%) and non-pregnant (4.3%) fertile women, but with no significant difference. Presence of thyroid hormones related clinical manifestation in infertile women was higher (26.9%) in comparison to pregnant fertile women and non-pregnant fertile groups. Abnormalities in menstrual cycles were much higher in infertile women (90.4%) than non-pregnant fertile women (27.7%). Serum level of IL-6 was with no significant differences among all study groups. In conclusion, AITD has a minor participation in the thyroid disorder–associated infertility and their menstrual abnormalities in comparison with the larger scale of the non-AITD participation. No role for the IL-6 in the thyroid associated primary infertility in women was detected.

Keywords: *Infertility, Thyroid disorders, TPO, IL-6, menstrual cycle.*

Introduction

Causes of female infertility are wide including anovulation due to hormonal disorders or follicle problem, polycystic ovary syndrome and others resulting in failure to produce mature eggs, malfunction of the hypothalamus and malfunction of the pituitary gland. Endometriosis is playing a major role in female infertility as 30-40% of patients with endometriosis are infertile, whereas other general factors may also participate in the infertility causes like age, smoking,, sexually transmitted infection and body weight and eating disorders [1, 2, 3].

Thyroid disorders and their effects on infertility are highly debatable, and the prevalence of hypothyroidism in women of reproductive age varies between 2% and 4% and is largely due to autoimmune thyroid diseases (AITD) in the presence or absence of autoantibodies [4]. Most of the studies have shown the association of thyroid disorder with menstrual disturbance and even anovulatory cycles as oligomenorrhea and menorrhagia and ovulatory dysfunction, due to numerous interactions of thyroid hormones with the female reproductive system [5]. In the study of Plowden *et al* (2016), it has been stated that women with subclinical hypothyroidism or thyroid autoimmunity are keeping their chances of conceiving and achieving a live birth and likely unaffected by marginal thyroid dysfunction [6], whereas in a Danish study it was stated that impaired fertility is associated with TSH, TPO antibodies, and mild (subclinical) hypothyroidism in a Danish population of women [7]. On the other hand, menstrual disturbances in hyperthyroidism had been described by Kakuno *et al* in 2010 and found that menstrual disturbances were with only severe cases of thyroid disorders but not mild or moderate and hence concluded that menstrual disturbances in thyroid disorders were less frequent than previously thought [8].

The underlying pathogenic mechanisms associating AITD and infertility remain largely speculative, as

Corresponding Author:
Madha Mohammed Saleh
Assistant Professor in Immunology, College of Health and Medical Technology, Baghdad, Iraq
e-mail: madhataiz2004@yahoo.com

neither animal models nor *in vitro* data on this issue are available. It was noted that in infertile women, thyroid autoimmunity features are significantly more frequent than in healthy fertile controls as represented by the level of anti-TPO antibodies [9].

The aim of the current study was first to estimate thyroid disorder markers; T_3, T_4, and TSH as well as IL-6 and prolactin levels in infertile women in comparison with pregnant fertile and non-pregnant fertile women. Second thing was to evaluate serum anti-TPO positivity in all study groups. This marker is an indicator of association strength between autoimmune thyroid disease and infertility after the exclusion of any other possible causes for infertility.

Materials and Method

Subjects: This study was conducted in the Teaching Hospitals of Al-Kut City/Iraq for the period from Oct. 2017 to Mar. 2018. A total of 52 primary infertile (PI) females were selected with an age range of 17-43 years. Two apparently healthy control women groups were included; 50 non-pregnant fertile (NPF) (with at least one previous reproducible pregnancies) and 47 of pregnant fertile (PF). Inclusion criteria for infertile subjects were: primary infertile female, no cause of infertility, and normal male factors. Exclusion criteria for infertile subjects were; age limitations, abnormal male factors, past or current history of infertility-related disease such as sexually transmitted infection (STI), uterine or pelvic pathology and others. Approximately 5 ml blood sample was collected from every participant and sera were separated.

Materials: The following kits were used in this study: Interlukin-6 (IL-6) kit (Immuntech, France), prolactin kit (Monobind, USA), thyroid stimulating hormone (TSH) kit (Monobind, USA), thyroid peroxide (TPO) kit (Human, Germany), thyroxin (T_4) kit (Monobind, USA), and triiodothyronine (T_3) kit (Monobind, USA).

Method: According to the manufacturer's instructions the following ELISA method were used: immuno-enzymometric assay for the estimation of (TSH) and prolactin (PRL), competitive enzyme immunoassay for the estimation of T3 and T4, indirect ELISA for the estimation of anti-TPO IgG antibody, and sandwich ELISA for the estimation of IL-6.

Statistical analysis*:* SPSS software, version 17 was employed for statistical analysis.

Results

Table 1 shows the thyroid hormones related clinical manifestations which were 26.9%, 16% and 12.8% in PI, PF and NPF women respectively with no significant differences. Table 1 also demonstrates the menstrual cycle status in PI & NPF women.

Table 1. Thyroid hormones-related clinical manifestation and menstrual cycle status as reported in study groups.

Study Groups	THRM				Menstrual Cycle status			
	Yes*		No		Normal (Regular)		Abnormal**	
	№	%	№	%	№	%	№	%
PI (n=52)	14	26.9	38	73.1	5	9.6	47	90.4
PF (n=50)	8	16.0	42	84.0				
NPF (n=47)	6	12.8	41	87.2	34	72.3	13	27.7
P value	PI X NPF =0.0001 (HS)***							

THRM = Thyroid hormones related manifestation (including; weight loss, heat intolerance, increase thirst, weight gain, cold intolerance, increased appetite, flushing, irritability and others). NS: not significant. at P> 0.05, *= The presence of at least three THRM manifestations, **Abnormal; Irregular, short, prolonged, amenorrhea, menorrhagia, oligomenorrhea and others.***HS: highly significant. at P < 0.01.

Thyroid function tests are depicted in Table 2. An abnormal thyroid hormones level was detected in 38.5% of the PI group including 34.6% with hypothyroidism and 3.9% with hyperthyroidism. Lower levels of thyroid hormones abnormalities were reported in the other study groups with significant difference.

Table 2. Thyroid status as evaluated in the study groups.

Thyroid Function Test		PI (n = 52)		PF (n = 50)		NPF (n = 47)		P value
		№	%	№	%	№	%	
Normal*		32	61.5	41	82.0	39	83.0	PI X PF = 0.022 (S) PI X NPF =0.018 (S)
Abnormal	Hypo**	18	34.6	3	6.0	5	10.6	
	Hyper***	2	3.9	6	12.0	3	6.4	
	Total	20	38.5	9	18.0	8	17.0	

Normal= T3, T4 and TSH serum levels are within normal range, Hypo= Hypothyroidism in which T3 and/or T4 serum level is below normal range and TSH serum level is above normal range, Hyper= Hyperthyroidism in which the T3 and/or T4 serum level is above normal range and TSH serum level is below normal range, S: Significant at P< 0.05

The menstrual cycle abnormalities in correlation with the thyroid status of PI women are tabulated in Table 3. The number of cases with abnormal menstrual cycle (including too short cycle, prolonged cycle and amenorrhea), was 47/52 (90.4%) which were distributed as 18/52 (34.6%), 28/52 (53.9%) and 1/52 (1.9%) on hypothyroidism, euthyroidism and hyperthyroidism respectively.

Table 3. Correlation of thyroid abnormalities and menstrual cycle as illustrated in infertile women.

Menstrual Cycle	**PI group (n=52) thyroid status**						**P value**
	Hypo		**Euo**		**Hyper**		
	№	**%**	**№**	**№**	**%**	**№**	
Normal (n= 5)	0	0.0	4	7.7	1	1.9	Hypo x Hyper = 0.05 (S) Hypo x Euo => 0.05 (NS) Hyper x Euo = 0.05 (S)
Abnormal (n= 47)	18	34.6	28	53.9	1	1.9	

NS: Non Sig. at P> 0.05; S : Sig. at P< 0.05; N = Normal menstrual cycle, Ab = Abnormal menstrual cycle.

Anti-TPO (IgG) antibody was detected in 9.6% of PI group compared to 4.0% and 4.3% in PF and NPF respectively with no significant differences (Table 4). Mean serum IL- 6 levels are shown in the same table with no statistical significant differences between all studied groups.

Table 4. Distribution of IgG TPO-Ab positivity and IL-6 level among study groups.

Study Group	**IgG TPO-Ab**				**IL- 6**				
	Positive		**Negative**		**Undetectable**		**Detectable**		**Mean±SD (Range)**
	№	**%**	**№**	**%**	**№**	**%**	**№**	**%**	
PI № =52	5	9.6	47	90.4	24	46.2	28	53.8	9.34± 7.98 (0.3-34.5)
PF № = 50	2	4.0	48	96.0	5	10	45	90	12.82±11.11 (1.1-30.0)
NPF № = 47	2	4.3	45	95.7	27	57.5	20	42.5	9.55±7.36 (4.7-27.8)
P value	PI X PF= 0.262 (NS)				PI X PF=0.052 (NS)				
	PI X NPF = 0.299 (NS				PI X NPF=0.388 (NS				

NS : Non Sig. at P< 0.05

Table 5 shows the correlation between thyroid status, prolactin (PRL) and anti-TPO antibodies in infertile women. For the total 18 cases with hypothyroidism there was 5/18 (27.8%) with elevated prolactin level. None of the euthyroid and hyperthyroid cases was with an elevated prolactin level. In the same table, 5.6%, 9.4% and 50% of the hypothyroid, euthyroid and hyperthyroid respectively were positive for anti- TPO antibodies.

Table 5. Correlation of thyroid status with PRL and anti-TPO Ab in infertile women

Test		Thyroid status in PI group						p-value
		Hypo		Euo		Hyper		
		№	%	№	%	№	%	
*PRL status	Normal	13	72.2	32	100	2	100	0.129 (NS)
	Elevated	5	27.8	0	0	0	0	
Anti-TPO Ab	Positive	1	5.6	3	9.4	1	50	
	Negative	17	94.4	29	90.6	1	50	

*PRL: prolactin

Discussion

The rational of this study was based on progressive analytical steps to find out the strength of correlation and/ or association between thyroid disorders and women's infertility.

The steps, in order, were first to examine the subjects of the infertile group for the presence or absence of any clinical manifestations that are related to the thyroid hormones abnormalities. The second and subsequent steps were to confirm or deny the menstrual cycle abnormalities, to evaluate the thyroid hormones level (T_3, T_4, and TSH) and to measure the IL-6 as a proinflammatory marker in the infertile women in comparison with the fertile women. The final step was to find out the prevalence of thyroid peroxidase antibodies (marker of autoimmune thyroid disease) and prolactin hormone.

In this study, there was a higher trend in thyroid hormones-related clinical manifestations in infertile group (26.9%) in comparison to the fertile groups (12.8 - 16.0 %) however this was not significant (Table 1).

In one study, TSH levels are significantly higher in a population of women without known thyroid dysfunction and with unexplained infertility as compared with a control group [10].

The higher prevalence of menstrual cycle abnormalities in PI group (90.4 %) compared to NPF group (27.7%) in the current study was expected and was similar to the results of other previous study [11]. The contribution of thyroid gland disorders in such abnormalities was indicated by the higher thyroid hormones-related manifestations among PI group (Table 1).Thyroid disorders were more evident in infertile group (38.5%) than fertile groups (17.0 to 18.0%) with a significant *P value* (34.6% hypothyroidism and 3.9% hyperthyroidism). These results would strengthen the possibility of the influence of thyroid disorder on the infertility status and comes in consistency with a previous study[11].

Tables 2 and 4 elucidate the effect of thyroid status on the menstrual cycle irregularities. The majority of infertile women (90.4%) were with abnormal menstrual cycle of which (34.6%) with hypothyroidism, (53.9 %) with euthyroidism and (1.9%) with hyperthyroidism. The impact of hypothyroidism on menstrual function and ovulation is related to numerous interactions of thyroid hormones with female reproductive system. At the cellular level, thyroid hormones synergize FSH to exert direct stimulatory effects on granulosa cell functions. Circulating thyroid hormone concentrations were associated with subtle differences in menstrual cycle function outcomes, particularly sex steroid hormone levels in healthy women. Results contribute to the understanding of the relationship between thyroid function and the menstrual cycle [12].

Morphological changes observed with follicles in hypothyroidism might be associated with elevated prolactin production that would inhibit secretion as

well as function of gonadotropin. Even in the absence of hyperprolactinemia, hypothyroidism by itself reduces the fertility since thyroid hormones are necessary for the optimal production of both estradiol and progesterone and thus in oocytes development [13].

Increased IL-6 production has been claimed as one of the mechanisms by which amiodarone exerts its toxic effect on the thyroid gland and in addition, albeit controversial, that IL-6 secretion is supported by TSH [14]. However, in this study, no significant differences were noticed in the IL-6 among all study groups which was similar to other study [15].

Hyperprolactinemia was only detected in the hypothyroid PI women (27.8% as seen in Table 5) a result that was in consistency with that of Turanker and his coworkers [16].

The main marker of AITD is the autoantibodies, including anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-Tg) and few others. In spite of that anti-TPO can also be found in sera of about 10 % of normal adults, this type of autoantibodies is more likely to be of pathogenic importance than other autoantibodies as it can fix complement and may directly damage thyroid cells [17].

Few studies have investigated the prevalence of AITD in infertile women [18]; however, the interpretation of these data is difficult because of the selection bias, the retrospective setting and the types of control population. Results from this study (Table 5) revealed no significant difference of anti-TPO among all study groups which was inconsistent with one other study [19]. However, in all studies (including the current one), the underlying pathogenic mechanisms linking AITD with infertility do not remain largely speculative, as neither animal models nor *in vitro* data on this issue are available.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Shin JJ, Jee BC, Kim H, Kim SH. Major Disease Prevalence and Menstrual Characteristics in Infertile Female Korean Smokers. J Korean Med Sci. 2017 Feb; 32(2):321-328.
2. Rajalakshmi R, Kalaivani S. Prevalence of asymptomatic infections in sexually transmitted diseases attendees diagnosed with bacterial vaginosis, vaginal candidiasis, and trichomoniasis. Indian J Sex Transm Dis. 2016 Jul-Dec; 37(2):139-142.
3. Comstock IA, Diaz-Gimeno P, Cabanillas S, Bellver J, Sebastian-Leon P, Shah M, Schutt A, Valdes CT, Ruiz-Alonso M, Valbuena D, Simon C, Lathi RB. Does an increased body mass index affect endometrial gene expression patterns in infertile patients? A functional genomics analysis. Fertil Steril. 2017 Mar; 107(3):740-748.e2.
4. Oiwa A, Minemura K, Nishio SI, Yamazaki M, Komatsu M. Implications of thyroid autoimmunity in infertile women with subclinical hypothyroidism in the absence of both goiter and anti-thyroid antibodies: lessons from three cases. Endocr J. 2019 Feb 28;66(2):193-198. doi: 10.1507/endocrj. EJ18-0350. Epub 2018 Dec 18.
5. Kumar S, Mahesh DM, Vignesh G, Sagili H, Dhanapathi H, Hamide A. Congenital hypothyroidism presenting as menorrhagia in adulthood. J Assoc Physicians India. 2013 ep; 61(9):660-1.
6. Plowden TC, Schisterman EF, Sjaarda LA, Zarek SM, Perkins NJ, Silver R, Galai N, DeCherney AH, Mumford SL. Subclinical Hypothyroidism and Thyroid Autoimmunity Are Not Associated With Fecundity, Pregnancy Loss, or Live Birth. J Clin Endocrinol Metab. 2016 Jun; 101(6):2358-65.
7. Feldthusen AD, Pedersen PL, Larsen J, Toft Kristensen T, Ellervik C, Kvetny J. Impaired Fertility Associated with Subclinical Hypothyroidism and Thyroid Autoimmunity: The Danish General Suburban Population Study. J Pregnancy. 2015; 2015:132718.
8. Kakuno Y, Amino N, Kanoh M, Kawai M, Fujiwara M, Kimura M, Kamitani A, Saya K, Shakuta R, Nitta S, Hayashida Y, Kudo T, Kubota S, Miyauchi A. Menstrual disturbances in various thyroid diseases. Endocr J. 2010; 57(12):1017-22.
9. Van Voorhis, B.J. & Stovall, D.W. Autoantibodies and infertility: a review of the literature. Journal of Reproductive Immunology, 1997; 33, 239–256.

10. Jokar TO,* Fourman LT, Lee H, Mentzinger K, and Fazeli PK. Higher TSH Levels Within the Normal Range Are Associated With Unexplained Infertility. J Clin Endocrinol Metab. 2018 Feb; 103(2): 632–639.
11. Roupa Z., Polikandrioti M, Sotiropoulou P, Faros E, Koulouri A, Wozniak G, Gourni M. Causes of infertility in women at reproductive age. Health Science Journal, 2009; 3(2): 80-87.
12. Melanie H J, Penelope P H, Lyndsey A D, Juliana W M, James S Kesner, Jessica B S, Metrecia L T and Michele M. Thyroid hormones and menstrual cycle function in a longitudinal cohort of premenopausal women. Paediatr Perinat Epidemiol. 2018 May; 32(3): 225–234.
13. Binita G, Suprava P, Mainak C, et al. Correlation of prolactin and thyroid hormone concentration with menstrual patterns in infertile women . J. Reprod. Infertil. 2009; 10 (3): 207 – 212.
14. Nakajima K, Yamazaki K, Yamada E, Kanaji Y, Kosaka S, Sato K, Takano K . Amiodarone stimulates interleukin-6 production in cultured human thyrocytes, exerting cytotoxic effects on thyroid follicles in suspension culture. Thyroid, 2001; 11:101–109.
15. Guven S et al .Serum IL-6 Level may have role in pathophysiology of unexplained infertility. American Journal of Reproductive Immunology, 2009; 62(4): 564-568.
16. Turankar S, Sonone K, Turankar A. Hyperprolactinaemia and its comparison with hypothyroidism in primary infertile women. J Clin Diagn Res. 2013 May; 7(5):794-6.
17. Poppe K, Glinoer D, Van Steirteghem A, Tournaye H, Devroey P, Schiettecatte J, Velkeniers B. Thyroid dysfunction and autoimmunity in infertile women. Thyroid. 2002; 12(11): 997-100.
18. Janssen O E, Mehlmaner N, Hahns S et al. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. Europ J Endocrinol. 2004; 150: 363-396.
19. Geva E, Lessing JB, Lerner-Geva L, Azem F, Yovel I, Amit A. The presence of antithyroid antibodies in euthyroid patients with unexplained infertility and tubal obstruction. Am J Reprod Immunol, 1997; 37: 184–186.