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TSH and AMH in Infertile Women

Hazem Mohamed Sammour, Wessam Magdi Abuelghar,
Nermine Essam El-Din Abd El-Salam, Haitham Hassan Ahmed
Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University

ABSTRACT

Introduction: Infertility is defined as the inability of a couple to achieve pregnancy over an average period of one year (in women under 35 years of age) or 6 months (in women above 35 years of age) of unprotected sexual intercourse. Infertility can be due to female, male reasons or both. It can be either primary or secondary. **Aim of the work:** The aim of this study is to evaluate the association between thyroid function and serum AMH levels. **Methodology:** Type of the study: this study was conducted a case control study. Study site: This study was carried out at Ain-Shams maternity hospital (outpatient infertility clinic). Duration of study: In the period between December 2015 and December 2016. Patients and study design: According to the sample size calculation by using the IBM© Sample Power© Software (IBM© Corp., Armonk, NY, USA), the study performed on 128 women divided into 2 equal groups as follows:
Group "1" (study group): 64 infertile women at reproductive age (20-35) years.
Group "2" (Control group): 64 normal fertile women aged (20– 35) years.
Result: TSH and patient age that were strongly correlated with AMH levels in 26 post-matched infertile patients using multivariate logistic regression. Both TSH levels and patient age significantly impacted AMH levels in infertile patients. **Conclusion:** AMH levels were inversely correlated with TSH levels in infertile women of reproductive age. **Recommendations:** The study should be done using larger sample sizes in a multicenter trial including both urban and rural areas to validate results. Autoimmune thyroid antibodies (thyroglobulin antibody and thyroid peroxidase antibody) could be assessed with TSH, FT4, FT3 and AMH as there is a strong association between infertility and autoimmune thyroid antibodies.
Keywords: TSH and AMH, Thyroid function, Infertile Women**.**

INTRODUCTION

Infertility is defined as the inability of a couple to achieve pregnancy over an average period of one year (in women under 35 years of age) or 6 months (in women above 35 years of age) of unprotected sexual intercourse. Infertility can be due to female, male reasons or both. It can be either primary or secondary [(1)].

Thyroid dysfunction and autoimmune thyroiditis are known adverse risk factors for pregnancy as well as fertility, regardless of the presence of disease in women of reproductive age [(2)]. In particular, hypothyroid women are at an increased risk of menstrual disorders and infertility because of altered peripheral estrogen metabolism, hyperprolactinaemia and abnormal release of gonadotropin-releasing hormone [(2)].

The prevalence of subclinical hypothyroidism characterized by aberrant high serum thyroid-stimulating hormone (TSH) levels with normal free thyroxin (FT4) levels in infertile women are reported to be approximately 20% and it is a primary cause of subfertility [(3)]. Indeed, average TSH levels in infertile women were reportedly higher than those in normal fertile women. And elevated serum TSH levels were associated with diminished ovarian reserve in infertile patients [(4)].

Moreover, although levothyroxine replacement therapy for subclinical hypothyroidism in infertile patients remains debatable, thyroxin supplementation may improve fertility to successful pregnancy [(5)].

This data suggests that hypothyroidism is strongly correlated with infertility [(5)].

On the other hand, female fecundity decreases with increasing age, primarily because of decreased ovarian function. Anti-mullerian hormone (AMH) is a dimeric glycoprotein belonging to the transforming growth factor-beta (TGF-B) super family, which act on tissue growth and differentiation. It is produced by the granulosa cells from pre-antral and small antral follicles. Ovarian research after oophorectomy showed that serum AMH levels were closely correlated with the number of primordial follicles; therefore, AMH is a suitable biomarker of ovarian age in women of reproductive age [(6)].Expectedly, ovarian function may be affected by impaired thyroid function; however this association has not been studied enough.

AIM OF THE WORK

The aim of this study is to evaluate the association between thyroid function and serum AMH levels.

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PATIENTS AND METHODS

Type of the study: This study was conducted a case control study.

Study site: This study was carried out at Ain-Shams maternity hospital (outpatient infertility clinic).

Duration of study: In the period between December 2015 and December 2016.

Patients and study design:

According to the sample size calculation by using the IBM© Sample Power© Software (IBM© Corp., Armonk, NY, USA), the study performed on 128 women divided into 2 equal groups as follows:

- ***Group "1" (study group)****:* 64 infertile women at reproductive age (20-35) years.
- ***Group "2" (Control group):*** 64 normal fertile women aged (20– 35) years.

Inclusion criteria

1) Age: 20-35 years.
2) BMI: 18-30 kg/m2
3) Diagnosed as primary infertility.
4) Duration of marriage more than 1 year.
5) Controls should be normal fertile women aged 20– 35 years had no history of treatment for infertility or thyroid disorders.

Exclusion criteria

1) Age: above 35 years old.
2) BMI: above 30 kg/m2
3) Women with ovarian dysfunction (PCOS, post ovarian surgery).
4) Treated thyroid dysfunction (Autoimmune thyroiditis, hypothyroidism).

All selected women for the study had giving an informed consent and were subjected to the following:

1) **History:**
   a) ***Personal history:*** Name, age, address, date of marriage
   b) ***Medical history:*** Anemia, diabetes mellitus, thyroid dysfunction, hypertension.
   c) ***Menstrual history:*** Date of last menstrual period, regularity, rhythm, duration of menses.
   d) ***Past history:*** Surgical operation as thyroidectomy and oophorectomy.
   e) ***Family history:*** Diabetes mellitus, hypertension, autoimmune throiditis, infertility, PCOS, premature ovarian failure.
2) **Examination:**
   a) ***General examination:*** Weight, hieght, blood pressure, pulse, temperature.
   b) Pelvic examination:
   - Inspection of external genitalia and vaginal discharge
   - Palpation of abnormal tenderness and swelling
3) **Investigation:**
   a) Serum Anti-Mullerian hormone level.
   b) Serum TSH level.
   c) Serum freeT3 level.

The study was done after approval of ethical board of Ain-Shams university and an informed written consent was taken from each participant in the study.

METHODS

Specimen collection and preparation

1. Plasma samples collected in tubes containing EDTA. Heparin or oxalate may interfere with test procedure and should be avoided.
2. Samples collected between 9 a.m. and 11 a.m.
3. Non-fasting blood samples drawn from the cubital vein of women in the supine position.
4. Collected blood samples done by universal precaution for venipuncture.
5. Allow samples to clot for 1 hour before centrifugation.
6. Prior to use, specimens should be capped and stored up to 48 hours at 2~8˚C. For longer storage, freeze the specimens at -20˚C. Thawed samples must be mixed prior to testing. Multiple freeze-thaw cycles should be avoided.

TSH level measurement using ELISA technique:

The assay system utilizes a unique monoclonal antibody directed against antigenic determinant on the intact TSH molecule.

Laboratory reference ranges for TSH and free T3 in the present study were 0.4-6 mIU/L [(7)] and 1.4-4.2 pg/ml [(8)] respectively according to used kit's references.

Anti-Müllerian Hormone level measurement using ELISA technique:

Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) using (AMH Gen II ELISA kit; Immunotech A Beckman Coulter Company, Brea, CA, U.S.A).

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

Statistical Methods

Data were analyzed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY, USA) and MedCalc© version 15 (MedCalc© Software bvba, Ostend, Belgium).

Normality of numerical data distribution was examined using the D'Agostino-Pearson test. Normally distributed numerical variables were presented as mean ± SD and intergroup differences were compared using the unpaired t test.

Receiver-operating characteristic (ROC) curve analysis was used to examine the value AMH, TSH, or FT3 for discrimination between cases and controls. The area under the various ROC curves (AUC) was compared using the DeLong method.

Correlations were examined using the Pearson product-moment correlation.

RESULTS

Table (1): Demographic of cases and controls

	Cases (n=64)		Controls (n=64)				
Variable	**Mean**	**SD**	**Mean**	**SD**	**t**	**df**	**p-value¶**
Age (years)	25.4	4.5	27.5	3.5	-2.901	119.325	**0.004**
BMI (kg/m^2)	21.2	2.6	24.5	3.0	-6.842	126	**<0.001**

t, t statistic; df; degree of freedom.

¶Unpaired t test.

Mean of age of patients included in the study group was 25.4 years while mean of age of control group was 27.5 years with p-value 0.004.

There was statistically a significance difference between the 2 groups as regard age.

Mean of BMI of patients included in the study group was 21.2 kg/m2 while mean of BMI of control group were 24.5 kg/m2 with p-value <0.001.

There was statistically a significance difference between the 2 groups as regard BMI.

Table (2): Results of hormonal assay in cases and controls

	Cases (n=64)		Controls (n=64)				
Variable	**Mean**	**SD**	**Mean**	**SD**	**t**	**df**	**p-value¶**
AMH (ng/ml)	0.32	0.07	2.22	0.32	-42.652	94.933	**<0.001**
TSH (mIU/l)	3.26	0.65	2.66	0.39	3.101	71.507	**0.003**
FT3 (pg/ml)	2.19	0.38	2.16	0.37	0.459	126	0.647

Mean of AMH of patients included in the study group was 0.32 ng/ml while mean of AMH of control group was 2.22 ng/ml with p-value <0.001.

There was statistically a significance difference between the 2 groups as regard AMH.

Mean of TSH of patients included in the study group was 3.26 mIU/l while mean of TSH of control group was 2.66 mIU/l with p-value 0.003.

There was statistically a significance difference between the 2 groups as regard TSH.

Mean of FT3 of patients included in the study group was 2.19pg/ml while mean of FT3 of control group was 2.16 pg/ml with p-value 0.647.

There was no statistically a significance difference between the 2 groups as regard FT3.

Table (3): Baseline characteristics of women with normal fertility and infertility.

Pre-matching				**Post-matching**		
	Fertility	**Infertility**	**p**	**Fertility**	**Infertility**	**p**
	Control group	**Case group**		**Control group**	**Case group**	
Patient No	64	64		26	26	
Age (years)	25.4 ±4.5	27±3.5	**0.004**	24.5±4.2	26.1±4.0	**0.004**
BMI (kg/m^2)	21.2 ±2.6	24.5±3.0	**<0.001**	20.3±2.2	20.2±2.1	0.94
AMH (ng/ml)	0.32 ±0.07	2.22±0.32	**<0.001**	0.29±0.06	0.12±0.02	**0.003**
TSH (mIU/l)	3.26±0.65	2.66±0.39	**0.003**	2.92±0.6	2.17±0.43	**0.004**
FT3 (pg/ml)	2.19±0.38	2.16±0.37	0.647	1.89±0.37	1.12±0.25	0.966

N.B: Infertile patients were matched with normal fertile women by age ± 1 year and by BMI ±1.0 kg/m^2

We used multivariate logistic regression model to evaluate independent variables strongly correlated with infertility in post-matched patients and included the following covariates in the model: patient age, BMI and AMH, TSH and FT3.

Both TSH levels and patient age significantly impacted AMH levels in infertile patients.

There was statistically a significance difference between the 2 groups as regard AMH levels in post matched infertile patients.

There was statistically a significance difference between the 2 groups as regard TSH levels in post matched infertile patients.

Table (4): Comparison of the receiver-operating characteristic (ROC) curves for discrimination between cases and controls using the AMH, TSH, or FT3 level

Predictor	**AUC**	**95% CI**		
AMH	1.0	0.972 to 1.000		
TSH	0.523	0.433 to 0.612		
FT3	0.519	0.429 to 0.608		
Comparison	**Difference between AUCs**	**95% CI**	**z statistic**	**p-value¶**
AMH versus TSH	0.477	0.370 to 0.584	8.764	**<0.0001**
AMH versus FT3	0.481	0.380 to 0.581	9.379	**<0.0001**
TSH versus FT3	0.004	-0.113 to 0.120	0.0615	0.951

AUC, area under the ROC curve. ¶DeLong method

Table "4" shows there were statistically significant differences between the AUC of the AMH versus TSH and AMH versus FT3 as P values<0.0001.

There were no statistically significant differences between the AUC of the TSH versus FT3 as p values =0.951.

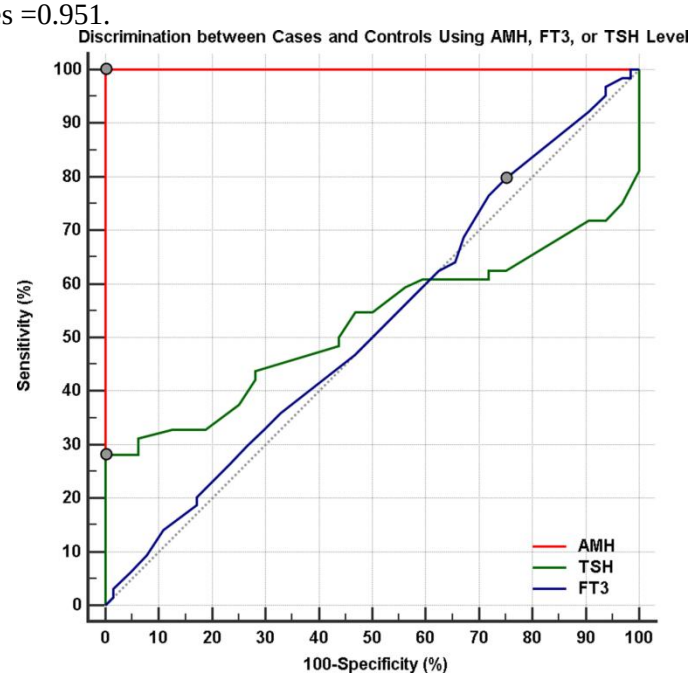

Figure (1): Comparison of the receiver-operating characteristic (ROC) curves for discrimination between cases and controls using the AMH, TSH, or FT3 level.

Table (5): Correlation among AMH, FT3, TSH, age, and BMI in the whole study population, cases, or controls.

		AMH		TSH		FT3		Age		BMI	
		r	**p-value**	**r**	**p-value**	**R**	**p-value**	**r**	**p-value**	**r**	**p-value**
All (n=128)	**AMH**	-	-	**-0.239**	**0.007**	0.067	0.450	**0.222**	**0.012**	**0.504**	**<0.0001**
	TSH	**0.239**	**0.007**	-	-	**0.319**	**<0.001**	-0.063	0.483	-0.097	0.275
	FT3	0.067	0.450	**0.319**	**<0.001**	-	-	-0.039	0.660	-0.055	0.534
Cases (n=64)	**AMH**	-	-	-0.112	0.378	0.005	0.968	-0.103	0.417	0.121	0.342
	TSH	0.112	0.378	-	-	**0.253**	**0.043**	0.001	0.992	0.064	0.614
	FT3	0.005	0.968	0.253	0.043	-	-	-0.080	0.532	-0.140	0.269
Controls (n=64)	**AMH**	-	-	**0.567**	**<0.0001**	**0.677**	**<0.0001**	-0.077	0.548	-0.053	0.680
	TSH	**0.567**	**<0.0001**	-	-	**0.817**	**<0.0001**	0.022	0.866	0.048	0.705
	FT3	**0.677**	**<0.0001**	**0.817**	**<0.0001**	-	-	0.035	0.786	0.050	0.693

r, Pearson correlation coefficient.

Table "5" show correlation between AMH and TSH in the whole study population as p value 0.007 and control group as p value<0.0001.

There was correlation between TSH and FT3 in the whole study population as p value <0.0001 and control group as p value<0.0001.

There was correlation between FT3 and AMH in the control group as p value <0.0001.

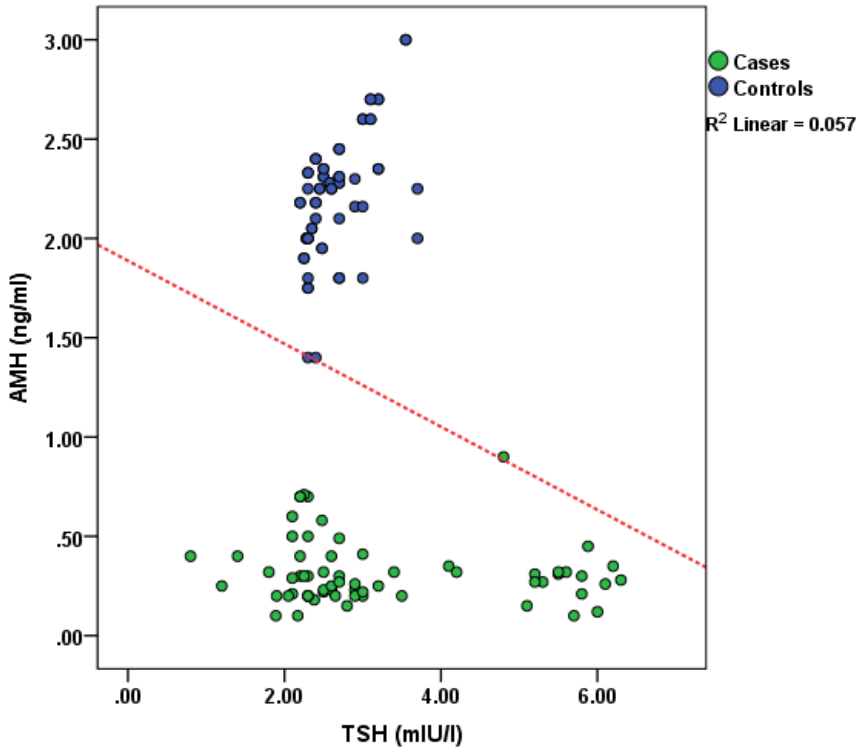

Figure (2): Scatter plot showing Inverse correlation between AMH and TSH.

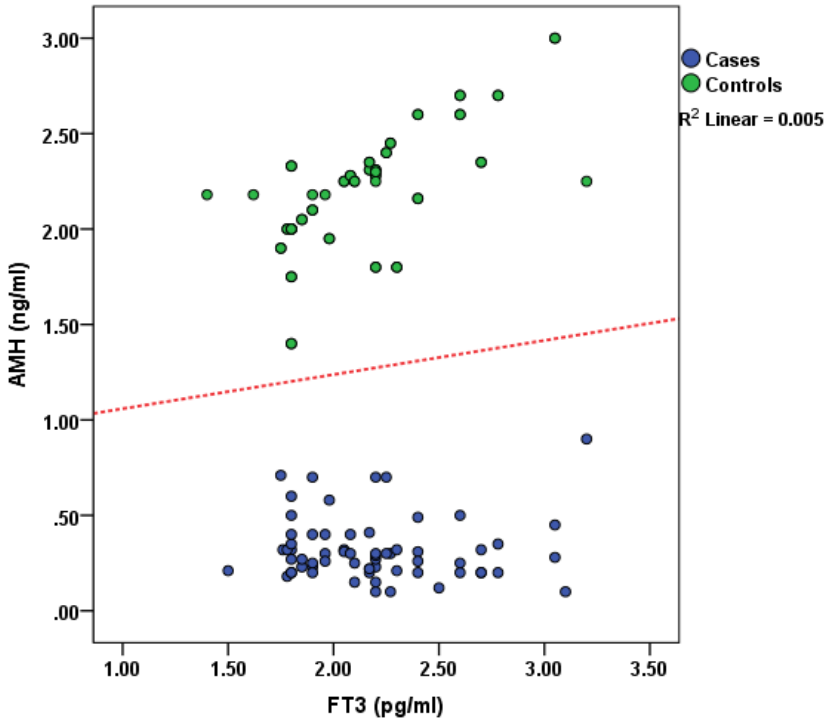

Figure (3): Scatter plot showing the correlation between AMH and FT3.

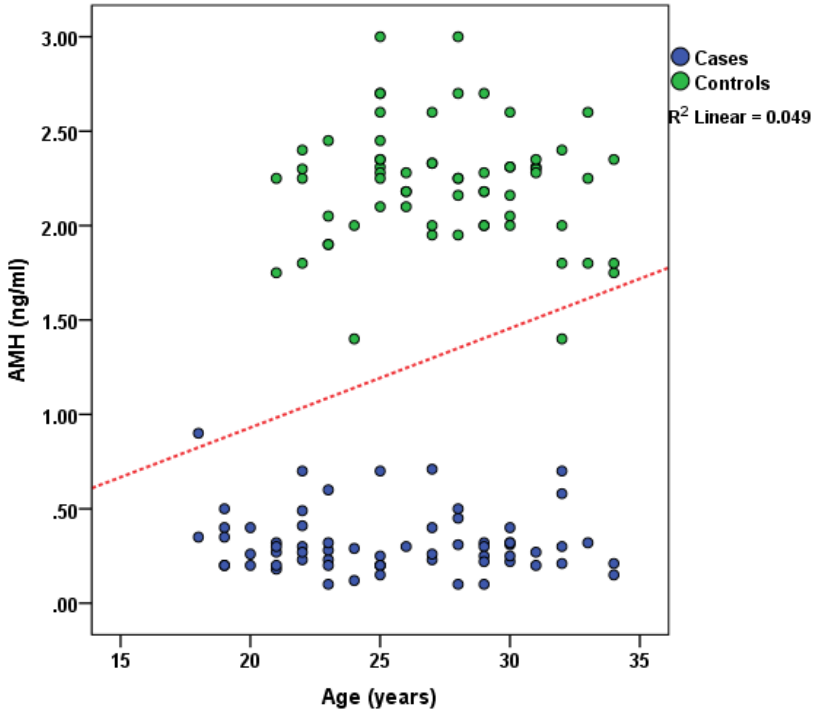

Figure (4): Scatter plot showing the correlation between AMH and age.

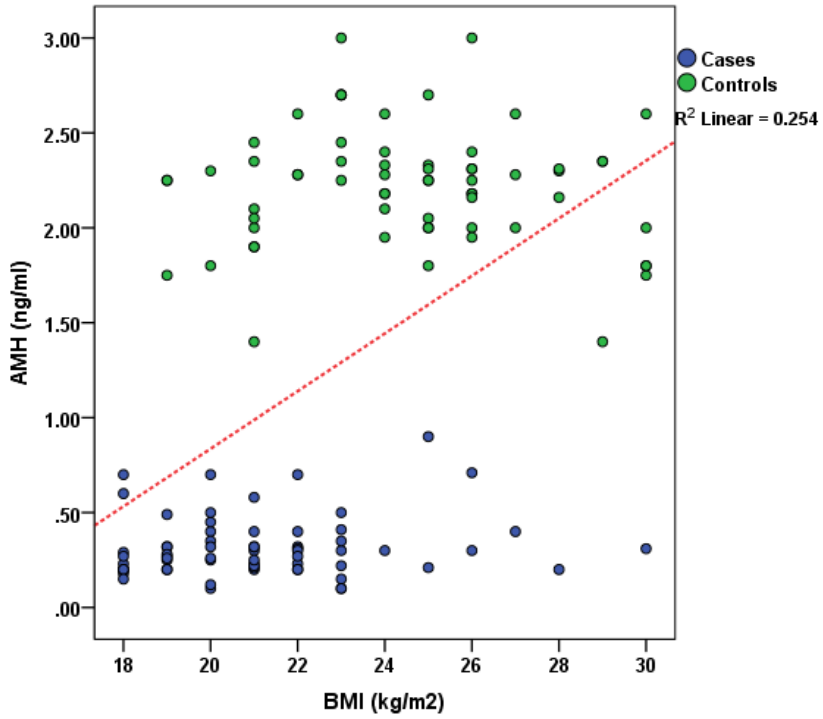

Figure (5): Scatter plot showing the correlation between AMH and BMI.

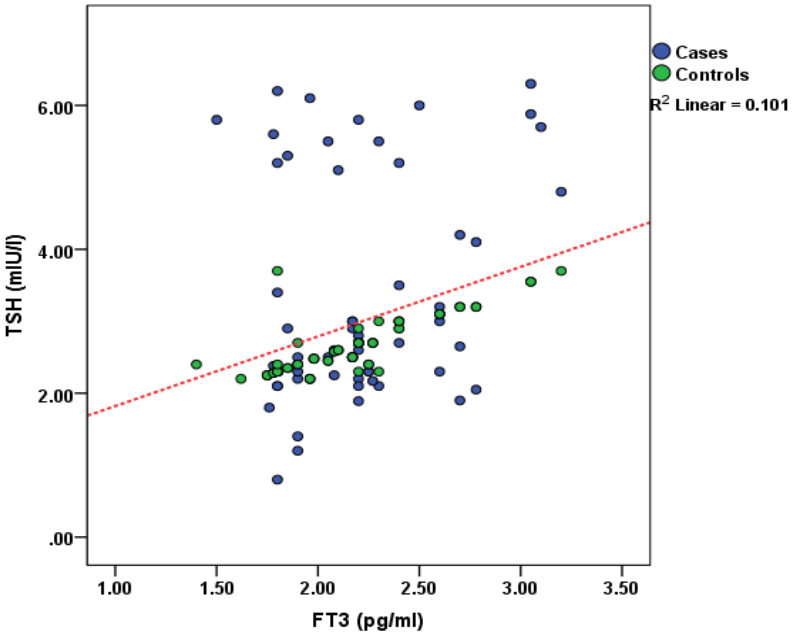

Figure (6): Scatter plot showing the correlation between TSH and FT3.

DISCUSSION

The current study showed there are statistically significance differences between the infertile women (case group) and the normal fertile women (control group) as regard Age 25.4 ± 4.5 and 27.5 ± 3.5 respectively (p-value<0.05).

Also, there are statistically significance differences between the infertile women (case group) and the normal fertile women (control group) as regard BMI 21.2 ± 2.6 and 24.5 ± 3.0 respectively (p-value<0.05). These results are consistent with the study performed by ***Krassas et al.*** [2] where was statistically significance difference between the infertile women (case group) and the normal fertile women (control group) as regard age and BMI.

On the other hand these results were not consistent with the study performed by ***Kuroda et al.*** [14] who found that no statistically significance

difference detected between the case group and the control group as regard age and BMI.

Michalakis et al. [4] study was inconsistent with our study, where was no statistically significance difference between the case group and the control group as regard age and BMI. This may be due to difference in inclusion criteria selection between the studies. The present study showed AMH levels were significantly lower in infertile women (case group) 0.32±0.16 ng/ml than in normal fertile women (control group) 2.22±0.32 ng/ml (p-value <0.001) with cut off value ≤0.9 ng/ ml.

Therefore, it was reasonable that a decrease in AMH levels was strongly correlated with infertility.

These results cope with study performed by ***Kuroda et al.***[14] who found that mean AMH levels in case group and control group were 2.60 ± 2.0ng/ml, and 4.49 ± 2.0ng/ml respectively, where the difference was highly statistically significant (p-value <0.001) with cut off value ≤3.5 ng/ml.

Also these results are supported by the study performed by ***Krassas et al.,***[2] who found that mean AMH levels in case group and control group was statistically significant difference (p-value <0.05).

Also these results are supported by the study performed by ***Gerhard et al.***[15]who found that that mean AMH levels in case group and control group was statistically significant difference (p-value <0.05). The present study showed mean TSH levels in case group and control group using unpaired t test as next, 3.26 ± 1.49mIU/l, and 2.66 ± 0.39mIU/l, respectively, where the difference was statistically significant (p-value<0.003) with cut off value >3.7 mIU/l . Finally we evaluated covariates (TSH and patient age) that were strongly correlated with AMH levels in 26 post-matched infertile patients using multivariate logistic regression. Both TSH levels and patient age significantly impacted AMH levels in infertile patients.

Post-matched women showed that both BMI, FT3 were not correlated with AMH levels,

In view of the results of this study, serum AMH levels in infertile patients were inversely correlated with patients' age and TSH levels. This finding has consistently been reported in numerous studies (2, 14).

Kuroda et al. [14] examined serum AMH and TSH levels in 67 infertile patients and 27 normal fertile women aged between 20-35 years without impact factors on thyroid and ovarian functions between 2012-2013.

They reported an AMH levels were significantly lower in infertile patients than in normal fertile women (p< 0.001)

They also confirm that serum AMH levels in infertile patients were inversely correlated with patients' age and TSH levels.

On the other hand, the result of our study showed that serum AMH levels in infertile patients were directly correlated with patients BMI and FT3 level. This finding has consistently been reported in numerous studies [2, 14].

These data revealed an inverse correlation between AMH and TSH levels in infertile women with decreased ovarian function without other factor affecting thyroid and ovarian functions.

SUMMARY AND CONCLUSION

In this study, Blood samples were collected from 128 women to measure serum concentrations of AMH and TSH. The levels of AMH and TSH were compared between infertile women and normal fertile women to evaluate the association between thyroid function and serum AMH levels, there were also statistically significance differences between the 2 groups as regard Age, BMI, AMH and TSH.

On the other hand there were no statistically significance differences between the 2 groups as regard FT3 level.

In this study, we found elevated serum thyroid-stimulating hormone was associated with decreased anti-Müllerian hormone in infertile women of reproductive age.

RECOMMENDATIONS

- The study should be done using larger sample sizes in a multicenter trial including both urban and rural areas to validate results.
- Autoimmune thyroid antibodies (thyroglobulin antibody and thyroid peroxidase antibody) could be assessed with TSH, FT4, FT3 and AMH as there is a strong association between infertility and autoimmune thyroid antibodies.

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