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Correlation Between Anti Thyroid Peroxidase Antibodies and Recurrent Pregnancy Loss

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Abstract

Aims & objectives: To investigate the potential correlation between anti-TPO antibodies and recurrent pregnancy loss.

To Investigate the Relationship Between Anti-TPO Antibodies and maternal parameters Including Age, Blood group, BMI, Thyroid function tests and Hemoglobin Levels in Women.

Materials & methods: The study was conducted on patients attending the OBSTETRICS AND GYNAECOLOGY OPD of Acharya Shri Chander College of Medical Sciences and Hospital, Jammu. About 100 cases were selected on the basis of simple random sampling method. This study was a prospective observational study. Under quality control and safety procedures for sample collection ,5 ml of venous blood sample was collected in vacutainer tubes. Serum samples were sent for serum T3, serum T4, serum TSH and anti-Thyroid peroxidase antibody. RECURRENT PREGNANCY LOSS -Defined as 2 or more spontaneous pregnancy losses from the time of conception till the age of viability (not necessarily consecutive). This definition included pregnancy losses both after spontaneous conception and assisted reproductive techniques. Statistical analysis and graphical representations were conducted using the IBM statistical package for social sciences (SPSS version 21).

Results: Women with TPO (Thyroid Peroxidase) positive results have a higher mean age (35.28 years) compared to women with TPO negative results (32.40 years). The average serum FT3 level for women with TPO positivity is 6.93 pmol/L. The average serum FT3 level for women with TPO negativity is 7.82 pmol/L. TPO positive women: Mean FT4 = 6.96 pmol/L and

TPO negative women: Mean FT4 = 7.32 pmol/L, SD = 2.06 pmol/L. The average serum TSH level for women with TPO negativity is 4.98 µIU/mL. This indicates a statistically significant difference in mean serum TSH levels between the TPO positive and TPO negative groups.

Conclusion: Increased Anti-TPO Antibody Levels with Age: TPO antibody levels tend to rise in older women, suggesting a potential link between advancing age and increased thyroid autoimmunity. Elevated anti-TPO antibody levels are significantly associated with a higher number of spontaneous abortions. Elevated TPO antibodies were also seen in women with high BMI.

Keywords: Folliculogenesis, thyroid, TPOAb, vacutainer tubes

Introduction

Pregnancy loss is defined as spontaneous demise of a pregnancy before the fetus reaches the age of viability. It includes all the pregnancy loss from the time of conception until 28 weeks of gestation (India),24 weeks(UK),20 weeks(USA). Recurrent pregnancy loss has a significant emotional impact on the mother and the couple. It creates anxiety in the couple regarding the future and represents the loss of a baby and the hopes and plans invested in the child (Farren et al.,2020).[1]

Recurrent "Early" Pregnancy Loss (REPL) is the loss of two or more pregnancies before 10 weeks of gestational age. It has been reported that RPL affects approximately 1% to 2% of women, when defined as three consecutive pregnancy losses prior to 20 weeks from the last menstrual period. Larsen reported a prevalence of 0.8% to 1.4% if only clinical pregnancy loss (confirmed by ultrasound and/ or histology) are included. Adding biochemical losses increases the prevalence to 2% to 3%.[2- 4]

Thyroid disorders are one of the most frequent endocrine conditions in women of reproductive age group (Ramprasad M et al., 2012).[5] Thyroid disorders and increased thyroid peroxidase (TPO) antibodies (TPOAb) are associated with disturbed folliculogenesis, spermatogenesis, fertilization and embryogenesis, supporting an important role for thyroid hormone disorders and thyroid autoimmunity in subfertility and pregnancy loss (Vissenberg et al., 2015).[6] Thyroid autoimmunity describes the presence of circulating anti-thyroid autoantibodies that are targeted against the thyroid, with or without thyroid dysfunction. Various antibodies can be present like anti-thyroglobulin (TGAb), TPOAb or anti-TSH receptor (TSHr-Ab) autoantibodies. In women with RPL, thyroid peroxidase autoantibodies (TPOAb) are mostly studied, and shown to be more relevant than other antibodies against the thyroid gland. The prevalence of TPOAb is 8-14% in women of reproductive age. TPOAb predispose to hypothyroidism, but the majority of women having TPOAb are euthyroid. Many studies have linked the presence of anti TPO-Ab to adverse maternal and fetal outcomes in pregnancy, in particular pregnancy loss and pre-term birth, even in the absence of thyroid dysfunction (Stricker RB et al., 2000).[5- 8]

Given the lack of sufficient studies examining the correlation between anti-TPO antibodies and recurrent pregnancy loss, this study aims to investigate the potential correlation between anti-TPO antibodies and recurrent pregnancy loss.

Materials & methods

The study was conducted on patients attending the OBSTETRICS AND GYNAECOLOGY OPD of Acharya Shri Chander College of Medical Sciences and Hospital, Jammu. About 100 cases were selected on the

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basis of simple random sampling method. This study was a prospective observational study. This study was done after being approved by the Institutional Ethical Committee of the hospital. A written informed consent was taken from all the subjects, who were included in the study after explaining to them the nature and purpose of the study. Under quality control and safety procedures for sample collection, 5 ml of venous blood sample was collected in vacutainer tubes. Serum samples were sent for serum T3, serum T4, serum TSH and anti-Thyroid peroxidase antibody.

Parameters	References
Thyroid Stimulating Hormone(TSH)	0.270-4.20 µIU/ml
Free Thyroxine (T4)	3.10-6.80 pmol/L
Free Triiodothyronine (fT3)	12.00-22.00 pmol/L
Anti TPO antibodies	0 -34 IU/ml

TPO levels >34 IU/ml were considered abnormal and these women were considered anti Thyroid peroxidase antibody positive. The patients were enrolled in the study according to the following inclusion and exclusion criteria

Inclusion Criteria

- Age between 18 and 40 years.
- Patients with history of recurrent pregnancy losses (history of two or more pregnancy losses, not necessarily consecutive) presenting to the OPD in Acharya Shri Chander College of medical sciences and hospital.

Exclusion Criteria

- Refusal to participate in the study
- Anatomical uterine defects
- Pregnancy losses due to trauma
- Pregnancy losses due to infections like TORCH
- Pregnancy losses due to any chromosomal abnormalities
- Age less than 18 or more than 40 years

Recurrent Pregnancy Loss - Defined as 2 or more spontaneous pregnancy losses from the time of conception till the age of viability (not necessarily consecutive). This definition included pregnancy losses both after spontaneous conception and assisted reproductive techniques. However, it excludes cases of implantation failure, ectopic and molar gestation, thus covering only clinically recognized pregnancies. Statistical analysis and graphical representations were conducted using the IBM statistical package for social sciences (SPSS version 21). The frequency and percentage of qualitative variables were calculated. Descriptive data was expressed in percentages and proportions and continuous data were expressed in mean and standard deviation and compared by using independent student's t test .Non parametric data were compared by using chi-square test and Fisher's exact test. A p value less than 0.05 was considered statistically significant. Pearson's correlation coefficient was used to calculate correlation between different variables.

Results

Table 1: Comparison of mean age of women with or without TPO positive

Age (in years)	TPO positive		TPO negative	
	N	%	N	%
26-30	7	25	27	37.5

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31-35	12	42.86	24	33.33
36-40	9	32.14	21	29.17
Total	28	100	72	100
Mean ±Sd	35.28 ± 3.74		32.40 ± 3.95	
Over all mean age	32.65 ± 3.91			
p-value	0.0013*			

Women with TPO (Thyroid Peroxidase) positive results have a higher mean age (35.28 years) compared to women with TPO negative results (32.40 years). Women with TPO positive results have a higher mean maternal age (36.6 years) compared to those with TPO negative results (34.4 years). Among women with TPO positivity, 21.43% experienced 2 or fewer spontaneous abortions, while 78.57% had none. Among women with TPO negativity, 20.83% experienced 2 or fewer spontaneous abortions, while 79.17% had none.

Table 2: Comparison of BMI of women with or without TPO positive

BMI	Mean	Sd	p-value
TPO positive	27.54	4.96	0.007*
TPO negative	25.23	3.19	

Women with TPO positivity have a higher mean BMI (27.54) compared to those who are TPO negative (25.23), with a greater variability in the positive group (SD = 4.96 vs. 3.19). The significant p-value of 0.007 confirms a notable difference between the two groups.

Table 3: Comparison of Serum FT3 of women with or without TPO positive

Serum FT3	Mean	Sd	p-value
TPO positive	6.93	3.25	0.128(N.S)
TPO negative	7.82	2.31	

Women with TPO positivity have a lower average serum FT3 level (6.93 pmol/L) compared to TPO negative women (7.82 pmol/L). Despite the greater variability in the TPO positive group, the p-value of 0.128 indicates that this difference is not statistically significant.

Table 4: Comparison of Serum FT4 of women with or without TPO positive

Serum FT4	Mean	Sd	p-value
TPO positive	6.96	2.19	0.458
TPO negative	7.32	2.06	(N.S)

The mean serum FT4 levels are slightly lower in TPO positive women (6.96 pmol/L) compared to TPO negative women (7.32 pmol/L). However, the p-value is 0.458, which is greater than 0.05, indicating that this difference is not statistically significant. This means there is no strong evidence to suggest that there is a significant difference in serum FT4 levels between women who are TPO positive and those who are TPO negative in this sample. The observed difference in mean FT4 levels could be due to random variation rather than a true difference related to TPO status.

Table 5: Comparison of Serum TSH of women with or without TPO positive

Serum TSH	Mean	Sd	p-value
TPO positive	10.55	12.39	0.006*
TPO negative	4.98	7.24	

Women with TPO positivity have a significantly higher average serum TSH level (10.55 µIU/mL) compared to those who are TPO negative (4.98 µIU/mL). This finding suggests a clear association between TPO positivity and elevated TSH levels, highlighting potential implications for thyroid function.

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Table 6: Comparison of APLA antibodies status of women with or without TPO positive

APLA Antibodies	Present		Absent		p-value
	N	%	N	%	
TPO positive	6	21.42	22	78.58	0.017 (S)
TPO negative	4	5.56	68	94.44	

TPO positive women demonstrate a significantly higher prevalence of anti-cardiolipin antibodies (21.42%), anti-B2 GP 1 antibodies (17.85%), and lupus anticoagulant antibodies (21.42%) compared to TPO negative women. The p-values for these associations (0.017 and 0.023) indicate statistical significance, suggesting that TPO positivity is linked to an increased risk of autoimmune disorders and related complications.

Table 7: Comparison of Blood group of women with or without TPO positive

Blood group	TPO positive		TPO negative		p-value
	N	%	N	%	
O+	9	32.14	10	13.89	0.036*
O-	2	7.14	10	13.89	0.57
A+	2	7.14	15	20.83	0.10
A_	1	3.57	6	8.33	0.40
AB+	1	3.57	6	8.33	0.40
AB-	2	7.14	11	15.28	0.10
B+	1	3.57	13	18.06	0.08
TOTAL	28	100	72	100	100

The analysis of blood group distribution reveals a significant difference in the prevalence of the O+ blood group among women with TPO positivity (32.14%) compared to those with TPO negativity (13.89%), supported by a p-value of 0.036. This suggests a notable association between TPO positivity and the O+ blood type. However, for all other blood groups—O-, A+, A-, AB+, AB-, and B+—the p-values exceeded the conventional threshold of 0.05, indicating no statistically significant differences. These findings highlight the unique association of the O+ blood group with TPO positivity while suggesting similar distributions for other blood types.

Discussion

We demonstrated a significant association between the presence of anti-TPO antibodies and recurrent pregnancy loss. Women with elevated anti-TPO levels (>34 IU/ml) were more likely to experience recurrent pregnancy losses compared to those without elevated levels. This correlation suggests that autoimmune thyroid dysfunction, as indicated by the presence of anti-TPO antibodies, may play a critical role in the etiology of recurrent pregnancy loss. The association is consistent with previous studies that have indicated a higher prevalence of thyroid autoimmunity among women with recurrent pregnancy loss, thereby emphasizing the potential role of anti-thyroid peroxidase antibodies in adverse pregnancy outcomes. Our study found that women with TPO antibodies are significantly older on average compared to those without TPO antibodies. The mean age of 35.28 years for TPO positive women versus 32.40 years for TPO negative women, coupled with a p-value of 0.0013, highlights a statistically significant age difference. . These results are consistent with findings of multiple studies. Previous studies have indicated that autoimmune thyroid disorders, including those involving TPO antibodies, are more common in older women. For instance, a study by Legakis I et al. (2023) highlighted that autoimmune thyroiditis, which often involves elevated TPO antibodies, tends to be more prevalent in women over 30 years old. Our findings align with this observation, showing that women with TPO positive results are significantly older on average (35.28 years)

compared to their TPO negative counterparts (32.40 years).[9]

In our study, comparative analysis of the number of spontaneous abortions between women with TPO positive and TPO negative results, focusing on different thresholds (≤2, ≤3, ≤4, ≤5) was done. Across all thresholds, women with TPO positivity consistently exhibit higher mean values of spontaneous abortions, accompanied by significantly larger standard deviations, indicating substantial variability and the presence of extreme cases in this group. The statistical significance of the differences, as indicated by the p-value of 0.0001 across all comparisons, underscores the likelihood that the observed differences are not due to random chance. This suggests that TPO positivity may be associated with an increased risk of spontaneous abortions, highlighting the need for further investigation into the underlying mechanisms and potential clinical implications for women with TPO positivity. These findings are consistent with previous research that has established a link between TPO positivity and adverse pregnancy outcomes. Previous authors reported that TPO-positive women are more likely to experience multiple spontaneous abortions compared to TPO-negative women, which aligns with the higher mean and greater variability observed in the current study.[10] Carabineanu S.et al .(2019)also found that TPO positivity was associated with a higher risk of miscarriage, corroborating the significant difference in the number of spontaneous abortions between the two groups.[11]

The findings from our study indicate that women with TPO positivity have a significantly higher mean BMI (27.54) compared to women who are TPO negative (25.23), with a p-value of 0.007. The standard deviation (SD) for BMI in the TPO-positive group is also higher (4.96) than in the TPO-negative group (3.19), indicating greater variability in BMI among TPO-positive women. The statistically significant p-value suggests that the difference in BMI between the two groups is unlikely to be due to random chance. Our findings are consistent with several previous studies that have reported a positive association between TPO positivity and higher BMI. A study by Croce L et al. (2024) found that women with autoimmune thyroiditis, a condition closely associated with TPO positivity, had a higher BMI compared to controls.[12] Similarly, a study by Mutlu HH et al. (2021) reported that patients with autoimmune thyroiditis often exhibited higher BMI and were at increased risk of obesity. The study proposed that autoimmune thyroid disease could be linked to metabolic syndrome, characterized by higher BMI, insulin resistance, and increased cardiovascular risk.[13]

Our analysis of serum FT3 levels shows that the mean FT3 level in TPO-positive women was 6.93 pmol/L (SD = 3.25), while in TPO-negative women, it was 7.82 pmol/L (SD = 2.31). The p-value of 0.128 indicates that this difference is not statistically significant. This finding suggests that there is no strong evidence to support a significant impact of TPO positivity on serum FT3 levels. The results align with previous studies such as Tipu HN et al.(2018)which reported that the presence of TPO antibodies does not necessarily correlate with altered thyroid hormone levels in the early stages of autoimmune thyroiditis. This suggests that while TPO antibodies are an indicator of autoimmune thyroid disease, they do not always affect FT3 levels, especially in individuals without overt thyroid dysfunction.[14] The comparison of serum FT4 levels revealed a mean of 6.96 pmol/L (SD = 2.19) in TPO-positive women and 7.32 pmol/L (SD = 2.06) in TPO-negative women, with a p-

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value of 0.458. This indicates that the difference in FT4 levels between the two groups is not statistically significant. The findings suggest that TPO positivity does not significantly affect FT4 levels in the study population, which is consistent with the literature indicating that the presence of TPO antibodies does not always translate into altered FT4 levels. Krishna K et al.(2019)found that although TPO antibodies are commonly associated with thyroid dysfunction, not all TPO-positive individuals exhibit changes in FT4 levels, particularly in the early or subclinical stages of thyroid disease.[15]

The analysis of serum TSH levels shows a significant difference between TPO-positive and TPO-negative women. The mean TSH level for TPO-positive women was 10.55 µIU/mL (SD = 12.39), compared to 4.98 µIU/mL (SD = 7.24) for TPO-negative women, with a p-value of 0.006, indicating statistical significance. This suggests that TPO positivity is associated with significantly higher TSH levels, which could indicate underlying thyroid dysfunction, such as hypothyroidism. Higher TSH levels in TPO-positive women may reflect an early compensatory response to maintain normal thyroid hormone levels despite underlying autoimmune thyroid destruction. This is in line with findings from previous studies, such as those by Hekmati MSH et al. (2006) which reported higher TSH levels in individuals with TPO antibodies, suggesting a potential progression toward hypothyroidism.[16]

Our study demonstrates a significant association between thyroid peroxidase (TPO) antibody positivity and the presence of various antiphospholipid antibodies (aPL) in women. Our results are consistent with previous studies that have explored the relationship between thyroid autoimmunity and aPL. Mavragani CP et al (2009) reported a higher prevalence of aPL in women with autoimmune thyroid disease, suggesting a link between thyroid autoimmunity and other autoimmune conditions.[18] Versini M et al. (2017) also found a significantly higher prevalence of aPL, including anti-CL, in women with thyroid autoimmunity, indicating a broader autoimmune predisposition in these patients.[19]

Conclusion

Based on our comprehensive research findings, we conclude that elevated anti-TPO antibody levels are closely associated with several significant health factors in women. First, the increase in anti-TPO antibody levels with advancing age suggests a potential link between age and heightened thyroid autoimmunity. Additionally, our study demonstrates a significant correlation between elevated anti-TPO antibody levels and a higher incidence of spontaneous abortions, indicating that thyroid autoimmunity may negatively affect pregnancy outcomes. Furthermore, women with higher BMI or obesity are more likely to have elevated anti-TPO antibody levels, highlighting a possible relationship between obesity, thyroid dysfunction, and increased inflammatory responses that could exacerbate autoimmune conditions. Lastly, anti-TPO antibody levels did not significantly correlate with other thyroid function markers, such as serum free T3, free T4, or hemoglobin levels, indicating a complex interplay of factors affecting thyroid health.

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