Section: Biochemistry

Original Research Article

SCREENING OF TPO GENE A2173C POLYMORPHISM AND THYROPEROXIDASE ANTIBODIES IN SUBCLINICAL HYPOTHYROIDSM

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Abstract

Background: The Aim is to evaluate the association between TPO gene polymorphisms and serum Anti TPO levels in sub clinical hypothyroidism. **Materials and Methods:** A case control study which included 66 subjects with 19-50 years of age. Out of these subjects, 33 were cases with subclinical hypothyroidism (SCH) –based on biochemical Thyroid function test reports.33 were normal individuals as controls. All individuals included in the study were subjected to full history taking, clinical examination and laboratory investigations including the Free T3, Free T4, TSH and Thyroid peroxidase antibody levels were measured by CLIA (Chemiluminescence Immunoassay Technique). Genotyping of TPO A2173C polymorphism was determined by PCR using premix tubes followed by Allele specific PCR analysis. Statistical analysis was done using SPSS software. **Result:** In our study we found that presence of C allele in SCH subjects and controls were 73% & 21% respectively, which showed a statistically significant p value of <0.00001. Mean anti-TPO antibody levels in persons with A allele was 130.75 IU/ml. whereas the mean anti-TPO antibody levels in persons with C allele was 192.25IU/ml. It shows that the presence of C allele increases the risk of developing hypothyroidism The genotypic frequencies of AA, AC, CC alleles were 33%, 49% and 27% respectively. (P value =0.235.) The distribution of genotype among cases and controls obeys Hardy Weinberg law and the distribution is in equilibrium. The analysis of logistic regression for C allele, the calculated odd's ratio for subclinical hypothyroidism was 10.60 at 95% confidence interval. Thus, it was suggested that individuals with C allele have 10 times higher risk of developing subclinical hypothyroidism than that of individuals with A allele. It shows a statistically significant p value of <0.0001.Thus, the risk of developing subclinical hypothyroidism can be predicted by identifying C allele. **Conclusion:** From the present study we found that there is a significant association of TPO gene polymorphism with presence of C allele for A allele at rs732609 and subclinical hypothyroidism. Genotyping rs732609 of TPO gene for the presence of C allele may help in early Detection of subclinical hypothyroidism. Early screening and appropriate management may prevent further progression to overt hypothyroidism.

INTRODUCTION

Subclinical Hypothyroidism (SCH) is a disorder affecting the thyroid gland, characterised by elevated levels of Thyroid stimulating hormone and normal levels of Free T3 & Free T4. The definition of SCH and overt Hypothyroidism are purely made by the biochemical criteria. SCH is commonly Seen as a laboratory finding in clinical practice. SCH may or may not progress to overt Hypothyroidism.[1] SCH is a mild thyroid failure, with prevalence of 3 to 15% in population without a known thyroid disease. The incidence of subclinical hypothyroidism is estimated to be 3% to 10% and increases to 18 to 20% in older individuals.[2] SCH has two categories, elevation in serum TSH Levels – slightly increased (4.0 to 10.0 mIu/L) and severely increased TSH level (>10

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mIu/L).[3] The clinical presentation of subclinical hypothyroidism is variable and the classical signs & symptoms of hypothyroidism may not be seen.[4]

Patients with SCH have a high rate to progression towards overt Hypothyroidism and this relates to Thyroid peroxidase antibodies (TPO). The rate of Progression is 2.6% every year if TPO antibodies are absent & 4.3% if TPO antibodies Are present.[5] The risk of incidence of hypothyroidism increases with age and is more Common among women. Besides age and sex, the risk increases among people with a Family history of thyroid disease or any autoimmune disease.[6]

Genetic predisposition is due to polygenic inheritance. It may be Characterised by single nucleotide polymorphism. Individuals with a susceptible genetic background are mostly prone to thyroid disease. The TPO gene polymorphism has its role in pathogenesis in hypothyroidism. Single Nucleotide Polymorphisms (SNPs) are seen in both thyroid functioning and thyroid autoimmune disorders.[7]

A2173C (rs732609) SNP characterized by the substitution of adenine by cytosine in the 2173rdposition of TPO gene located at the chromosome 2p25, it is responsible for substitution of amino acid proline instead of threonine at 725th amino acid in the TPO protein.[8]

The presence of C allele has been shown to increase the risk of subclinical hypothyroidism. Understanding the genetic etiology of the disease is essential to establish the pharmacological targets for intervention. Therefore early screening and proper management is required to prevent the progression of the disease and its complication.[4]

TPO gene polymorphism have been implicated in the pathogenic mechanism of anti-TPO levels in Subclinical Hypothyroidism.[8] Thus the aim of this study is to examine the relationship between TPO gene polymorphisms and serum Anti-TPO levels in sub clinical hypothyroidism.

MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry, Trichy SRM Medical College Hospital and Research Centre Trichy for a duration of 6 months, after seeking the approval from the institution ethical committee.

Study design: case control study

This study included 66 subjects with 19-50 years of age. Out of these subjects, 33 were cases with subclinical hypothyroidism (SCH) –based on biochemical Thyroid function test reports.33 were normal individuals as controls. Informed written consent were obtained from the subjects. All individuals included in this study were subjected to full history taking, clinical examination and laboratory investigations including The Free T3, Free T4, TSH and Thyroid peroxidase antibody levels were measured by CLIA (Chemiluminescence Immunoassay Technique) and Genotyping for TPO Gene polymorphism was determined by Polymerase chain Reaction (PCR). PCR reactions were carried out by Thermocycler, followed by Allele specific PCR analysis.

Inclusion criteria

Age (19-50years)33 cases with subclinical Hypothyroidism-based on biochemical Thyroid function test reports.33 Healthy individuals as the control

Exclusion Criteria

1. Patients with known autoimmune thyroid disease and overt Hypothyroidism.
2. Patients who are on levothyroxine.
3. Patients with other autoimmune conditions like Rheumatoid Arthritis, Diabetes and etc.
4. Patients recovering from non-thyroidal illness.
5. Patients recovering from post-partum thyroiditis.
6. Patients who had undergone radioactive ablation and neck surgery.

Laboratory and molecular diagnosis:

Laboratory investigations including The Free T3, Free T4, TSH and Thyroid peroxidase antibody levels were measured by CLIA (Chemiluminescence Immunoassay Technique).

For the analysis of TPO gene polymorphisms blood samples were collected in EDTA tubes & stored at -20° C. DNA extraction was done using proteinase K column method according to the protocol of the manufacturer (HELINI pure fast human blood DNA mini spin prep kit). The extracted DNA was amplified by polymerase chain reaction using master mix tubes (HELINI).

PCR- a technique used to make multiple copies of a segment of DNA. It is very precise and can be used to amplify a specific DNA target from a mixture of DNA molecules using primers.

In allele specific PCR, oligonucleotide primers were designed that forms a 3’ end. The amplification occurs only if the nucleotide present at 3’ end of each primer complemented the base at the mutant –type or wild type DNA sample. DNA isolated for thyroperoxidase gene was amplified by polymerase chain reaction using forward and reverse primers. Two forward primers specific for each allelic variant of SNP were used

Forward Primers

rs732609 A	5’-TGCCCATGGATGCCTTCCAAG-3’
rs732609 C	5’-TGCCCATGGATGCCTTCCCAG-3’
Reverse primer: rs732609 R	5’GCTCTCTGGGAAGCCACACT- 3’

For every sample, two reaction mixtures were made and allowed to run in parallel.one with A allele primer mix and the other with C allele primer mix. mismatches would result in delayed or no amplification. Genotyping was carried out for these two alleles with differential amplification

Reaction mixture for each sample was prepared, which was amplified in PCR thermocycler, in which DNA templates were denatured at 95° C for 5 minutes, amplification, consisting of 35 cycles at 95°

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C for 45 seconds, 58° C for 1 min & 72 ° C for 45 seconds with a final extension at 72 ° C for 5 minutes. The amplified PCR products were made to run in a submarine gel electrophoresis and visualised under UV light.

Interpretation

Homozygous A/A: 190 bp
Homozygous C/C: 440bp
Heterozygous A/C: 190 & 440 bp
The PCR products were run in submarine gel electrophoresis & the bands were visualised from which the results were interpreted.

RESULTS

33 individuals with subclinical hypothyroidism based on biochemical reports and 33 healthy control with normal thyroid function test were included in the study. They were analysed for genotype distribution of TPO gene and its association with subclinical hypothyroidism.

Statistical analysis:

1. Statistical products and services solution (SPSS) package were used for standard statistical analysis of the data.
2. The biochemical parameters between subjects with subclinical hypothyroidism and healthy control were tested by using the students t test.
3. By using Chi –square (χ 2), the genotype distribution frequency was compared between cases and controls.
4. By applying Hardy –weinberg law, the genotype frequency was analysed to find whether the distribution is in Hardy Weinberg equilibrium.
5. By calculating, Odd's ratio and 95% CI – confidence intervals, logistic regression analysis was done.
6. The significance level for p value was set:

If p value < 0.05 – it shows statistical significance
If p value < 0.001– statistical significance level is high.
[Table 1] The mean age of controls – 31.2 ± 8.72 years, while the mean age of cases 33.3±9.28 years.The risk of developing hypothyroidism increases as the age increases.(p value <0.05).
[Table 2] the mean values of TSH in cases and controls were 10.8±4.01 and 3.24±1.67 µIU/mL respectively and shows significant difference in TSH values between the cases and control groups with a p value = 0.015.
[Table 3] Shows the mean value of serum FT4 in cases and controls were 1.27 ±0.27 and 1.17 ±0.22 ng/dl respectively with no difference in serumFT4 levels between the study group. p value =0.072 which is statistically insignificant.
The mean value of serum T3 in cases and controls were 1.17 ±0.31 and 1.08±0.26 ng/ml respectively with no difference in serumT3 levels between the study group. p value =0.091 which is statistically insignificant.

In the study population, the genotype frequency of AA, AC, CC alleles are 22(33%), 26(49%) and 18(27%) respectively.
By using chi-square test, χ2= 2.8889 and p value =0.235. The distribution of genotype among cases and controls obeys Hardy Weinberg law and the distribution is in equilibrium.

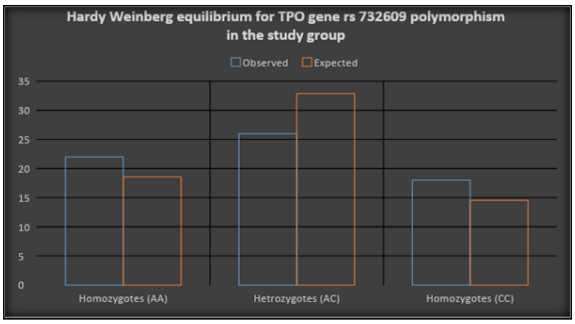

Figure 1: Genotype distribution of TPO gene at rs 732609

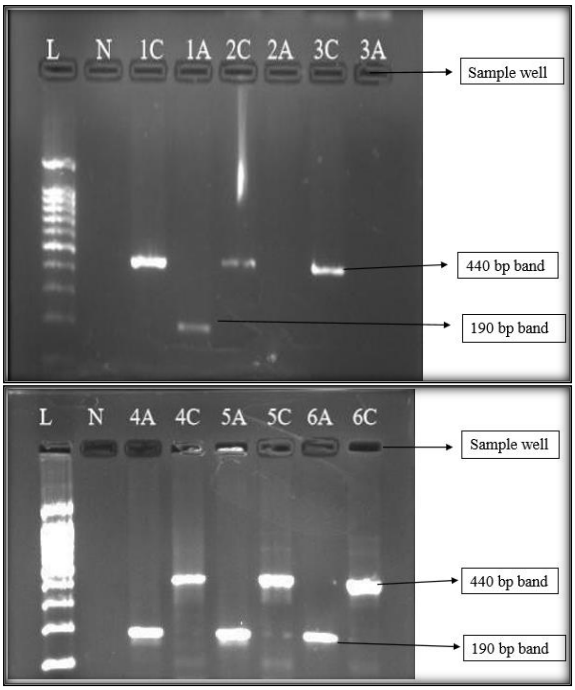

Figure 2: PCR products under UV transillumination – cases

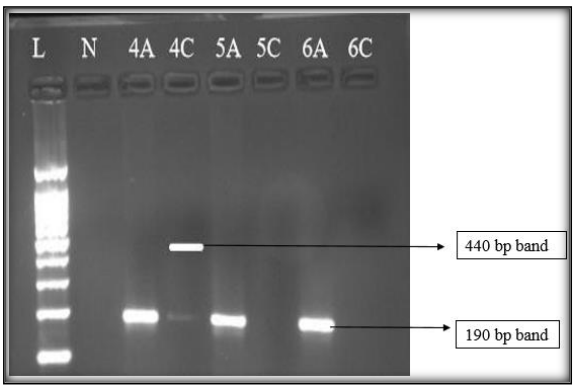

Figure 3: PCR Products Under UV Transillumination Control

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[Table 4] shows the genotype distribution frequency among subjects with subclinical hypothyroidism and individuals with normal thyroid function tests.
AA genotype was found to be present in 6 & 60 % of cases and controls respectively.AC genotype was found to be present in 42 & 36 % of cases and controls respectively. CC genotype was found to be present in 51 & 4 % of cases and controls respectively. Pearson chi square test was done and the value was 29.1033 which shows strong statistical significance with a p value <0.00001
[Table 5] The frequency of allele distribution among cases with subclinical hypothyroidism and control with normal thyroid function test. A allele was observed in 27% of patients when compared to that of 79% in control group. C allele was observed in 73% of patients when compared to that of 21% in control group. Chi square test was done and the value was 35.2325 which shows strong statistical significance with a p value 0f <0.00001.

[Table 6] Mean anti-Tpo antibody levels in persons with A allele was 130.75 IU/ml whereas the mean anti-Tpo antibody levels in persons with C allele was 192.25IU/ml By using the student t test , statistical analysis was done and it shows that the presence of C allele increases the risk of developing hypothyroidism.
[Table 7] Odd's ratio for subclinical hypothyroidism was 10.60. Thus, individuals with C allele were observed to have 10 times higher risk of developing subclinical hypothyroidism than that of individuals with A allele. Therefore this association was considered as highly significant with a p value of <0.0001. Thus, it can be stated that by identifying C allele, the risk of developing subclinical hypothyroidism can be predicted.

Interpretation:
Homozygous A/A: 190bp
Homozygous C/C:440bp
Heterozygous A/C: 190 & 440 bp

Table 1: Comparison of age between cases and controls..

Variables	Study Group	N	Mean	Standard deviation	t- value	p- value
Age (years)	Cases	33	33.3	9.28	0.062	0.007
	Controls	33	31.2	8.72		

Table 2: comparison of TSH between cases and controls.

Variables	Study Group	N	Mean	Standard deviation	t- value	p-value
TSH	Cases	33	10.8	4.01	11.7	0.015
	Controls	33	3.24	1.67		

Table 3: Comparison of FT4&T3 between cases and controls.

Variables	Study Group	N	Mean	Standard deviation	t- value	p-value
FT_4	Cases	33	1.27	0.27	131.1	0.072
	Controls	33	1.17	0.22		
T_3	Cases	33	1.17	0.31	112.6	0.091
	Controls	33	1.08	0.26		

Table 4: Hardy Weinberg equilibrium for TPO gene rs 732609 polymorphism in the study group

Genotypes	Observed	Expected	Chi square test	p value
Homozygotes (AA)	22	18.56	2.8889	0.235
Heterozygotes (AC)	26	32.87		
Homozygotes (CC)	18	14.56		

Table 5: Genotype distribution of TPO gene at rs 732609 polymorphism in the study group

TPOrs 732609 Genotype	Cases (N%)	Controls (N%)	Total (N%)	Chi square test	p value
AA	2 (6)	20 (60)	22(33%)	29.1033	<0.00001
AC	14 (42)	12 (36)	26(49%)		
CC	17 (51)	1 (4)	18(27%)		

Table 6: Allelic distribution of TPO gene at rs 732609 among cases and controls

rs 732609 allele	Cases (N%)	Controls (N%)	Total (N%)	Chi square test	p value
A allele	18(27%)	52(79%)	70(53%)	35.2325	<0.00001
C allele	48(73%)	13(21%)	61(47%)		

Table 7: Comparison of TPO antibody levels among A and C allele

Variables	Allele	Mean	Standard deviation	t-value	p-value
Anti TPO levels	A	130.75	378.28	15.0	0.00001
	C	192.25	393.58		

Table 8: Logistic regression of C allele for predicting subclinical hypothyroidism as dependent variable

Variable (independent) Allele	Odd's ratio for SCH	95% CI for OR	p value
A	1	-	<0.0001
C	10.60	4.7255 – 24.0774	

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DISCUSSION

Subclinical hypothyroidism is a mild thyroid failure with a prevalence rate of 3-8% in the population without any known thyroid diseases.[2] The prevalence rate increases with the age and is more common in females. Prevalence in men increases after sixth decade of life 9.80 % of patients with subclinical hypothyroidism shows the presence of anti-thyroid antibodies.[5] This study was carried out among subjects with subclinical hypothyroidism, based on thyroid function tests and individuals with normal thyroid function tests to analyse the genotype with respect to rs732609 SNP.

Age is considered as one of the risk factor for hypothyroidism. Jusmita dutta et al. showed that the anti –TPO mean value was higher among females between 31-40 years of age. They concluded that anti-tpo levels were higher in cases of hypothyroidism and autoimmunity is the cause of hypothyroidism.[10] Antibody against thyroperoxidase is associated with hypothyroidism and is more common among females in reproductive age group.[11]

In our study the mean age of individuals with SCH was 33.3 years whereas the mean age of control group was 31.2 years. Individuals in the age group of 19-50 were included in the study. The mean age was slightly higher among the cases with SCH when compared to the control groups with statistically significant p value <0.05. According to (NHANES III). National Health & Nutrition Survey, global prevalence of hypothyroidism is 4.6%, out of which 0.3% - overt & 4.3% for the subclinical type of hypothyroidism resulting as the most frequent endocrine disease among the elderly.[12] The prevalence in females are higher than males. This study is in concordance with Calsolaro et al, who proposed that the prevalence rate of thyroid diseases increases with aging.[13]

Several studies have shown that the positive anti-TPO levels were associated with the higher rate of progression towards overt hypothyroidism.[14] Liu H et al showed that there was increased risk of miscarriages in anti-TPO positivity in SCH mothers.[15] Okuroglu N et al suggested that anti-TPO positive individuals may require higher dosage of levothyroxine compared to antibody negative individuals.[16] So, it is important to measure anti-TPO levels in individuals with SCH. Mean anti-Tpo antibody levels in cases & control were 244.91 and 15.24 respectively.

H.engler et al stated that a marked variability in anti-TPO levels was noted in hypothyroidism. Some patients showed a marked reduction in the anti-TPO levels during levothyroxine therapy.[17]

Reham M. El shabrawy et al. concluded that the TPO gene polymorphism (A2173C) may be considered as risk factor for developing autoimmune thyroiditis in patients with bronchial asthma and allergic rhinitis. These patients should be screened regularly for hidden thyroiditis.[18] Shashikala Bhat et al, concluded that the presence of anti-TPO may increase the risk of miscarriage, gestational hypertension in pregnant females. studies are needed to explore the association between anti-TPO levels, adverse pregnancy outcomes and postpartum thyroid dysfunction.[19]

The objective of this study is to determine the association of rs732609 A/C SNP in subclinical hypothyroidism. In the present study we found that the distribution of CC allele (homozygous) to be much higher (51%) in individuals with SCH compared to that of the controls (4%) with the p value of <0.00001 which is considered to be statistically significant. this correlates with the study conducted by Amirhoseinkoshi et al.[20]

According to Amirhoseinkoshi et al, the presence of C allele in the exon 17 of TPO gene at 2173 position increases the risk of developing subclinical hypothyroidism. A2173C (rs732609) SNP is characterized by the substitution of adenine by cytosine in the 2173rd position of TPO gene located at the chromosome 2p25, it is responsible for substitution of amino acid proline instead of threonine at 725th amino acid in the TPO protein.[20] This alterations of amino acid Threonine to Proline may change the structure and activity of Thyroperoxidase enzyme. However genetic variations may lead to changes in its structure, thus TPO enzyme is recognized as foreign body and antibody are produced against it.

Reduced activity or expression of TPO impairs thyroid follicular cell function by reducing iodide trapping and impairing thyroid hormone synthesis.[8]

Hanan S.Ahmed et al found an association of rs732609A/C polymorphisms with autoimmune hypothyroidism and correlated the anti-TPO levels with different genotypes (AA,CC and AC) in hypothyroid patients. Also they have an association of rs732609A/C polymorphism with the disease severity. They concluded that patients with CC genotype and the C allele are more prone for subclinical and clinical hypothyroidism than the controls.[21]

In our study we found that presence of C allele in SCH subjects and controls were 73% & 21% respectively, which showed a statistically significant p value of <0.00001. Mean anti-TPO antibody levels in persons with A allele was 130.75 IU/ml whereas the mean anti-TPO antibody levels in persons with C allele was 192.25IU/ml. It shows that the presence of C allele increases the risk of developing hypothyroidism. This correlates with study conducted by Amirhoseinkoshiet al and Hanan S.Ahmed et al.[20,21]

Balmiki et al. have demonstrated that TPO gene polymorphism such as Asp666Asp(rs1126797) & Thr725Pro (rs732609)& found the significant correlation with Hypothyroidism in Indian population.[7]

The genotypic frequencies of AA, AC, CC alleles were 33%, 49% and 27% respectively. (p value

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=0.235.) The distribution of genotype among cases and controls obeys Hardy Weinberg law and the distribution is in equilibrium.

In this study, we did analysis of logistic regression for C allele, the calculated odd's ratio for subclinical hypothyroidism was 10.60 at 95% confidence interval. Thus, it was suggested that individuals with C allele have 10 times higher risk of developing hypothyroidism than that of individuals with A allele. It shows a statistically significant p value of <0.0001. Thus, the risk of developing subclinical hypothyroidism can be predicted by identifying C allele.

CONCLUSION

From the present study we found that there is a significant association of TPO gene polymorphism with presence of C allele for A allele at rs732609 and anti-TPO levels in subclinical hypothyroidism. Genotyping rs732609 of TPO gene for the presence of C allele may help in early Detection of subclinical hypothyroidism. Early screening and appropriate management may prevent further progression to overt hypothyroidism.

REFERENCES

1. Schultheiss UT, Teumer A, Medici M, et al. A genetic risk score for thyroid peroxidase antibodies associates with clinical thyroid disease in community-based populations. J ClinEndocrinolMetab. 2015; 100(5): E799-E807.
2. Ertugrul O, Ahmet U, Asim E, Gulcin HE, Burak A, Murat A, Sezai YS, Biter HI, Hakan DM. Prevalence of Subclinical Hypothyroidism among Patients with Acute Myocardial Infarction. ISRN Endocrinol. 2011;2011:810251. doi: 10.5402/2011/810251. Epub 2011 Jul 28. PMID: 22363888; PMCID: PMC3262623.
3. Khan MA, Ahsan T, Rehman UL, Jabeen R, Farouq S. Subclinical Hypothyroidism: Frequency, clinical presentations and treatment indications. Pak J Med Sci. 2017 Jul-Aug;33(4):818-822. doi: 10.12669/pjms.334.12921. PMID: 29067046; PMCID: PMC5648945
4. Faam, B., Daneshpour, M.S., Azizi, F., Salehi, M., Hedayati, MAssociation between TPO gene polymorphisms and Anti-TPO level in Tehranian population: TLGSGene.2012;498:1.
5. Fatourechi, Vahab.Subclinical hypothyroidism: an update for primary care physicians.Mayo Clinic proceedings .2009;84: 65-71.
6. Arafah BM. Increased need for thyroxine in women with hypothyroidism during estrogen therapy. N Engl J Med. 2001;344(23):1743-1749.
7. Balmiki N, Bankura B, Guria S, et al. Genetic analysis of thyroid peroxidase (TPO) gene in patients whose hypothyroidism was found in adulthood in West Bengal, India. Endocr J. 2014;61(3):289-296
8. HedayatiM,etal,Association between serum level of anti-TPO titer and polymorphisms G1193/C Exon 8 and C2145/T Exon 12 of thyroid peroxidase gene in an Iranian population.Int J EndocrinolMetab. 2010:64-67.
9. Karmisholt J, Andersen S, Laurberg P. Variation in thyroid function tests in patients with stable untreated subclinical hypothyroidism. Thyroid. 2008 Mar;18(3):303-8.
10. Dutta J, Jain S, Jain A, Jain S, Jain S. An association of anti-thyroid peroxidase antibodies in clinical and subclinical hypothyroidism. Int J Clin Biochem Res 2019;6(3):415-420.
11. Jantikar AM. A study on relationship between thyroid peroxidase antibodies (Anti-TPO antibodies) and thyroid dysfunction patients. Int J Clin Biochem Res 2020;7(2):238-242.
12. Wyne KL, Nair L, Schneiderman CP, Pinsky B, Antunez Flores O, Guo D, Barger B, Tessnow AH. Hypothyroidism Prevalence in the United States: A Retrospective Study Combining National Health and Nutrition Examination Survey and Claims Data, 2009-2019. J Endocr Soc. 2022 Nov 10;7(1):bvac172. doi: 10.1210/jendso/bvac172. PMID: 36466005; PMCID: PMC9706417.
13. Calsolaro, V., Niccolai, F., Pasqualetti, G., Calabrese, A. M., Polini, A., Okoye, C., Magno, S., Caraccio, N., &Monzani, F. Overt and Subclinical Hypothyroidism in the Elderly: When to Treat?. Frontiers in endocrinology.2019;10:177
14. Kabadi UM. 'Subclinical hypothyroidism'. Natural course of the syndrome during a prolonged follow-up study. Arch Intern Med. 1993;153:957-61.
15. Liu H, Shan Z, Li C, Mao J, Xie X, Wang W, Fan C, Wang H, Zhang H, Han C, Wang X, Liu X, Fan Y, Bao S, Teng W. Maternal subclinical hypothyroidism, thyroidautoimmunity, and the risk of miscarriage: a prospective cohort study. Thyroid. 2014;24:1642-9.
16. Okuroglu N, Ozdemir A, Sertbas Y, Sancak S. The relationship between thyroid antibody titer and levothyroxine dose in patients with overt primary hypothyroidism. Ann Saudi Med. 2017;37:189-193.
17. Engler H, Riesen WF, Keller B. Anti-thyroid peroxidase (anti-TPO) antibodies in thyroid diseases, non-thyroidal illness and controls. Clinical validity of a new commercial method for detection of anti-TPO (thyroid microsomal) autoantibodies. Clin Chim Acta. 1994 Mar;225(2):123-36. doi: 10.1016/0009-8981(94)90040-x. PMID: 8088002.
18. Reham M. El Shabrawy, Amal H. Atta, Nearmeen M. Rashad .Serum Anti-TPO and TPO Gene Polymorphism as a Predictive Factor for Hidden Autoimmune Thyroiditis in Patient with Bronchial Asthma and Allergic Rhinitis. THE EGYPTIAN JOURNAL OF IMMUNOLOGY Vol. 23 (1), 2016 Page: 77-86
19. Shashikala Bhat1 , AP Ashwini2 , AP Suma3 , Shashikiran Umakanth4 Effect of Thyroid Peroxidase Antibody on Pregnancy Outcome among Hypothyroid Women. Journal of Clinical and Diagnostic Research. 2018 Aug, Vol-12(8): QC01-QC03
20. Amirhosein Khoshi,1 Alireza Sirghani,2 Mehran Ghazisaeedi,3 Ali Zarei Mahmudabadi,2 Amir Azimian4 Association between TPO Asn698Thr and Thr725Pro gene polymorphisms and serum anti-TPO levels in Iranian patients with subclinical hypothyroidism.HORMONES 2017;16(1):75-83
21. Ahmed HS, Nsrallah AAM, Abdel-Fatah AH, Mahmoud AA, Fikry AA. Association of Thyroid Peroxidase Gene Polymorphisms and Serum Anti- TPO Levels in Egyptian Patients with Autoimmune Hypothyroidism. Endocr Metab Immune Disord Drug Targets. 2021;21(4):734-742. doi: 10.2174/1871530320666200715101907. PMID: 32669082.

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