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RESEARCH ARTICLE

AN INVESTIGATION INTO THE POLYMORPHISM OF *TPO* GENE AND ITS ASSOCIATION WITH HYPOTHYROIDISM IN KASHMIRI POPULATION.

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Abstract

TPO gene is pivotal for the normal functioning of the thyroid gland. A mutation in this gene is associated with several disorders of thyroid hormonogenesis including hypothyroidism. In this study, we investigated the role of TPO codon T2229C genetic variants in hypothyroidism. Blood samples were taken from a total of 445 participants (220 cases and 225 healthy subjects), aged 16-70 years, which were randomly selected from among the Kashmiri population. All the necessary information on the thyroid disease and other lifestyle and environmental exposure were gathered by questionnaire and the patient's endocrinologist or general practitioner verified the diagnosis. The polymorphism of the codon T2229C of the TPO gene was identified by polymerase chain reaction-based restriction analysis and direct sequencing. Statistical analysis suggested a single nucleotide polymorphism that is a C-to-T substitution at codon 2229 of the TPO gene. The frequency of its variant genotype T (combined CT and TT genotype) as noted in cases was associated with a borderline increased risk of hypothyroidism (OR = 1.62; 95% CI = 1.07-2.43; P = 0.02) as compared to healthy subjects. Our study also observed a positive association of the TPO gene polymorphism with certain environmental exposures, lifestyle changes, smoking habits and in subjects with high basal metabolic Index (BMI). This observation suggests a possible role of this polymorphism in the etiology of hypothyroidism in Kashmir.

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Introduction:-

The thyroid gland is very necessary for the normal functioning of the human body as it plays a crucial role in almost all the tissues of the human body through its secretion of two major thyroid hormones; thyroxine (T₄) and 3-3'-5-tri-iodo thyronine (T₃) (1,2). These thyroid hormones control growth, differentiation, regulation of metabolism and other physiological functions in the human body (3). Under normal conditions, thyroid gland output is about 90% of T₄ and 10% T₃ (4).

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An underproduction of the thyroid hormones T₃ and T₄ leads to hypothyroidism. It may happen as a result of congenital thyroid abnormalities, AITD (auto-immune thyroid disorders) where the immune system mistakes healthy thyroid tissue as a foreign body and attacks it, such as Hashimoto's thyroiditis (5) the surgical removal of the thyroid gland as a treatment for severe hyperthyroidism and/or thyroid cancer. The prevalence of thyroid disorders as seen throughout the world is 25% in females and 0.6% in males. It has been estimated that about 42 million people in India have thyroid dysfunction (6). A hospital based study on the prevalence of thyroid disorders in Srinagar reported an overall 40.36% of Kashmir population suffering from thyroid disorders (7). Subclinical hypothyroidism was found to be the most prevalent thyroid disorder (33.7%) followed by an overt hypothyroidism (5.1%) and hyperthyroidism (1.63%). In women, the maximum prevalence of subclinical hypothyroidism was seen in the reproductive age group. According to the age-wise prevalence of thyroid disorders, maximum cases of subclinical hypothyroidism belong to 21–30 years age group. With respect to overt hypothyroidism, maximum cases were observed between the age 21 and 50 years.

The Jammu and Kashmir state is situated along the Himalayan range and is surrounded by the mountains. It is reported that in mountainous areas and hilly region, living things are deficit of iodine content due to years washing of the soil by heavy rains and recurrent floods. (8). Diet has also been observed to play an important role in the development of thyroid disorder. The staple diet of Kashmir valley is rice, fresh green leafy vegetables and non-vegetarian foods (9). There are some food types that contain goitrin, thiocyanates and flavonoids called goitrogens or goitrogenic foods, for example, turnip, radish, cabbage, cauliflower, corn, soy, etc., which are known to cause goiter and other disorders of the thyroid gland. The consumption of such food items is said to make it difficult for the thyroid gland to absorb iodine by decreasing the activity of the enzyme thyroid peroxidase, which promotes the insertion of iodine into thyroid hormone (10,11). In healthy adult subjects, thyroid stimulating hormone (TSH) has considerable variability between individuals, whereas this variability is much less in the same individual when TSH is measured repeatedly over an extended period of time (12). Therefore, understanding variation in TSH levels and the genes responsible may be particularly important in a population at risk for abnormal TSH levels. Thyroid peroxidase (TPO) is the key enzyme in the synthesis of thyroid hormones and its (TPO) mutations is one of the most common causes of thyroid dyshormonogenesis. TPO is a 110 KD glycoprotein that uses heme as a cofactor (13). It has 933 amino acids, which are encoded by mRNA of 3048 nucleotides (14). The TPO gene is located on the short arm of chromosome 2, locus 2p25 consists of 17 exons (15,16). Autoimmune thyroid diseases may occur due to several reasons, such as total absence of TPO activity, non-binding of cofactor heme to TPO, inability to interact with the Tg substrate and abnormal subcellular localization (17). There are no data for the codon T229C of the TPO gene polymorphisms among hypothyroidic patients in Kashmir valley. The current study was designed to determine whether this TPO gene polymorphism is associated with hypothyroidism in Kashmir study population.

Materials and Methods:-

This study was approved by the Human Research Ethics Committee at the Sheri Kashmir Institute of Medical Sciences and Informed consent was obtained from each subject. The subjects for the current case-control study were 445 adults (220 cases and 225 controls), These include 174 men and 271 women, aged 16-70 years, who were randomly selected from among the Kashmiri population. Subjects with clinically confirmed thyroid disorders (hypothyroidic) were assessed and included as cases in the study while as controls were healthy individuals with no history of any disease/disorder. Subjects with any type of malignancy, diabetes, high cholesterol levels and abnormal lipid profile and those who do not want to participate were excluded. Controls were individually matched to cases in all respects (age \pm 10 years). Samples were obtained from the community centers and other departments of SKIMS. At study entry, all subjects completed a questionnaire, covering data on demographic, biochemical factors, smoking habits, the basal metabolic index including weight and height, type of food intake etc.

Genotypic Analysis:-

The Genomic DNA was extracted from whole blood samples using modified phenol - chloroform method (18). The polymorphism of the TPO gene codon T229C was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The primers for the TPO gene codon T229C along with their PCR cycling conditions, restriction enzymes, and restriction digestion fragments are listed in Table 1. PCR was performed in a total volume of 25 μ l carried out in 0.2ml PCR tubes (axygen). The PCR reaction mixture consisted of 50-100ng of genomic DNA templates. 200 μ M of deoxynucleotidetriphosphate [dNTPs] (Biotools), 0.5 μ M of each primer (Fermentas), 2.5mM MgCl₂ and 2.0U of Taq Polymerase with 2.5 μ l 10x reaction buffer (Biotools). The codon 2229 SNP in the exon 12 of TPO gene was also assessed by the PCR technique, thereby yielding 177 bp product (Fig. A). The PCR product was subjected to restriction enzyme analysis by digestion at 37°C for overnight

with 1 unit of the Eco571 restriction endonuclease. 10 μ l of the digested product was then analyzed by loading onto a 3% agarose gel stained with ethidium bromide and separated by electrophoresis. The PCR-RFLP results obtained from the study genes were validated by direct sequencing PCR products (Sequence scanner software BioEdit was used for comparing sequences between the original PubMed gene sequence) (Fig. B & C).

Table 1:- List of Primers, PCR conditions, restriction enzymes, and restriction digestion fragments of the genes of interest.

Gene	Primers	PCR conditions	RE*	AP ^a & DP ^b (bp ^c)
TPO	FP- 5'- CTGTCTCGGGTCATCTGT G -3' RP-5'- GTAACGTGGTGTGAGAGGAGAC-3'	95°C - 5 min 60°C - 60 sec 35 cycles 72°C - 1 min	Eco 571	AP= 177 W= 177 H= 177, 95, 82 M= 95, 82

*RE - Restriction enzyme used.

^aAP - Amplified products, ^bDP - Digested products, ^cbp - base pairs

W - Wild allele, H - Heterozygous mutant, M - Mutant allele

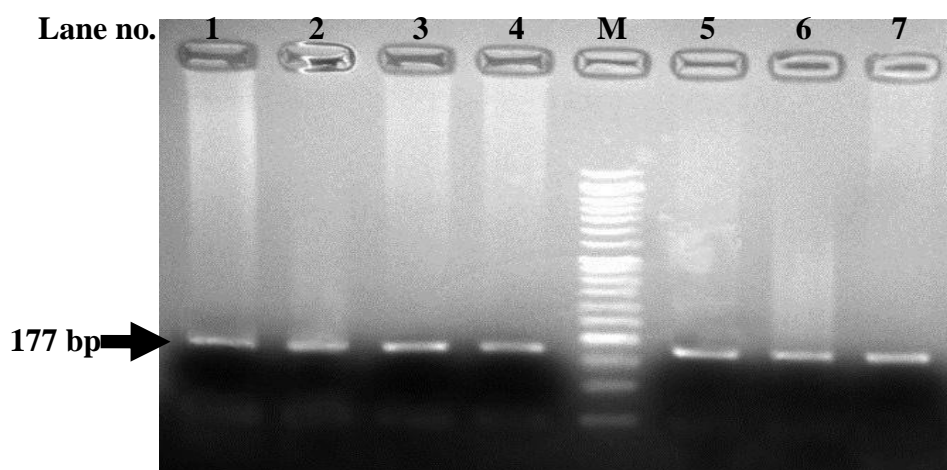


Figure A: - Representative gel picture (1.5%) showing 177 bp PCR product of TPO gene, lane 1-7 represent amplicon of codon T2229C and lane M (50bp) represents a molecular weight marker.

Lane no: 1 TT 2 CT 3 CC 4 M 5 CC 6 CT 7 CC 8 CC 9 TT

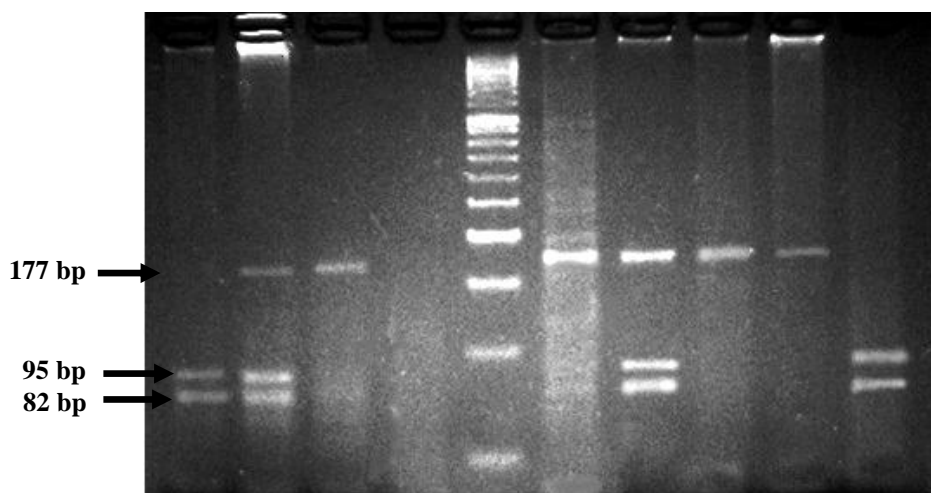
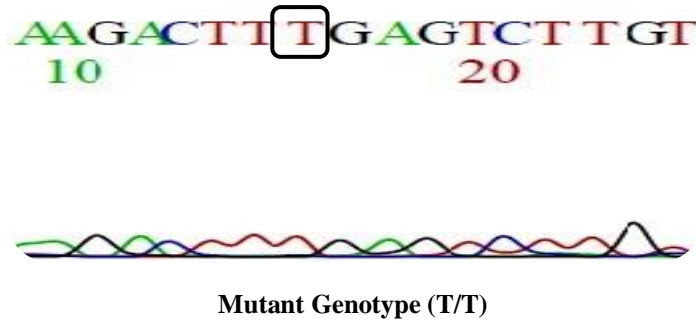


Figure B: - Representative gel picture showing RFLP analysis of TPO gene Codon T2229C: Lane no. 2 and 6 - CT (heterozygous variant genotype), Lane no. 3, 5, 7 and 8 - CC (wild/normal genotype) and Lane no. 1 and 9 represent homozygous mutant genotype TT. Lane M is the DNA size marker (50bp).

(A)



(B)

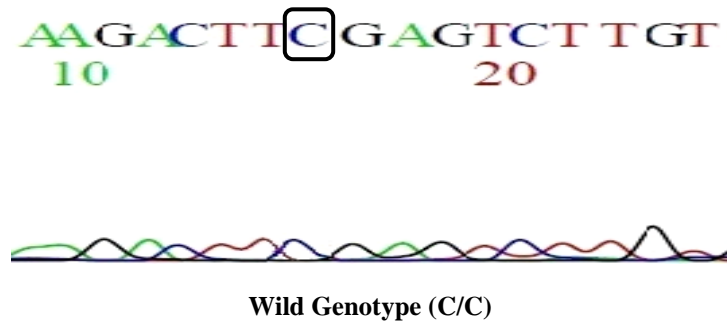


Figure C:- Chromatogram of *TPO* codon T2229C genotype showing; (A): C/T transition and (B): C/C genotype.

Statistical Analysis:-

The allelic frequencies were estimated by gene counting and genotypes were scored. The chi-square (χ^2) test was used to examine differences in demographic variables, distribution of genotypes with those expected for a population in the Hardy-Weinberg equilibrium and to test the significance of the differences of observed alleles and genotypes between groups. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by using a Yates' continuity corrected chi-square (χ^2) test and based on this calculation, the risk of thyroid disorders was estimated. Statistical analysis of the data was performed using Graph Pad Prism version 6.0 software. The criterion for statistical significance was defined as $P < 0.05$.

Results:-

In this study, a total of 445 blood samples were included, 220 thyroid disorder cases and 225 healthy controls from Kashmir were recruited. These include 81 men and 139 women for cases and 93 men and 132 women for controls. The frequency of cases and controls was found to be higher in females (63.18 vs. 58.66) as compared to males (36.81 vs. 41.33) and in the age group of 27-37 years old (31.81 % and 32.44 %) respectively. Overweight/obese subjects with an increased basal metabolic index (BMI) were seen to be at higher risk of developing hypothyroidism. Subjects who consume goitrogenic food like turnip, cauliflower, soy, etc. were also observed to be at higher risk of developing hypothyroidism as compared to those who do not consume such food item/s. The screening of the *TPO* gene at locus 229 resulted in 3 genotypes; CC, CT, and TT. The frequency of these genotypes was 65.7% (CC), 20.13% (CT) and 14.09% (TT). The frequency of variant genotypes (CT + TT) was significantly higher in cases as compared with that in the healthy control group (O.R = 1.62, 95% CI = 1.07 - 2.43, $P = 0.02$). The frequency of T allele was seen to be significantly higher in hypothyroidic cases as compared to healthy controls, suggesting that the subjects carrying at least one T allele had an increased risk for the development of hypothyroidism compared with subjects carrying the C allele (O.R = 1.57, 95% CI = 1.13 - 2.17, $P = 0.008$). As TT genotype was relatively infrequent, it was combined with CT genotype as a group for analysis. The distribution of the *TPO* genotypes and allelic frequencies between hypothyroidic cases and healthy control subjects are shown in Table 2.

Analyzing the effect of the TPO genotypes by stratifying it against potential variables such as gender, age, dwelling, smoking status, goitrogenic food consumption, etc. Statistically significant results were obtained between this gene polymorphism and age group of 16-59 years old, both genders, smoking, goitrogenic food consumers, high basal metabolic index, urban dwelling. On the contrary, no statistically significant results were obtained for 60-70 years old age group, subjects with low basal metabolic index and other important factors determining predisposition to this type of thyroid disorder. Table 3 & 4 shows an association between TPO genotypes T2229C and different clinical risk factors.

Table 2: - Genotypic and allelic frequencies of TPO gene codon T2229C between the *hypothyroidic* cases and healthy controls.

Genotypes	Cases N=220 (%)	Controls N=225 (%)	* χ^2	^a OR (95%CI) ^b	P value
Wild (CC)	142 (65.7)	168 (78.6)	-	Referent	-
Heterozygous (CT)	47 (20.13)	36 (14.4)	2.65	1.54 (0.95-2.51)	0.10
Mutant (TT)	31 (14.09)	21 (6.8)	2.87	1.74 (0.97-3.17)	0.09
Variants (CT+ GT)	78 (34.2)	57 (21.3)	4.92	1.62 (1.07-2.43)	0.02
C allele	331 (75.8)	372 (86.14)	-	Referent	-
T allele	109 (24.16)	78 (13.8)	6.97	1.57 (1.13-2.17)	0.008
Total	440	450			

a; OR - Odds Ratio.

b; CI - Confidence Interval.

*; χ^2 - Chi square test.

Table 3: - Genotypic and allelic frequencies of TPO gene codon T2229C among *hypothyroidic* cases and healthy controls stratified by different age groups.

Genotypes/Variables	Cases N=220 (%)	Controls N=225 (%)	* χ^2	OR ^a (95%CI) ^b	P value
16 - 26 years age group					
Wild (CC)	36(16.3)	42 (18.6)	-	Referent	-
Heterozygous (CT)	12 (5.4)	8 (3.5)	0.73	1.75 (0.64-4.75)	0.40
Mutant (TT)	7 (3.1)	1 (0.44)	3.44	8.16 (0.95-69.59)	0.06
CT+TT	19 (8.63)	9 (4.0)	3.06	2.46 (0.99-6.11)	0.08
27 -37 years age group					
Wild (CC)	39 (17.7)	58 (25.7)	-	Referent	-
Heterozygous (CT)	15 (6.8)	9 (4.0)	3.02	2.48 (0.98-6.22)	0.08
Mutant (TT)	16 (7.2)	6 (2.6)	6.37	3.96 (1.42-11.03)	0.01
CT+TT	31 (14.10)	15 (6.66)	2.86	3.07 (1.47-6.43)	0.004
38 - 48 years age group					
Wild (CC)	32 (14.5)	39 (17.3)	-	Referent	-
Heterozygous (CT)	12 (5.4)	8 (3.5)	0.86	1.82 (0.66-5.02)	0.35
Mutant (TT)	2 (0.90)	5 (2.2)	0.19	0.48 (0.08-2.68)	0.65
CT+TT	14 (6.36)	13 (5.77)	0.14	1.31 (0.54-3.19)	0.70
49 - 59 years age group					
Wild (CC)	25 (11.3)	21 (9.3)	-	Referent	-
Heterozygous (CT)	7 (3.1)	5 (2.2)	0.0006	1.17 (0.32-4.25)	0.93
Mutant (TT)	3 (1.3)	7 (3.1)	1.09	0.36 (0.08-1.57)	0.3
CT+TT	10 (4.54)	12 (5.33)	0.18	0.70 (0.25-1.94)	0.67
60 - 70 years age group					
Wild (CC)	10 (4.5)	8 (3.5)	-	Referent	-
Heterozygous (CT)	1(0.45)	6 (2.6)	2.01	0.13 (0.013-1.34)	0.15
Mutant (TT)	3 (1.3)	2 (0.8)	0.11	1.20 (0.16-9.01)	0.74
CT+TT	4 (1.81)	8 (3.55)	0.67	0.40 (0.08-1.82)	0.41

a; OR - Odds Ratio.

b; CI - Confidence Interval.

*: χ^2 - Chi square test.

Table 4:- Genotypic and allelic frequencies of TPO gene codon T2229C among *hypothyroidic* cases and healthy controls stratified by gender, smoking, resident, Basal Metabolic Index (BMI), goitrogenic food intake, family history, boiled water intake.

Genotypes/Variables	Cases N=220 (%)	Controls N=225 (%)	* χ^2	OR ^a (95%CI) ^b	P value
Gender (Male)					
Wild (CC)	61 (27.7)	75 (33.3)	-	Referent	-
Heterozygous (CT)	16 (7.2)	11 (4.8)	1.34	1.78 (0.77-4.13)	0.24
Mutant (TT)	4 (1.81)	7 (3.11)	0.05	0.70 (0.19-2.51)	0.81
CT+TT	20 (9.10)	18 (8.0)	0.44	1.36 (0.66-2.81)	0.50
Gender (Female)					
Wild (CC)	81 (36.8)	93 (41.3)	-	Referent	-
Heterozygous (CT)	31 (14.09)	25 (11.1)	0.98	1.42 (0.77-2.61)	0.32
Mutant (TT)	27 (12.2)	14 (6.2)	4.20	2.21 (1.08-4.51)	0.04
CT+TT	58 (26.3)	39 (17.3)	3.85	1.70 (1.03-2.82)	0.04
Smoke (never)					
Wild (CC)	101 (45.9)	117 (52.0)	-	Referent	-
Heterozygous (CT)	29 (13.18)	15 (6.6)	4.85	2.24 (1.13-4.41)	0.27
Mutant (TT)	12 (5.45)	5 (2.2)	2.81	2.78 (0.94-8.16)	0.09
CT+TT	41 (18.6)	20 (8.8)	7.50	2.37 (1.30-4.31)	0.006
Smoke (Moderate)					
Wild (CC)	35 (15.90)	31 (13.7)	-	Referent	-
Heterozygous (CT)	10 (4.5)	12 (5.3)	0.13	0.74 (0.28-1.94)	0.71
Mutant (TT)	13 (5.90)	14 (6.2)	0.04	0.82 (0.33-2.01)	0.84
CT+TT	23 (10.0)	26 (11.5)	0.21	0.78 (0.37-1.64)	0.64
Smoke (Heavy)					
Wild (CC)	6 (2.7)	20 (8.8)	-	Referent	-
Heterozygous (CT)	8 (3.6)	9 (4.0)	1.71	2.96 (0.79-11.08)	0.19
Mutant (TT)	6 (2.7)	2 (0.88)	5.12	10.0 (1.58-63.13)	0.02
CT+TT	14 (6.3)	11 (4.8)	4.50	4.24 (1.27-14.18)	0.03
Resident (Rural)					
Wild (CC)	61 (27.7)	74 (32.8)	-	Referent	-
Heterozygous (CT)	16 (7.2)	19 (8.4)	0.018	1.02 (0.48-2.15)	0.90
Mutant (TT)	9 (4.09)	13 (5.7)	0.02	0.84 (0.33-2.18)	0.88
CT+TT	25 (11.3)	32 (14.2)	9.85	0.94 (0.50-1.76)	0.99
Resident (Urban)					
Wild (CC)	81 (36.8)	94 (41.7)	-	Referent	-
Heterozygous (CT)	31 (14.09)	17 (7.5)	4.34	2.11 (1.09-4.10)	0.03
Mutant (TT)	22 (10.0)	8 (3.5)	6.45	3.19 (1.34-7.56)	0.01
CT+TT	53 (24.1)	25 (11.1)	9.31	2.46 (1.40-4.31)	0.002
Basal Metabolic Index (Underweight)					
Wild (CC)	4 (1.81)	3 (1.3)	-	Referent	-
Heterozygous (CT)	2 (0.90)	2 (0.88)	0.16	0.75 (0.06-8.84)	0.68
Mutant (TT)	1 (0.45)	3 (1.3)	0.16	0.25 (0.01-3.77)	0.68
CT+TT	3 (1.36)	5 (2.22)	0.05	0.45 (0.05-3.57)	0.80
Basal Metabolic Index (Normal)					
Wild (CC)	89 (40.4)	117 (52.0)	-	Referent	-
Heterozygous (CT)	30 (13.6)	17 (7.5)	5.73	2.32 (1.20-4.47)	0.01
Mutant (TT)	15 (6.8)	7 (3.11)	4.04	2.81 (1.10-7.20)	0.04
CT+TT	45 (20.4)	24 (10.6)	9.16	2.46 (1.39-4.34)	0.002
Basal Metabolic Index (Overweight)					
Wild (CC)	49 (22.2)	48 (21.3)	-	Referent	-

Heterozygous (CT)	15 (6.8)	17 (7.5)	0.02	0.86 (0.38-1.92)	0.87
Mutant (TT)	15 (6.8)	11 (4.8)	0.18	1.33 (0.55-3.20)	0.66
CT+TT	30 (13.6)	28 (12.4)	0.0004	1.05 (0.54-2.01)	0.98
Goitrogenic Food Intake (Yes)					
Wild (CC)	55 (25.0)	164 (72.8)	-	Referent	-
Heterozygous (CT)	27 (12.2)	31 (13.7)	9.11	2.60 (1.42-4.73)	0.002
Mutant (TT)	11 (5.0)	17 (7.5)	1.87	2.0 (0.85-4.37)	0.17
CT+TT	38 (17.2)	48 (21.3)	9.71	2.36 (1.40-4.0)	0.001
Goitrogenic Food Intake (No)					
Wild (CC)	87 (39.5)	4 (1.7)	-	Referent	-
Heterozygous (CT)	20 (9.1)	5 (2.2)	4.67	0.18 (0.04-0.74)	0.03
Mutant (TT)	20 (9.1)	4 (1.7)	2.71	0.23 (0.05-0.99)	0.09
CT+TT	40 (18.1)	9 (4.0)	5.81	0.20 (0.06-0.70)	0.01
Family History (Yes)					
Wild (CC)	63 (28.6)	35 (15.5)	-	Referent	-
Heterozygous (CT)	27 (12.2)	15 (6.6)	0.037	1.0 (0.47-2.12)	0.84
Mutant (TT)	6 (2.7)	6 (2.6)	0.42	0.55 (0.16-1.85)	0.51
CT+TT	33 (15.0)	21 (9.33)	0.04	0.87 (0.44-1.73)	0.83
Family History (No)					
Wild (CC)	79 (35.9)	133 (59.1)	-	Referent	-
Heterozygous (CT)	20 (9.09)	21 (9.3)	1.46	1.60 (0.81-3.14)	0.22
Mutant (TT)	25 (11.3)	15 (6.6)	7.8	2.80 (1.40-5.64)	0.005
CT+TT	45 (20.4)	36 (16.0)	7.30	2.10 (1.25-3.53)	0.007
Boiled Water Intake (Yes)					
Wild (CC)	92 (41.8)	121 (53.7)	-	Referent	-
Heterozygous (CT)	29 (13.18)	22 (9.7)	2.57	1.73 (0.93-3.21)	0.10
Mutant (TT)	17 (7.7)	7 (3.11)	5.56	3.19 (1.27-8.02)	0.01
CT+TT	46 (20.1)	29 (12.8)	6.60	2.08 (1.21-3.57)	0.01
Boiled Water Intake (No)					
Wild (CC)	50 (22.7)	47 (20.8)	-	Referent	-
Heterozygous (CT)	18 (8.1)	14 (6.2)	0.06	1.21 (0.54-2.70)	0.79
Mutant (TT)	14 (6.3)	14 (6.2)	0.005	0.94 (0.40-2.18)	0.94
CT+TT	32 (14.5)	28 (12.4)	0.002	1.07 (0.56-2.04)	0.95

a; OR - Odds Ratio.

b; CI - Confidence Interval.

*; χ^2 - Chi square test.

Discussion:-

Endocrine disorders are significant contributors to abnormal human physiological functions. Hypothyroidism is a most common type of endocrine disorder, other than diabetes mellitus (19). This study demonstrates the association between two genetic variations in the TPO gene (Thyroid peroxidase) and enhanced hypothyroid susceptibility. The results suggested that there may be an increased risk of developing hypothyroidism in the presence of some alleles in the studied polymorphisms. This is the first study showing that the TPO gene codon T2229C exon 12 variant genotypes are associated with an increased risk of thyroid disorders in a Kashmiri population. The frequency of cases and controls was found to be higher in the age group of 27-37 years old (30.6% and 31.85%) followed by 38-48 years age group (20.0% and 20.4%) respectively. Subjects with an increased basal metabolic index (BMI) were also seen to be at an increased risk of developing hypothyroidism. All the subjects (patients as well as healthy group) in our study were non-vegetarian, i.e., their main diet was red meat, fish, chicken, etc. According to a survey on the prevalence of thyroid disorder induced by demography and food habits in a South Indian population; the relative risk of developing a thyroid disorder is quite high in non-vegetarian population and excessive intake of iodine rich food can also lead to an auto-immune thyroid disorder. (6). Another research article reported that a high-fiber diet can cause fluctuation of thyroid hormones (20). A single nucleotide polymorphism of thyroid peroxidase gene (TPO) codon T2229C was also analyzed as an approach to the association of genetic characterization with a thyroid disorder in Kashmiri population. The result suggested that the variant genotypes (C/C + T/T) or the presence

of the T allele in T2229C polymorphism has a positive association with hypothyroidism. It was also significantly associated with different risk determinants. TPO variant genotypes (CC+TT) were observed in females, urban residents, goitrogenic food consumers as well as non-consumers, never as well as heavy smokers (≥ 1 pack per day) and subjects with normal basal metabolic Index (BMI). Such distribution of vulnerable genotypes among different determinants is because of unknown biological reason. Genetic variations in thyroid peroxidase gene (TPO) in association with anti-TPO levels have been reported earlier by some studies. Anti-TPO levels can be found in over 90% of patients with autoimmune hypothyroidism and graves disease. TPO antibodies together with TG antibodies are the predominant antibodies found in autoimmune hypothyroidism (21, 22). In this case-control study, the results obtained by sequencing the TPO gene suggested that this gene variation is associated with an increased risk of hypothyroidism. According to a family based study, the genetic variation in the TPO gene with an increasing anti-TPO level lead to congenital hypothyroidism (23). Another study reported that this genetic variation in the exon 10 of the TPO gene has an important effect on the thyroid peroxidase enzyme activity (24). Analysis of these variations in TSH levels and the gene/s responsible may be particularly important in a population at risk for abnormal TSH levels.

Conclusion:-

This is the first observational study to examine the association between the single nucleotide polymorphisms (SNP) of the TPO gene and risk of hypothyroidism in Kashmir valley. Our study demonstrated a significant association between the TPO genetic polymorphism and hypothyroidism in Kashmiri population, suggesting a possible role for TPO T2229C polymorphism in determining the risk of hypothyroidism in our valley. Specifically, this study emphasized the effect of different lifestyle and environmental factors, e.g. age, sex, smoking status, residence, food intake, BMI, etc. as the potential confounders which could modify the association between the TPO gene SNP and hypothyroidism.

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Competing Interests:-

The authors have declared that no competing interest exists.

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