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Unraveling the Molecular Mechanisms of XRCC1 Gene SNPs in Thyroid Cancer Pathogenesis

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Abstract

This study aims to find the association of polymorphism Arg194Trp (rs1799782) with thyroid cancer in Southern Punjab. For this purpose, 60 samples of cases and 60 samples of controls were collected from the Multan Institute of Nuclear Medicine and Radiotherapy (MINAR) along with associated data. Factors included in this study were gender, age, smoking status, alcohol consumption, family history of cancer, family history of thyroid cancer, diet, exposure to radiation in childhood, and diabetes. Clinicopathological characters included in the study were types of thyroid cancer and grade of tumor. In the case of women, the number of pregnancies, use of oral contraceptives, and menstruation history were included. DNA extraction was done by using the salt extraction method and for amplification of DNA tetra ARMS PCR was done. The frequency of three genotypes CC, TT, and CT of Arg194Trp (rs1799782) was found to be 49, 3, and 8 in patients while it was 43, 7, and 10 in controls respectively. The allelic frequency of C and T was 0.8 and 0.2 in controls respectively. While in patients' allelic frequency of C and T was 0.9 and 0.1 respectively. For statistical analysis, SPSS version 20 was used. After statistical analysis, Arg194Trp polymorphism was not found to be associated with thyroid cancer. However, the association of this cancer was found with gender, family history of cancer, diet, oral contraceptives, and menstruation history.

Keywords: XRCCI, SNPs, DNA, Genotype, Thyroid Cancer,

INTRODUCTION

The thyroid gland is the single largest organ that is specific for the production of endocrine hormones. Regarding anatomy, the thyroid gland comprises 2 lobes. These two lobes are attached by an isthmus which is a thinner tissue band and a capsule surrounds it, as shown in Figure Depending on gender, body weight, and iodine supply, average weight of thyroid gland in adults is 15-25g (Hubbard *et al.*, 2009). Structurally thyroid gland is made up of two main types of parenchymal cells i.e. parafollicular (C cells) cells and thyroid follicular cells. The thyroid cells or thyroid follicular cells make follicles, spherical structures that are structural unit of thyroid. The follicles and thyroid cells use for concentrating iodine, storage, secretion and production of hormones of thyroid gland (Werner *et al*., 2005). The parafollicular cells secretes calcitonin, a hormone that control calcium homeostasis, but in humans physiological role of calcitonin is not apparent (Carling *et al.*, 2014).

The term thyroid cancer (TC) is referred as the abrupt growth of cells within the thyroid gland (Keefe *et al*., 2010). It is the one of well-known malignancy of endocrine organ and it represents about one percent of all tumors furthermore 0. 2% of deaths and its occurrence increase rapidly in recent years (Jemal *et al*., 2011). According to the recent report of global cancer statistics between 2006-2010, the occurrence ratio of TC in all races is 6.2 and 18.2 per 100,000 males and females respectively (Ai *et al*., 2014).

Thyroid follicular epithelial cells give rise to large no. of thyroid tumors while C cells or parafolliluar cells give rise to 3-5% of cancers. Cancers originated from follicular cells are subdivided into follicular carcinoma, anaplastic (undifferentiated) carcinoma, well-differentiated papillary carcinoma and poorly differentiated carcinoma (also called insular carcinoma) (DeLellis *et al*., 2004). Some follicular carcinomas arise from follicular adenoma that is a benign tumor. Anaplastic carcinoma and poorly differentiated carcinoma that are enlisted as less-differentiated thyroid cancer, can originate de novo, while many of them originate by gradual dedifferentiation of follicular and papillary carcinoma (Nikiforov *et al*., 2011).

Previous irradiation is the most familiar risk factor for PTC which reports to be 80% of all thyroid cancers. Deficiency and excess of iodine, history of benign thyroid disorder for example autoimmune thyroid disorder and nodules besides family history are risk factors of papillary thyroid cancer (Hemminki *et al*., 1997). Women are more susceptible to TC than man and the ratio is 3:1. This difference of gender in occurrence of thyroid cancer propose that risk factor of the disease may be associated to reproductive and hormonal function, And this practice is seen over ethnicity and geographic location (Blot *et al*., 1997).

In BER pathway X-ray repair cross complementation group 1 (XRCC1) protein play key role (Hung *et al*., 2005). It is required in cell for efficient repairing of bases and breaks in single strand (Hung *et al*., 2005). While testing on mice it was found that presence of this protein is essential for existence of mammals because absence of this protein is lethal to embryonic

development. The initiation of DNA repairing after genetic mutation may undergo polymorphism and this will lead to cancer (Arizono *et al*., 2008).

XRCC1 is found to have association with gap-filling DNA polymerase β (POLβ), DNA ligase 3a (LIG3a), poly (ADP-ribose) polymerase 1 (PARP-1) and DNA 30phosphatase (PNKP) in base excision repair (BER) pathway (El-Khamisy *et al*., 2003). According to a recent study transcription factor E2F1 enhance DNA repair and regulates XRCC1 (Chen *et al*., 2008). There are 2 BRCA1 carboxylterminal (BRCT) domains i.e. BRCT1 at center and BRCT2 at C-terminal of XRCC1. The function of BRCT2 is to stabilizing and binding of DNA ligase III and responsible for single-strand breaks and gaps repair (SSBR), especially during the G0/G1 phases of cell cycle (Moore *et al.*, 2000). PARP1 is a single-strand breaks and gaps recognition protein that is responsible for SSBR during the two phases of cell cycle (G1 and S/G2) The center of BRCT1 binds to PARP1 and down regulate it. The location of XRCC1 is at domain that separates but connects BRCT and XRCC1 NH2 terminal. By mutation of Arg194Trp the function of XRCC1 may not be changed but the structure of XRCC1 will change. It is reported in some studies that the polymorphism of XRCC1 Arg194Trp is associated with risk of cancer (Feng *et al*., 2014).

The stabilization of OGG1 on AP site is done by XRCC1 on double stranded DNA until the binding of APE1 to DNA. This will allow the migration of substrate DNA from DNA glycosylase to AP endonuclease. XRCC1 and APE1 interact with Polβ. XRCC1 play dominant role in BER and to stabilize LIG. There are chances that the four proteins (XRCC1, LIG3α, PARP-1 and Polβ) may bind and form a complex that arbitrate XRCC1 and its BER allies (Norjmaa *et al*., 2016). The location of XRCC1 gene is 19q13.3. It is 33 kb long and consisting of seventeen exons, and encodes a transcript of 2.2 kb which produces X-ray cross-complementing group enzyme which involves in BER pathway (Wang *et al*., 2014). Polymorphism of XRCC1 disturb the interaction between other enzymatic protein and XRCC1 as a result DNA repair capacity overcome, leading to carcinogenesis and genetic instability (Forat-Yazdi *et al*., 2015).

Three polymorphisms of XRCC1 genes are observed at: Codon 194 (rs1799782, C > T substitution at position 26304, exon 6, Arginine to Tryptophan), codon 280 (rs25489, C > T substitution at position 43552260, exon 9, Arginine to Histamine) and codon 399 (rs25487, G > A substitution at position 28152, exon 10, Arginine to Glutamine) (Santos *et al*., 2012; García-Quispes *et al*., 2011; Halkova *et al*., 2016) shown in Figure 3. Recent studies have elaborated the association of TC with polymorphism of XRCC1 (Nedooshan *et al*., 2017). The cells comprising XRCC1 194 Arg/Arg genotype exhibit greater damage to DNA when exposed to bleomycin orbenzo[a]pren-diol–epoxide while the cells having XRCC 194 Trp allele shows less exposure to damage (Wang et al., 2003). Moreover, it is also observed in studies that the patients are more affected by DNA damage (chromosomal breaking) who have genotype XRCC1 194 Arg/Arg

than the individuals having 194Trp allele (Tuimala *et al*., 2004). Inspite of this, there are some conflicting reports that shows the higher frequency of change in chromosome in patients having XRCC1Trp allele (Seibold *et al.*, 2015).

By analyzing the biochemical properties of XRCC1 it is expected that Trp allele is associated with risk of all type of cancer (Wang *et al*., 2009). According to result of a meta-analysis Arg194Trp polymorphism is linked with breast cancer risk, glioma risk in Asians and lung cancer risk in Caucasians. Additionally Arg194Trp polymorphism is associated with some other types of cancer such as pancreatic, gastric, nasopharyngeal and prostate cancer (Feng *et al*., 2014). Another meta-analysis suggest that polymorphism XRCC1 rs1799782 is associated with thyroid cancer development (Nedooshan *et al*., 2017). The aim of present study was to find out the genotypic frequency of XRCC1 gene polymorphism rs1799782 in patients and controls. In this study we also evaluate the allelic frequency of alleles C and T in patients and controls and determine the relationship of genetic factors with TC.

MATERIAL AND METHODS

Data collection and Sampling

The study comprised of 60 blood samples of patients of thyroid cancer visited Multan Institute of Nuclear Medicine and Radiotherapy (MINAR) Nishtar Hospital Multan recruited from November 2018 to April 2019. Blood sample (3ml) was taken from each thyroid cancer patient into 5ml vacutainer tube containing EDTA (Ethylene diamine tetra acetate) to a concentration of 1.5-2.0 mg/ml blood. To prevent the coagulation of blood, blood was mixed with EDTA by repeating tilting of tube. Then sample was stored at -20°C in deep freezer untill further procedure.

All patients signed a written consent before being entered into the study, and they were interviewed to fill the proforma regarding risk factors including demographic factors, genetic factors and clinical pathology of thyroid cancer. Risk factors such as, history of any other disease, family history of cancer, diet of patients, exposure to radiation, lifestyle of patients, age, gender, grade of cancer, obesity, smoking status, dwelling and diabetes. In addition in case of women use of contraceptive pills, no. of births and no. of pregnancy were included in the questionnaire. Biopsy proven thyroid cancer patients of any age and gender were involved in this study.

Controls subjects

Study consisted of 60 controls subjects who came for regular medical check up at laboratory of Nishtar hospital Multan and MINAR. 3ml blood was collected into EDTA tube and stored at -20°C for extraction of DNA. Age and gender matched persons with no cancer history were taken as controls.

Extraction of DNA

The extraction of DNA was carried out from either groups (control and test) by salt extraction method (Helms, 1990).

Procedure

3ml of blood, 3ml of cold RBCs lysis buffer (0.32M sucrose, 5mM $MgCl_2$, 10 mM Tris HCl, 1% Triton X-100) and 6 ml of sterile, cold, distilled water were added in falcon tube (15 ml). The tubes were inverted and samples were incubated on ice for 15 minutes.

After incubating on ice, centrifugation of samples was carried out in centrifuge machine (model no universal 320R Hettich, zentrifuge) for 15 minutes at 2205×g rpm and 4 degree centigrade. Taking care of pellet, supernatant was discarded from the other side of pellet in bleach solution (10%).

Cold buffer solution A (2ml) and distilled water (6ml) were added to pellet and vortexed the solution at medium speed to resuspend pellet.

After resuspension Solution was centrifuged at 2205×g for fifteen minutes at four degree centigrade. Taking care of pellet, supernatant was discarded from the opposite side of pellet in bleach solution (10%) carefully.

Step 3 and 4 were repeated until creamy white pellet appeared.

To the pellet 5ml of buffer B (20mM Tris HCl, 4mM Na_2EDTA, 100mM NaCl) and 500µl of 10 percent SDS was added and vortexed vigorously to resuspend pellet. In each tube 25 µl of fresh, cold solution of proteinase K ((20mg/ml)) was introduced. Incubation of samples was carried out overnight at 45°C and then samples were taken from incubator and incubated on ice for 10 minutes. Then 4ml of 5.3 molar NaCl was added in each sample, the solution turned cloudy. It was vortexed for 15 seconds. Solution was centrifuged at 3645×g for 20 min at four degree centigrade. Supernatant was poured off into a new tube of 15 ml. Supernatant was recentrifuged as in the previous step. Supernatant was poured off to a new falcon tube (50ml) and an equal volume of isopropanol (ice cold) was added in tube. For precipitation of DNA tubes were inverted gently 5-6 times. After precipitation of DNA, the centrifugation of solution was carried out at 3645×g for twenty min at 4 degree centigrade. The washing of DNA pellet was done with 1ml of 70% ethanol. Tubes were inverted 5-6 times gently and centrifugation was done at 3645×g for 10 min. After centrifugation, the position of DNA pellet was marked after discarding supernatant. The drying process of DNA pellet was carried at 37°C in incubator for about 20 min. To DNA pellet 300µl of low TE (1M Tris HCl, 0.5M EDTA) was added to dissolve it and for overnight left it at room temperature. DNA was shifted to 1.5ml eppendorf tubes and heated

in water bath at 70°C for 1 hour (so that any remaining nucleases are inactivated). Extracted DNA was kept and stored at -20°C in deep freezer.

Gel Electrophoresis

Agarose gel electrophoresis was used to visualize DNA. TAE buffer was used for gel running. 50X TAE buffer was prepared by dissolving 242g of Tris base in H_2O and 57.1ml of glacial acetic acid was added in it. Stirred on magnetic stirrer and volume of the solution was made up to the mark of 1000ml with distilled H_2O. Twenty ml of 50X TAE buffer and 980 ml of distilled water was dissolved in volumetric flask to make 1X TAE buffer and shaken the solution well. Agarose gel (1%) was prepared by mixing 0.25 gram of agarose powder in 20ml of 1X TAE buffer, heated in oven for few seconds to dissolve agarose powder. The gel solution was cooled to about 60 degree centigrade and then 0.5 micro gram per milliliter of ethidium bromide was added in gel. The prepared gel solution was poured in gel casting tray and comb was inserted in it. The gel was left at room temperature for 30 min to polymerize it. The gel was left at room temperature for 30 min to polymerize it. After polymerization of gel, it was shifted to gel tank. Comb was removed carefully. 1X TAE buffer was added in gel tank in a way that gel was submerged in 1X TAE buffer. 6 microliter of DNA sample was mixed with 2µl of DNA loading dye (6X) and sample was loaded into wells of gel carefully. The gel was run at 80V for 5 min and at 100V for 20 min with power supply to resolve DNA under the presence of electric field. The observation of gel was done by using UV transilluminator and saved in gel documentation system.

Qualitative and quantitative assessment of DNA

Quantity and quality and of DNA was measured by spectrophotometer by measuring optical density (OD) / absorbance at 260nm and 280nm. Optical density at 260nm showed the quantity of DNA while the ratio of OD at 260 and OD at 280 depicted the quality of DNA. If the ratio was greater than 1.7, the quality of DNA was good while if the ratio was less than 1.7, the quality of DNA was bad. Aliquots of DNA (50ng/µl) were made and stored at -20°C.

Amplification of DNA by tetra arms PCR

Amplification of SNP rs1799782 was done by tetra arms PCR by using G-storm (GS4822) thermocycler. Primers details used for PCR are given in table below (Salimi *et al., 2014).*

Table I: Primers for rs1799782 gene XRCC1 analysis by tetra primer arms PCR (Salimi *et al., 2014).*

Gene	SNP No.	Primer ID	Sequence (5'-3')	Product size (bp)
XRCC1	rs1799782	FI	CGGGGGCTCTCTTCTTCATCC	Outer:47

	(Arg194Trp)	RI	CACCTGGGGATGTCTTGTTGATACA	1 Arg:297 Trp:219
		FO	CGTCCCAGGTAAGCTGTAC	
		RO	CACTCCTATCTATGGGACACAG	

Tetra Arms PCR

Master mix was prepared by mixing PCR buffer, $MgCl_2$, 5U/µl of Taq DNA polymerase, dNTPs, DMSO, reverse outer primer, forward outer primer, forward inner primer, reverse inner primer and sterile water to make final volume of 18µl. In each tube 2µl of DNA was taken and then 18µl of PCR master mix was added and run in thermocycler.

Table II: Components of PCR

Components of PCR	Final Concentraion	Volume(µl)
10X Buffer	1X	2
25mM $MgCl_2$	2.5 mM	2
DMSO	8%	1.6
Taq DNA polymerase	5U/ µl	0.3
2.5Mm dNTPs	200 µM	2
10 µM Primers (forward inner and reverse inner)	7.5 µM	0.75
10 µM Primers (forward outer and reverse outer)	10 µM	1
Template DNA (50ng/ µl)	100ng	2 µl
H_2O		7.1

Cycle conditions of PCR

For PCR amplification Initial denaturation of DNA was done at 95 degree centigrade for 5 minutes, followed by 35 cycles (95°C for thirty seconds and annealing was occurred at 60°C for

thirty seconds, extension at 72°C for thirty seconds), final extension at 72°C for seven minutes and finally hold at 4°C (Figure1).

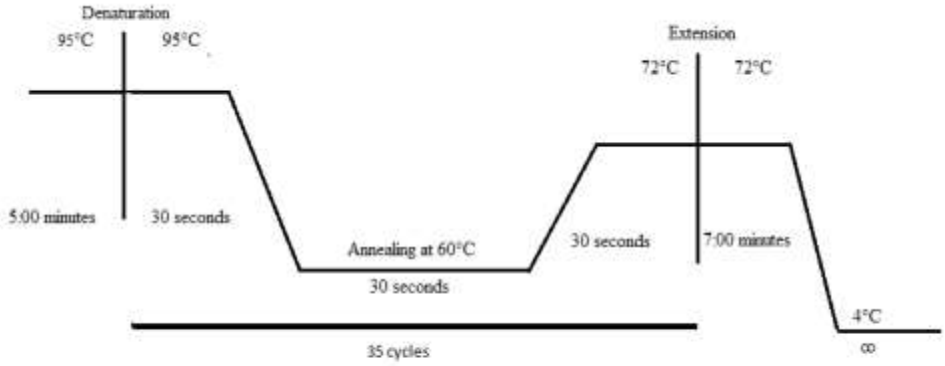


Figure 1: Amplification conditions for PCR

Visualization of PCR products by agarose gel electrophoresis

2% gel was prepared by mixing 1.8g of agarose powder in 90ml of 1X TAE buffer, agarose solution heated in oven to dissolve agarose powder. After cooling the gel solution to about 60°C, 6µl of ethidium bromide was added in it. Gel solution was shifted in gel casting tray and comb was introduced in it. Gel was allowed to polymerize at room temperature. After the polymerization of gel, comb was removed. 2µl of loading dye was added in each sample. In gel, samples were loaded. DNA ladder was also loaded in gel as a reference to compare the product size. Gel was run on 80 V for initial 5 min and 100V for approximately 20 min. It was observed using UV transilluminator and saved in gel documentation system.

Statistical Analysis

Data collected from patients and controls was tabulated in the form of Microsoft excel sheet for statistical analysis of association of risk factors, genotypic and allelic frequency of polymorphism rs1799782 by using SPSS software version 20 with thyroid cancer patients and controls. P values ≤ 0.05 was considered to be statistically significant.

RESULTS

Characteristics of study population

Study population consisted of 120 subjects, including 60 thyroid cancer patients and 60 controls. In southern Punjab thyroid cancer was more prevalent in women as compared to men. Minimum and maximum age for cases was 7 and 75 and for controls it was 7 and 70. Mean age for thyroid

cancer patient was 42 and divided in two age groups i.e. ≤42 and >42. Other risk factors for thyroid cancer included in this study were gender, age, smoking status, alcohol consumption, family history of cancer, family history of thyroid cancer, exposure to radiation in childhood, diabetes and diet i.e. divided in three sub groups (meat fonder, vegetarian and mixed). In case of women following data was collected: number of pregnancy was divided in two groups (0-5 and 6-10), menstruation was divided in four groups (regular, irregular, menopause and no i.e. premenarche). Clinical characteristics of thyroid cancer patients included in this study were grade of tumor (1, 2, and 3 grade are observed in cases) and types of thyroid cancer (PTC and FTC are found in studied cases.

Table III: Association of demographic factors of cases and controls

Risk factors	Cases (n=60)	Controls (n=60)	P-value
Gender			
Male	13 (21.6%)	29 (48.33%)	0.002
Female	47 (78.33%)	31 (51.66%)	
Age			
≤42	31 (51.66%)	31 (51.66%)	0.572
>42	29 (48.33%)	29 (48.33%)	
Smoking Status			
Smokers	5 (8.33%)	3 (5%)	0.359
Non Smokers	55 (91.66%)	57 (95%)	
Alcohol Consumption			
Yes	5 (8.33%)	0 (0%)	0.29
No	55 (91.66%)	60 (100%)	
Family history of cancer			
Yes	24 (4%)	5 (8.33%)	0.000
No	36 (60%)	55 (91.66%)	
Family history of thyroid cancer			
Yes	10 (16.66%)	5 (8.33%)	0.135
No	50 (83.33%)	55 (91.66%)	
Diet			

Meat	5 (8.33%)	3 (5%)	0.031
Vegetarian	25 (41.66%)	13 (21.66%)	
Mixed	30 (50%)	44 (73.33%)	
Exposure to radiation in childhood			
Yes	19 (31.66%)	23 (38.33%)	0.283
No	41 (68.33%)	37 (61.66%)	
Diabetes			
Yes	16 (26.67%)	14(23.33%)	0.417
No	44 (73.33%)	46(76.67%)	
In In case of women			
No. of pregnancy			
0-5	17 (36.17%)	5 (16.12%)	1.000
6-10	21 (44.68%)	9 (29.03%)	
Use of oral contraceptives			
Yes	4 (8.51%)	8 (13.33%)	0.001
No	43 (91.48%)	22 (36.66%)	
Menstruation			
No	3 (6.38%)	1 (3.2%)	0.000
Regular	18 (38.29%)	21 (67.74%)	
Irregular	13 (27.65%)	1 (3.2%)	
Menopause	13 (27.65%)	7 (22.58%)	

Clinical characteristics of thyroid cancer patients

Table IV: Grade of tumor

Grade of tumor	Frequency	Percentage
1	13	21.7%
2	32	53.3%
3	15	25%

Table V: Types of thyroid cancer

Types	Frequency	Percentage
FTC	12	20%
PTC	48	80%

Isolation and quantification of DNA

DNA of 60 cases samples and 60 controls samples were isolated by using salt extraction method of DNA and analyzed on 1% agarose gel. Quantity and quality of DNA was measured by spectrophotometer. DNA isolated from cases and controls is shown in Figure 5.

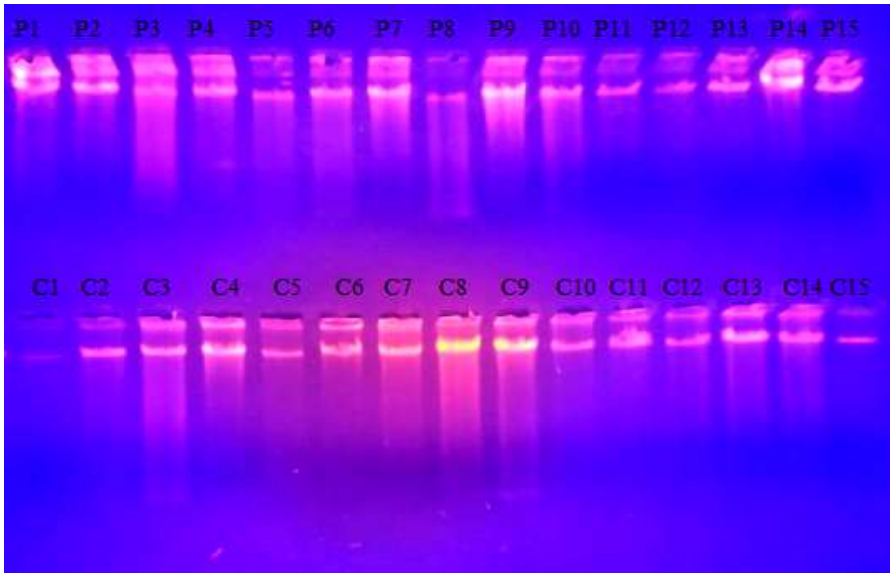
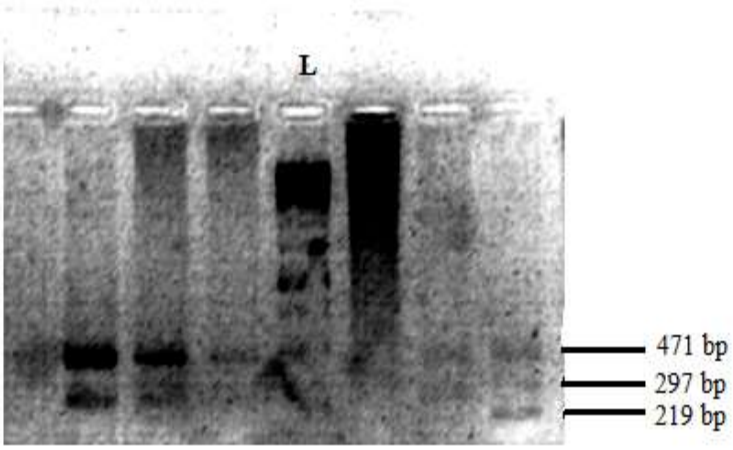


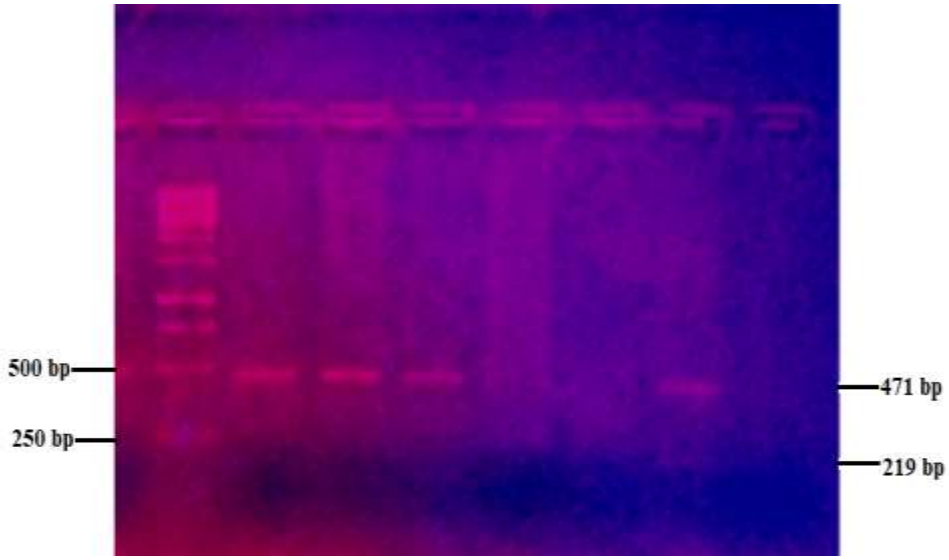
Figure 5: Representative agarose gel (1%) agarose gel showing human genomic DNA. Upper lane: DNA samples of patients (P1-P15). Lower lane: DNA samples of controls (C1-C15)

Genotypic analysis of DNA by PCR

The region of XRCC1 gene containing codon 194 (rs1799782, exon 6) was amplified by Tetra ARMS PCR in cases and controls and analyzed on 2% agarose gel in which 1kb DNA ladder is used for comparison of size, as shown in Figure 6.



(a)



(b)

Figure 6: Representative Agarose gel (2%) showing the amplification of XRCC1 gene polymorphism (rs1799782). Outer band (471bp), product size of C allele (297bp), product size for T allele (219bp). L showing the ladder (1kb). (a) Cases (b) Controls.

Gender

Data analysis of controls and cases showed that there were 13 (21.6%) males, 47 (78.33%) females in thyroid cancer patients and 29 (48.33%) males, 31 (51.66%) females in controls. Statistical analysis of gender gave a P-value of 0.002 which showed that gender is significantly associated with thyroid cancer. The data for gender is shown in Figure 7 as bar graph in cases and controls.

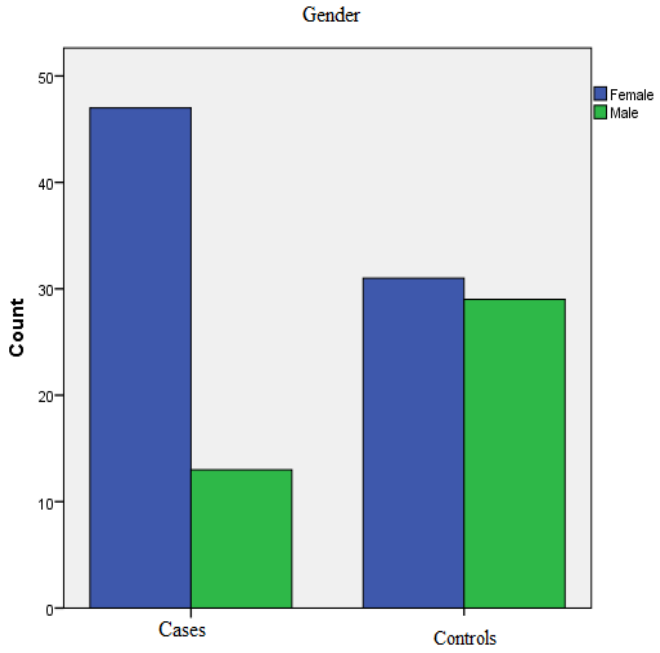


Figure 7: Graphical presentation of gender in cases and controls.

Smoking Status

Regarding smoking status it was analyzed that out of 60 controls and cases, 57 (95%) controls were non-smokers and 3 (5%) were smokers. Among cases 55 (91.66%) were non-smokers and 5 (8.33%) were smoker. Statistical analysis of smoking status gave a P-value of 0.359 which showed that smoking status is not associated with thyroid cancer. Comparison of smoking status of cases and controls is shown in Figure 8 as bar graph.

Alcohol Consumption

By comparing the data of controls and cases it was analyzed that in cases 5 (8.33%) were consuming and 55 (91.66%) were not consuming alcohol, while in controls no individual consumed the alcohol. Statistical analysis for alcohol consumption showed a P-value of 0.29 which shows that alcohol consumption is not associated with thyroid cancer (Figure 9).

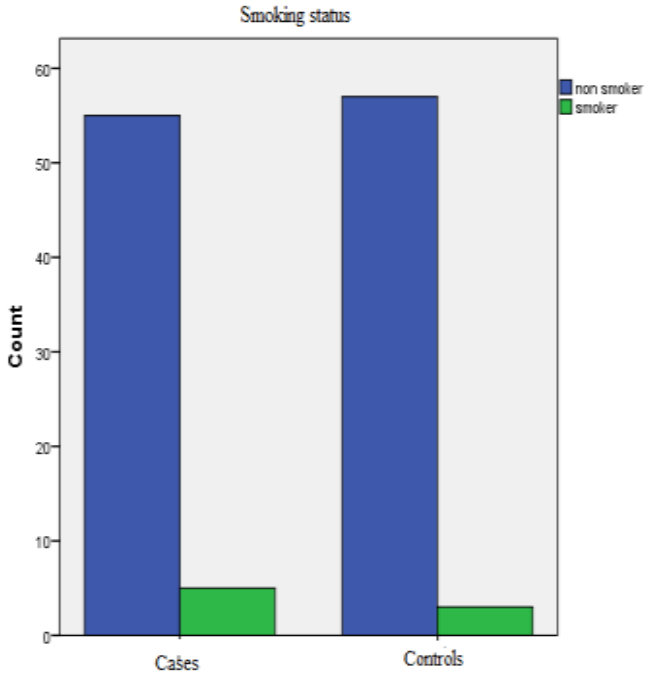


Figure 8: Graphical presentation of smoking status in cases and controls.

Family history of cancer

In cases 24 (40%) were with and 36 (60%) were without family history of cancer. In controls 5 (8.33%) were with and 55 (91.66%) were without family history of cancer. Statistical analysis for family history of cancer gave a P-value of 0.000 which showed that family history of cancer is significantly associated with thyroid cancer. Bar graph of family history of cancer in cases and controls is shown in Figure 10.

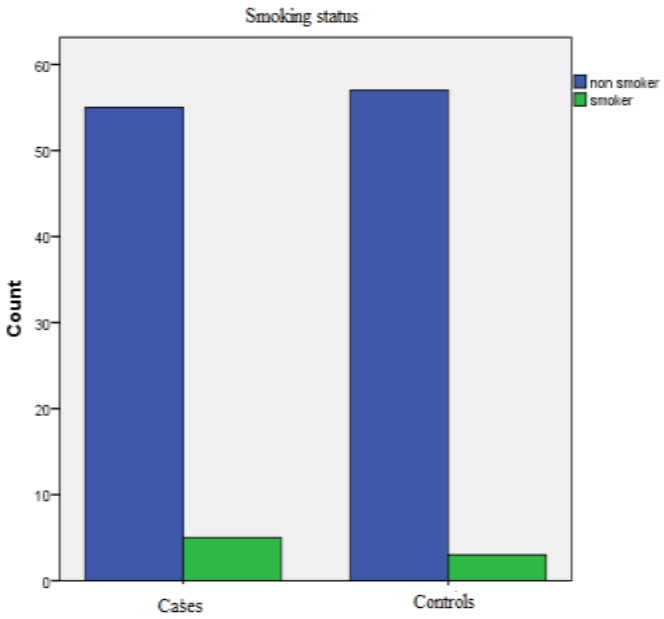


Figure 9: Graphical presentation of alcohol consumption in cases and controls.

Family history of thyroid cancer

In cases 10 (16.66%) individuals were having family history of thyroid cancer while 50 (83.33%) were with no family history of thyroid cancer. In controls, 5 (8.33%) were with family history of thyroid cancer, and 55 (91.66%) were with no family history of thyroid cancer. Statistical analysis for family history for thyroid cancer showed a P-value of 0.135 which shows that family history of thyroid cancer is not associated with thyroid cancer. Bar graph of family history of thyroid cancer of controls and cases is shown in Figure 11.

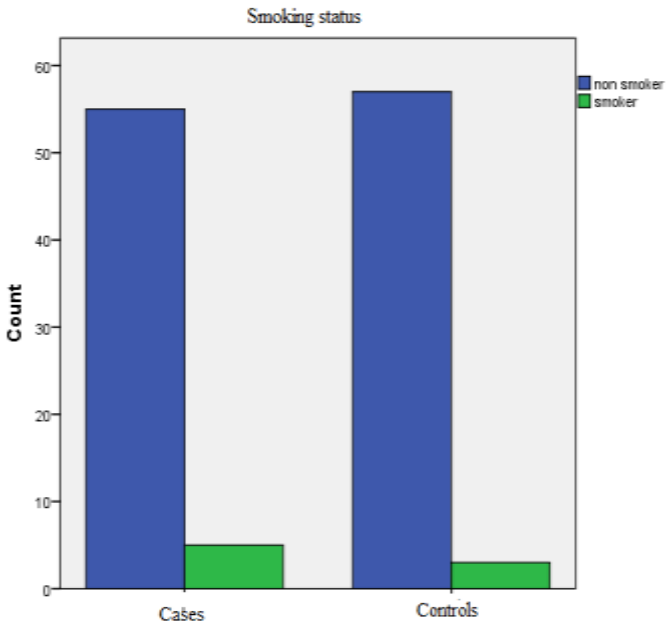


Figure 10: Graphical presentation of Family history of cancer in cases and controls.

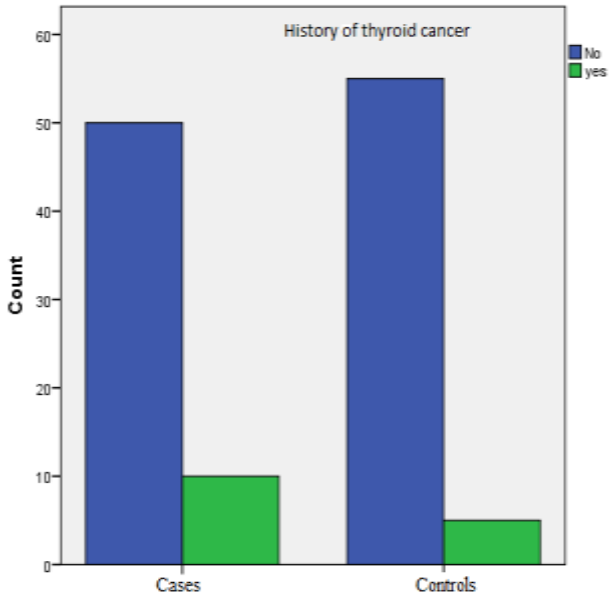


Figure 11: Graphical presentation of Family history of thyroid cancer in cases and controls.

Diet

Diet is divided into three groups i.e. meat fonder, vegetarian and mixed. In cases the distribution for meat fonder, vegetarian and mixed diet is 5 (8.33%), 25 (41.66%) and 30 (50%) respectively.

While for controls the distribution is 3 (5%), 13 (21.66%) and 44 (73.33%) respectively. Statistical analysis for diet showed a P-value of 0.031 which show that diet is significantly associated with thyroid cancer. Bar graph showing the distribution of diet (Figure 12).

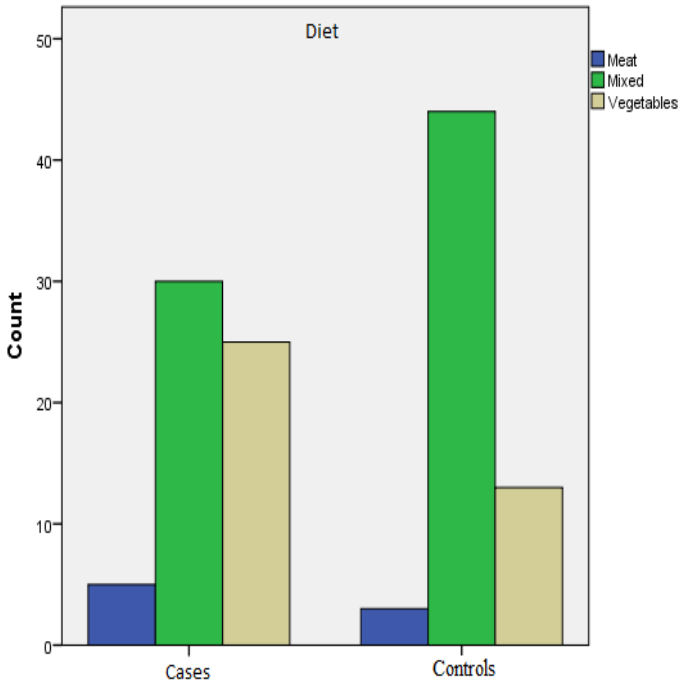


Figure 12: Graphical presentation of Diet in cases and controls.

Exposure to radiation in childhood

In cases, 19 (31.66%) individuals were exposed to radiation while 41 (68.33%) were not exposed to radiation. In controls 23 (38.33%) were exposed to radiation while 37 (61.66%) were not exposed to radiation. Statistical analysis for radiation exposure showed a P-value of 0.283 which shows that radiation exposure is not significantly associated with thyroid cancer. Figure 13 represents the association of radiation exposure with thyroid cancer.

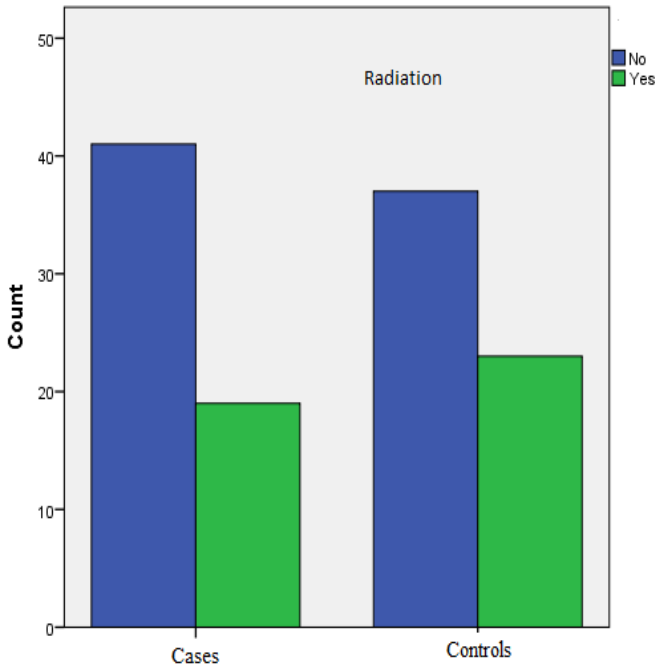


Figure 13: Graphical presentation of radiation exposure in cases and controls.

Diabetes

Data analysis of cases and controls showed that there were 16(26.67%) diabetic and 44(73.33%) non diabetic individuals in thyroid cancer patients. In controls there were 14(23.33%) diabetic and 46(76.67%) non diabetic. Statistical analysis for diabetes showed a P-value of 0.417 which shows that diabetes is not significantly associated with thyroid cancer. The data of diabetes is shown in Figure 14 as bar graph in cases and controls.

Use of oral contraceptives

In cases, out of 47 females oral contraceptives were used by 4 (8.51%) but 43 (91.48%) not used oral contraceptives. In controls out of 31 females, 8 (25.80) of them used oral contraceptives and 22 (70.96) had no use of contraceptives (Figure 15). Statistical analysis for oral contraceptives showed a P-value of 0.04, which shows that this factor is significantly associated with thyroid cancer.

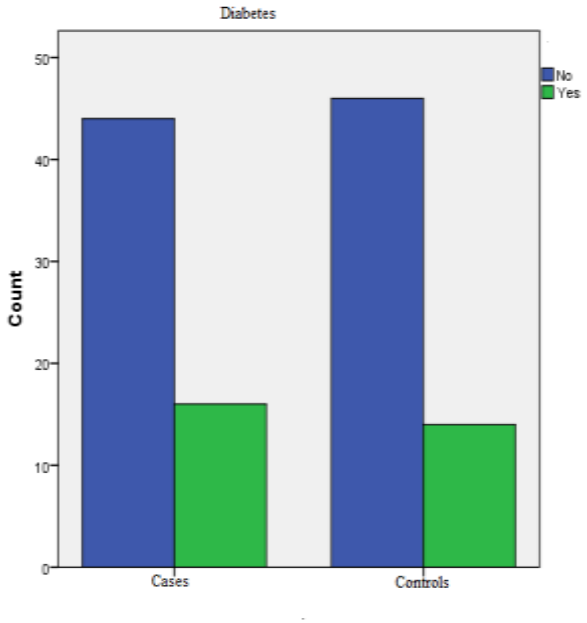


Figure 14: Graphical presentation of diabetes in cases and controls.

Menstrual history

Menstrual history was divided into 4 groups regular, irregular, menopause and no (premenarchal). In cases out of 47 females the distribution is 18 (38.29%), 13 (27.65%), 13 (27.65%) and 3 (6.38%) while in controls 21 (67.74%), 2(6.45%), 7 (22.58%) and 1 (3.2%) respectively. Statistical analysis of menstrual history showed a P-value of 0.00 which shows that this factor is significantly associated with thyroid cancer (Figure 16).

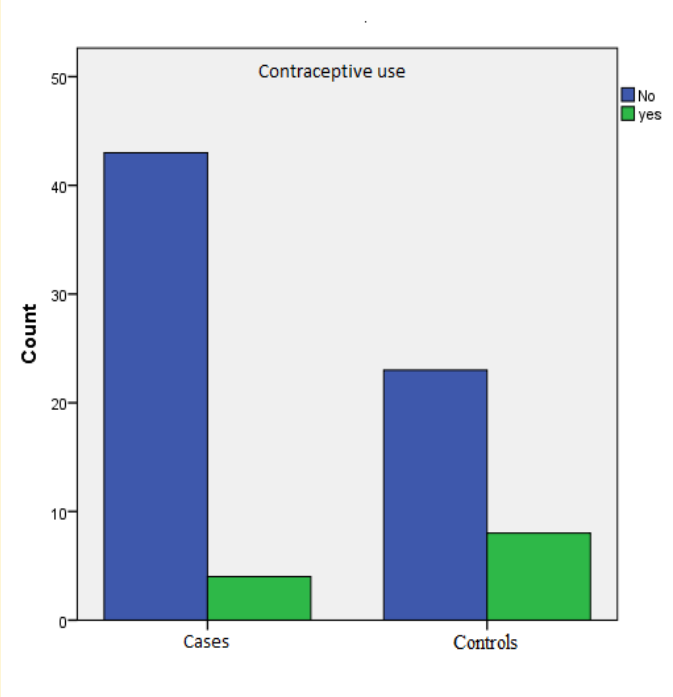


Figure 15: Graphical presentation of oral contraceptive status in cases and controls.

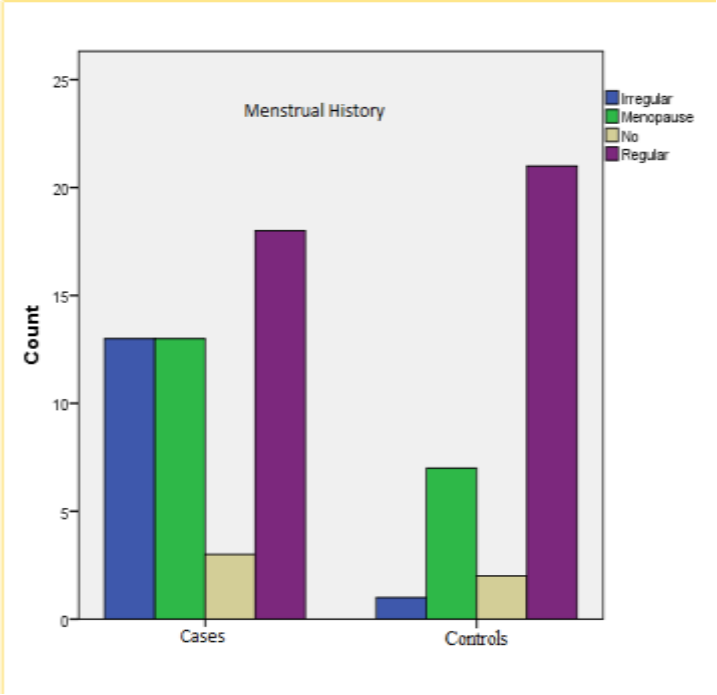


Figure 16: Graphical presentation of menstrual status in cases and controls.

Types of thyroid cancer

Regarding types of thyroid cancer, Follicular thyroid carcinoma was seen in 12(20%) patients, while papillary thyroid carcinoma was seen in 48(80%) patients. Shown in Figure 17.

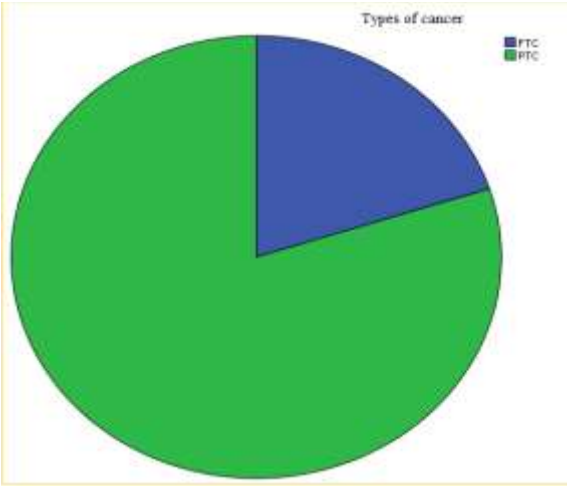


Figure 17: Graphical presentation of types of thyroid cancer.

Association of types of thyroid cancer and Genotype:

Genotype CC was found in 40 PTC and 9 FTC cases. Genotype CT was found in 6 PTC and 2 FTC cases. Genotype TT was found in 2 PTC and 1 FTC cases. (Table VI)

Table VI: Association of types of cancer and Genotype

Types	Genotyping			Total
	CC	CT	TT	
FTC	9	2	1	12
PTC	40	6	2	48
Total	49	8	3	60

Genotyping

Genotyping of XRCC1 SNP rs1799782 (Arg194Trp) was done by Tetra Arms PCR. P-value for genotyping data is 0.331 which show that these genotypes are not significantly associated with thyroid cancer. Genotypic distribution of cases and controls is shown in table VII.

Table VII: Genotypic frequency of SNP (rs1799782) of cases and controls

Genotype	Cases	Controls	P-value
CC	49	43	0.331
TT	3	7	
CT	8	10	

Allelic Frequency

Allelic frequency of controls and patients is given in table below VIII.

Table VIII: Allelic frequency in cases and controls

Allele	Cases N(%) Total=120	Controls N(%) Total=120	Frequency	
			Cases	Controls
C	106 (88.33)	96 (80)	0.9	0.8
T	14 (11.67)	24 (20)	0.1	0.2

DISCUSSION

Thyroid tumor is caused by uncontrolled multiplication of cells of thyroid gland. Thyroid tumor is most frequent tumor of endocrine organ. Globally thyroid cancer incidence is continuously increasing in US and other industrialized countries it has increased three times in last thirty years (Davies *et al*., 2006; Colonna *et al*., 2007).

According to our data the frequency of female (78.33%) is higher as compared to males (21.6%). And P-value is 0.002 which show that gender is significantly associated with thyroid cancer. In New Caledonia its incidence is very high among women (71.4/100,000 person per years). In year 2012 the incidence rate in men is 4.1/100,000, and in women it was 12.6/100,00 (Tcheandjieu *et al*., 2016). According to some studies, thyroid cancer is more diagnosed in women as compared to men and the ratio is 3:1 (Gilliland *et al*., 1997; Kilfoy *et al*., 2009). Albasri *et al*., 2014, reported that in women of Saudia Arabia thyroid cancer is 2nd most frequent tumor. In this area the female to male ratio is 29:4. In Pakistan the female to male ratio is 4:1, and the ratio is 2.2:1 in Karachi, the observed age range for this frequency is 30-60 (Zuberi *et al*., 2004). In Baluchistan the incidence is high among females as compared to males 58 out of 87 were females, 53 percent from these 58 females were between 21 to 40 year age (Iftikhar *et al*., 2011; Bilal[a,b] *et al.,* 2024).

According to our data the mean age of cases is 42 and patients belonged to all ages except for a specific age range that is frequently found among patients. Our data suggests that age range is not significantly associated with thyroid cancer. In contrast to our study, two studies reported that for well differentiated thyroid cancer age is an important prognostic indicator (Gilliland *et al*., 1997; Hundahl *et al*., 1998). According to some studies in females the incidence based on age increases as the reproductive years starts. In case of women the peak age is between 40 to 49 years, In men the peak age is between 60 to 69 year (Ortega *et al*., 2004; Kilfoy *et al*., 2009). In

women the onset of disease is at early age while in men the disease is aggressive at diagnosis (Kilfoy *et al*., 2009b).

In our study, smoking is not found to be associated with thyroid cancer, P-value is 0.359(Table III) which indicates that smoking is not significantly associated with thyroid cancer. Similar to our results, some studies reported no association of smoking and thyroid cancer (Zivaljevic *et al*., 2004; Cho *et al*., 2018). Rather cigarette smoking may cause antiestrogenic effect as Picchi *et al*., 2001, found that cigarette smoking may be a cause for reducing thyroid neoplasia. According to a report, in current smoker females and previously smoker women risk of thyroid cancer is significantly decreased (Boquist, 1993). Galanti *et al*., 1996, observed a marked reduction in risk of thyroid cancer in women who started smoking before 15 years of age. In smokers Level of thyroid autoantibodies and TSH is low and associated with TC risk (Soldin *et al*., 2009). Smoking can also influence the risk of thyroid cancer by altering level of sex steroid hormone (Brand *et al*., 2011). Because of proliferative effect on thyroid (Chen *et al*., 2009), estrogen effect thyroid malignancy and its incidence is higher in women than men (Kilfoy *et al*., 2009). Therefore, smoking because of its anti-estrogenic effect could influence the risk of thyroid cancer (Brand *et al*., 2011).

In our study alcohol consumption is not associated with thyroid cancer with a p-value of 0.29. Similar to our study, some studies reported no association of alcohol consumption with thyroid cancer risk (Rossing *et al*., 2000; Mack *et al*., 2003). In contrast to our study one study reported that alcohol consumption may increase the risk of TC by increasing the level of TSH (Williams, 1976). According to one study alcohol consumption reduced the risk of TC (Huang *et al.*, 2018). There is a direct toxic effect of alcohol on thyroid cells, i.e. reduction of thyroid volume. Thyroid gets some benefits from this toxic effect of thyroid. It is suggested that alcohol consumption work against development of thyroid nodule and goiter (Balhara *et al*., 2013).

Our study suggests that family history of cancer is significantly associated with thyroid cancer. In cases, 24% patients were those who had family history of cancer. P-value for family history of cancer is 0.000 which shows that this factor is significantly associated with thyroid cancer. Similar to our result, a study reported a significant association between family history of cancer and thyroid cancer with odds ratio of 2.630, which showed that chance of getting disease is 2.630 for person who have family history of cancer (Asif *et al.*, 2015). Generally, family aggregation of cancer related to genetic and environmental factor and family members have same habits because they share same environment. In familial risk of different malignancies, genetic characteristics has great importance. It is reported that in family members of thyroid cancer patients higher occurrence of any cancer play a role in risk of various forms of thyroid cancer (Vlajinac *et al*., 1997; Jawad et al., 2023). In contrast to our study Galanti *et al*., 1997, reported that cancer history in parents was not associated with differentiated non-medullary thyroid carcinoma.

According to our data family history of thyroid cancer is not significantly associated with thyroid cancer. P-value is 0.135 which shows that family history of thyroid cancer is not significantly associated with thyroid cancer. In contrast to our study a study reported that individuals with family history of thyroid cancer had 4.5 times increased risk of thyroid cancer than with those who had no family history of thyroid cancer (Brindel *et al*., 2010; Noor *et al.,* 2024; Bilal[a,b] *et al.,* 2024).

In our data Diet showed a significant association with thyroid cancer. The frequency of intake of vegetables is more in our data which suggest that it may be a risk factor for thyroid cancer. Some studies found that intake of cruciferous vegetables cause thyroid cancer (Bosetti *et al*., 2002; Cho *et al*., 2015). In contrast to our study Zamora-Ros *et al*., 2018, found no association between intake of cruciferous vegetables and thyroid cancer.

In our study, P-value for radiation exposure is 0.283, which showed that radiation exposure is not significantly associated with thyroid cancer. In contrast to our study, a study of Dal Maso *et al*., 2009, reported that radiation exposure at early childhood is major cause of thyroid cancer. In Chernobyl after nuclear power plant accident the occurrence of thyroid cancer largely increased. Similar to our results, Kitahara *et al*., 2018, showed no association of thyroid cancer with occupational radiation exposure.

According to our study, diabetes showed a P-value of 0.417 which is not significantly associated with thyroid cancer. Similar to our study, USRT cohort and another study did not show association between diabetes and thyroid cancer (Meinhold *et al.,* 2010). While in contrast to our study NIHAARP cohort showed a positive association in women, not in men with thyroid cancer (Aschebrook-Kilfoy *et al.,* 2011). Diabetes and thyroid cancer are diseases of endocrine glands. According to some studies thyroid cancer patients usually have diabetes (Shih *et al*., 2012). Insulin can stimulate thyroid cancer growth directly through reduced apoptosis or increasing proliferation of cancer cell or indirectly by stimulating other hormone production, like estrogen, TSH and insulin-like growth factor (Hursting *et al*., 2008).

This study showed no association of number of pregnancy with thyroid cancer, P-value is 1.00 (Table III). Similar to our study one study reported that number of birth is not associated with thyroid cancer risk (Dal Maso *et al*., 2009). In contrast to our study, Mannathazhathu *et al*., 2019, showed that parity greater than two is significant risk for thyroid cancer.

In this study, use of oral contraceptive is found to be significantly associated with thyroid cancer. According to a study use of contraceptive pills is associated with thyroid cancer (La Vecchia *et al.,* 1999). In contrast to our study, a study reported no association of use of oral contraceptives with thyroid cancer (Sakoda *et al*., 2002). In contrast to our study Mannathazhathu *et al.,* 2019, observed protective effect of oral contraceptives for thyroid cancer.

Arg194Trp polymorphism is located in regions that are evolutionarily conserved, that's why it is functionally significance. If genotype Arg/Arg occur, the chances of chromosomal break increases (Vodicka *et al*., 2007; Afzal *et al.*, 2024). Six studies analyze the association of thyroid cancer and Arg194Trp polymorphism. Two out of six studies were from Asian population, two from mixed population and two from Caucasian population. No association was found for this SNP with TC in total population (Wang *et al*., 2014). The 194Trp polymorphism cause significant increase in differentiated thyroid carcinoma. A study of Kazakhstan showed that minor allele of rs1799782 associated with decreased risk of thyroid nodule, while the common allele relate to increased risk of thyroid nodule (Sigurdson *et al*., 2009).

According to our study genotyping of the SNP rs1799782 is not significantly associated with thyroid cancer, which may be due to small sample size. It can be further analyzed on larger sample size to have more elaborated results. Similar to our study, some studies reported there is no significant association between risk of DTC and XRCC1 194 C> T (Zhu *et al*., 2004; Adampourezare *et al.*, 2017). In another study it is reported that T allele of Arg194Trp is associated with increased PTC risk (Zhu *et al*., 2018). Wang *et al*., 2015, reported that in Chinese population specifically in smokers and drinkers rs1799782 may be associated with thyroid cancer.

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