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PJMLS OPEN ACCESS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v8i3.3262	Copyright © All rights are reserved by Corresponding Author
Vol. 8 No. 3, 2025: pp. 683-692	
www.readersinsight.net/pjmls	**Revised:** June 09, 2025 **Accepted:** June 20, 2025
Submission: March 22, 2025	**Published Online:** September 30, 2025

GENETIC AND SOCIO-EPIDEMIOLOGICAL ASSESSMENT OF HYPOTHYROIDISM IN QUETTA DISTRICT

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Abstract

Hypothyroidism, a prevalent endocrine disorder, is characterized by insufficient production of thyroid hormones. Those hormones are essential for growth, regulating metabolism, and other body functions. Untreated hypothyroidism can lead to cognitive impairment, infertility, hypertension, etc.

In this study, hypothyroidism in the local vicinity of the Quetta district was evaluated with respect to socio-economic factors (age, gender, ethnicity, area, etc.) and the genes (TSHR, TPO, TITF,1 and PAX8) involved in hypothyroidism. The study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology, Quetta. For this study, two sampling methods were used: retrospective sampling through a structured questionnaire to evaluate the socio-economic factors and biological sampling through the collection of blood for the identification of genes involved in hypothyroidism. A total of 120 samples were collected from Heart and General Hospital, Quetta, out of which 89 were normal, 25 were hypothyroid, and 6 were hyperthyroid on the basis of thyroid hormones levels. Basically, 25 patients were diagnosed positive with hypothyroidism; out of these, 21 (84%) were female and 4 (16%) were male. The findings revealed that the females are generally at higher risk to this disease as compared to males. Other findings revealed that the age group of 26-45 showed more susceptibility as compared to other age groups. Most patients were from the Quetta district and belonged to the Pathan families, and the majority of the affected patients were housewives. On the other side, molecular analysis showed that the rate of existence of the TPO gene showed the highest occurrence in individuals, 81% in females and 75% in males, followed by the TSHR and PAX8 genes. However, the TITF1 gene was least common among the patients.

Keywords: *DNA Extraction, Genetic analysis, Hypothyroidism, Polymerase chain reaction, Thyroxine, Thyroid peroxidase*

INTRODUCTION

Among the endocrine glands, the thyroid is considered an important gland and is located near the base of the neck, and it looks like a butterfly. The thyroid gland is situated underneath the larynx on the sides and anterior of the trachea (1). In all vertebrates, the thyroid and its hormonal secretions have a range of functions that affect the development of organs along with the homeostatic control of different biological processes like body growth and energy expenditure (2).

In addition, cardiovascular, mental disorders, eye problems, and other diseases are linked to thyroid problems (3). There are two main kinds of thyroid disease: hyperthyroidism and hypothyroidism. Hypothyroidism is a disorder marked by unusually low thyroid hormone production (4). On the other hand, hyperthyroidism is a condition in which the thyroid gland produces more thyroid hormone than the body requires (5).

The concentrations of two hormones, called thyroxine and TSH, may be influenced by genes. These two hormones are linked to the majority of thyroid-related conditions. Thyroxine is a product of the thyroid

that aids in controlling metabolism and other body functions, whereas thyroid-stimulating hormone, or TSH, is a hormone that controls the thyroid's ability to generate thyroxine (6). The stability of thyroid hormone levels in the blood is maintained by this negative feedback process that is essential for biological operations of every organ (7).

Possible genes that impact the thyroid function include phosphodiesterase 8B, iodothyronine deiodinase 1, *TSHR* (TSH receptor gene), etc. (8, 9). Thyroid hormone and TSH levels vary between individuals, and the genes that cause these variances are divided into the following categories: (i) genes, i.e., *VEGFA, INSR, IGFBP2, FOXA2,* and *FGF7*, are involved in growth factor; (ii) genetic factors (genes) encode proteins involved in the metabolism (*DIO1, DIO2,* and *DIO3OS*), synthesis (*TG, TPO,* and *CAPZB*), and transportation of thyroid secretions (*MCT8*); and (iii) genes for the proteins that are involved in the thyroid-stimulating hormone receptor signalling pathway *PDE8B, TSHR, PDE10A* etc. (6).

Hypothyroidism is defined as the failure of the thyroid gland to produce sufficient thyroid hormones to meet the metabolic demands of the body. Untreated hypothyroidism can lead to cognitive impairment, infertility, hypertension, and neuromuscular dysfunction. The National Health and Nutrition Examination Survey reported that about one in 300 persons in the United States have hypothyroidism (10). It is considered the most common endocrine disorder in the United States (11). Underactive thyroid disease, or hypothyroid condition, results from alterations in the *PAX8* gene (12) as well as alterations in *TSHR* or *DUOX2* genes. The condition has a dominant autosomal inheritance mode; this indicates that the condition can be caused by one altered replica of the gene in every cell (7, 13, 14). Hypothyroidism may result in various symptoms and clinical signs. These symptoms include depression, fatigue, constipation, muscle pain or weakness, irregular menstrual cycle, dry skin, and poor concentration (10).

The aim of this study was to evaluate healthy individuals with hypothyroid patients in terms of socio-economic characteristics, particularly in the Quetta district, as well as to identify genes involved in hypothyroidism.

MATERIALS AND METHODS

STUDY CENTER

The research was conducted at the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan. The duration of the study was approximately 6 months.

SAMPLING

In this study, two different types of sampling were employed for the collection of data; these methods include retrospective sampling and biological sampling. For the collection of retrospective data, a structured questionnaire was developed to find socio-economic/demographic characteristics (age, gender, ethnicity, etc.) of individuals with hypothyroidism as well as healthy individuals. The blood samples were collected from Heart and General Hospital Quetta through the hypodermic needle and syringe method from different age groups in EDTA (ethylene di-amine tetra-acetic acid) blood tubes. EDTA is used as an anticoagulant that prevents the blood from clotting (15). Samples were stored in the freezer at -20°C.

DNA EXTRACTION

Genomic DNA was extracted using the standard phenol–chloroform method. Frozen whole-blood samples were thawed at room temperature, and 200 µL of each sample was transferred into labeled Eppendorf tubes. A freshly prepared lysis buffer (Tris-HCl 0.121 g, NaCl 0.05 g, EDTA 0.372 g, SDS 1 g; pH 8.0) was added at a volume of 200 µL along with 10 µL of Proteinase K. The tubes were vortexed thoroughly and incubated at 65 °C for 3 hours. Following incubation, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, mixed by vortexing, and centrifuged at 14,000 rpm for 10 minutes. The upper aqueous layer was transferred to a new tube, and twice the volume of chilled absolute ethanol was added to precipitate the DNA. Samples were stored overnight at −20 °C. The next day, tubes were thawed and centrifuged again at 14,000 rpm for 10 minutes. The supernatant was discarded, and the pellet was washed

with 100 µL of 70% ethanol, vortexed, and centrifuged under the same conditions. After air-drying, the DNA pellet was dissolved in 100 µL of TE buffer, vortexed gently, and stored at −20 °C until further analysis. DNA integrity and quality were confirmed using agarose gel electrophoresis.

IDENTIFICATION/AMPLIFICATION OF GENES

Polymerase chain reaction (PCR) was performed to amplify the target genomic DNA using DNA polymerase. For each sample, a 25 µL reaction mixture was prepared containing 1 µL of forward primer, 1 µL of reverse primer, 5 µL of extracted genomic DNA, 10 µL of master mix, and 8 µL of PCR-grade water. The PCR tubes were placed in a thermal cycler and subjected to gene-specific cycling conditions as outlined in Table I. Upon completion of amplification, PCR products were analyzed by agarose gel electrophoresis, and bands were visualized using a gel documentation system (16).

Table I. PCR conditions for the amplification of hypothyroid-associated genes

Genes	Base pair	PCR Conditions	Primers
TSHR	910	Initial denaturation: 95°C for 5 minutes Denaturation: 95°C for 1 minute Annealing: 52 to 58°C for 1 minute Extension: 72°C for 1 minute Final extension: 72°C for 10 minutes Cycles: 35 cycles	Forward GAATTCACCATGAGGCCGGCGGACTTGCTG Reverse CCGCGGTTACAAAACCGTTTGCATATACTC (17)
TPO	238	Initial denaturation: 95°C for 5 minutes Denaturation: 95°C for 1 minute Annealing: 58 to 62°C for 1 minute Extension: 72°C for 1 minute Final extension: 72°C for 10 minutes Cycles: 30 cycles (18)	Forward CCATGTCCAGAGGAAAGGAG Reverse CAGACTCAGGCAGGACAACC (18)
TITF1	204	Initial Denaturation: 95°C for 5 minutes Denaturation: 95°C for 1 minute Annealing: 58 to 62°C for 1 minute Extension: 72°C for 1 minute Final extension: 72°C for 10 minutes Cycles: 30 cycles	Forward TACAAGAAAGTGGGCATGGA Reverse CAGGTTGCCGTTGCAGTAG (19)
PAX8	174	Initial denaturation: 95°C for 3 minutes Denaturation: 95°C for 30 seconds Annealing: 55 to 60°C for 1 minute Extension: 72°C for 1 minute Final extension: 72°C for 10 minutes Cycles: 35 cycles (20)	Forward GATCAGGATAGCTGCCGACT Reverse GTTGTACCTGCTGCCTTTG (19)

RESULTS

SOCIO-ECONOMIC/ DEMOGRAPHIC CHARACTERISTICS OF DATA

In this study, the socioeconomic/demographic characterstics were investigated among the individuals in Quetta District. Quetta has a diverse group of populations, with different ethnicities i.e. Baloch, Pathan, Punjabis, and Hazaras residing in the area. Due to hilly area there is a limited access to iodine-rich foods, the region may have environmental factors contributing to hypothyroidism.

CONCENTRATION OF THYROID HORMONE LEVELS

The dataset in Table II showed the average values of T3 (triiodothyronine), T4 (thyroxine), and TSH (thyroid-stimulating hormone) in three different groups: normal, hypothyroidism, and hyperthyroidism patients, and also demonstrates the count of each group. The total number of individuals was 120, out of which 89 were normal, 25 were hypothyroid, and 6 were hyperthyroid on the basis of thyroid hormones levels.

Table II. Thyroid hormone levels in normal, hypothyroid, and hyperthyroid patients

Groups	TSH (μLu/ml)	T4 (μg/dl)	T3 (ng/ml)	Number
Normal	2.53±0.77	8.47±1.84	1.49±0.85	89
Hypothyroidism	13.10±8.44	7.51±0.24	1.37±0.81	25
Hyperthyroidism	0.18±0.14	19.78±3.59	1.35 ±1.11	6

AGE

The age group distribution of the 120 individuals is represented in Table III. The individuals were categorised into 4 groups, i.e., 1-25 years, 26-40 years, 41-60 years, and above 60 years. The total number of individuals was 120, of which 9 (7.5%) were in the 1-25 years age group, 107 (89%) in the 26-45 years age group, 3 (2.5%) in the 46-60 years age group, and 1 (0.83%) in the above 60 years age group. In 120 individuals, 25 (20.83%) were positive for hypothyroidism, out of which 21 (84%) were female and 4 (16%) were male, and most positive patients were in the 26-45 years age group. Overall, the data indicate the occurrence of hypothyroidism was higher in the 26-45-year-old age group.

Table III. Age group distribution of the collected data

Age groups	Frequency	Percentage%
1 to 25	9	7.5
26 to 45	107	89
46 to 60	3	2.5
Above 60	1	0.83
Total	120	100

GENDER

The data was collected from Heart and General Hospital, Quetta. The total number of datasets was 120 individuals, out of which 110 (91.66%) were females and 10 (8.33%) were male, as shown in Table IV. In 120 individuals, 25 were positive for hypothyroidism, out of which 21 (84%) were female and 4 (16%) were male, as shown in Table V. The data indicates that the occurrence of hypothyroidism is higher in females than in males.

Table IV. Gender-wise distribution of total collected data

Gender	Frequency	Percentage%
Male	10	8.33
Female	110	91.66
Total	120	100

Table V. Gender-wise distribution of hypothyroid patients

Gender	Frequency	Percentage%
Male	4	16
Female	21	84
Total	25	100%

ETHNICITY

The ethnic group distribution of 120 individuals is represented in Table VI. The total number of individuals was 120, of which 63 (52.5%) were Pathan, 33 (27.5%) Baloch, 2 (1.67%) Sindhi, 16 (13.33%) Punjabi, 3 (2.5%) Hazara, 2 (1.6%) Urdu-speaking, and 1 (0.83%) Christian. Mostly positive patients were of Pathan ethnicity. Overall, the study indicates that all positive patients with hypothyroidism belonged to the Pathan ethnic group.

Table VI. Distribution of collected data in different ethnic groups

Ethnic Group	Frequency	Percentage%
Pathan	63	52.5
Baloch	33	27.5
Sindhi	2	1.6
Punjabi	16	13.33
Hazara	3	2.5
Urdu speaking	2	1.6
Christain	1	0.83
Total	120	100

AREA

The area distribution of 120 individuals in the data is represented in Table VII. The total number of individuals was 120, of which 109 (90.83%) were from Quetta, 6 (5%) were from Pishin, 2 (1.67%) were from Killa Abdullah, 1 (0.83%) was from Duki, and 2 (1.67%) were from Khanozai. The total number of individuals was 120, out of which 25 were positive with hypothyroidism, 21 were female, and 4 were male. Out of those individuals, 23 (92%) were from Quetta, and 2 (8%) were from Pishin. Overall, most patients with hypothyroidism were from Quetta, followed by Pishin.

Table VII. Area-wise distribution of collected data

Area	Frequency	Percentage%
Quetta	109	90.83
Pishin	6	5
Killa-Abdulla	2	1.67
Duki	1	0.83
Khanozai	2	1.67
Total	120	100

OCCUPATION

The occupation distribution of 120 individuals in the data is represented in Table VIII. Occupations were categorised into 4 groups: government job/private job, housewives, students, and labour. Out of 120 individuals, 94 (78.33%) were housewives, 9 (7.5%) had private jobs, 14 (11.66%) were students, and 3 (2.5%) were labourers. The total number of individuals was 120, out of which 25 (20.83%) were positive with hypothyroidism; out of those, 19 (76%) were housewives, 2 (8%) had private jobs, 3 (12%) were students, and 1 (4%) was a labourer. The data indicates most positive cases were housewives.

Table VIII. Occupation-wise distribution of hypothyroid patients

Occupation	Frequency	Percentage%
Gov job/ private job	9	7.5
House wives	94	78.33
Student	14	11.66
Labor	3	2.5
Total	120	100%

GENETIC IDENTIFICATION OF COLLECTED DATA AND DNA EXTRACTION

Polymerase chain reaction was used for amplifying the nucleic acid/DNA by using the DNA polymerase enzyme. The DNA extraction method was used to separate DNA from its cellular components, membranes, and proteins. In this study, DNA was extracted through the phenol-chloroform method and confirmed by a gel electrophoresis. The results were shown in Fig. 1.

IDENTIFICATION OF HYPOTHYROID-ASSOCIATED GENES (*TSHR, TPO, TITF1, AND PAX8*) THROUGH POLYMERASE CHAIN REACTION

The hypothyroid-associated genes *TSHR, TPO, TITF1*, and *PAX8* were identified after PCR amplification. The *TSHR* gene was amplified on 910 bp; the band was shown in Fig. 2a. The *TPO* gene band

was shown on 238 bp (Fig. 2b), the *TITF1* gene was amplified on 204 bp; the band was shown in Fig. 2c, and the *PAX8* gene band was shown on 174 bp (Fig. 2d).

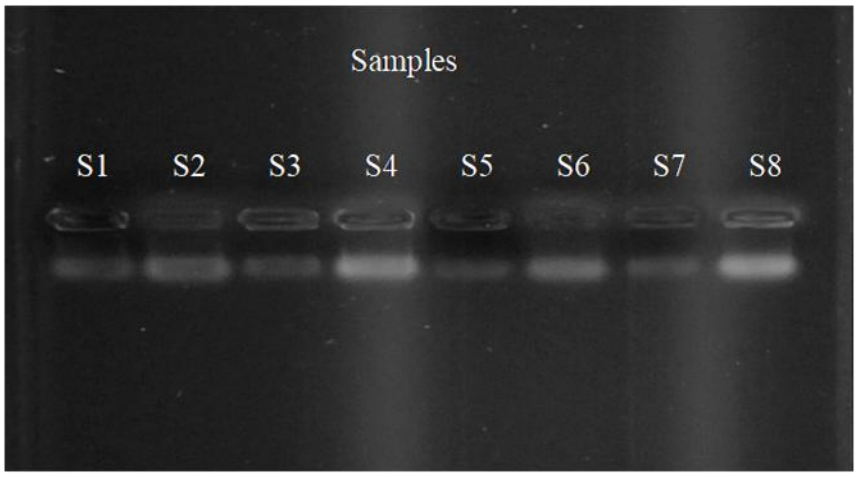

Fig. 1. The electrophoresis pattern of genomic DNA

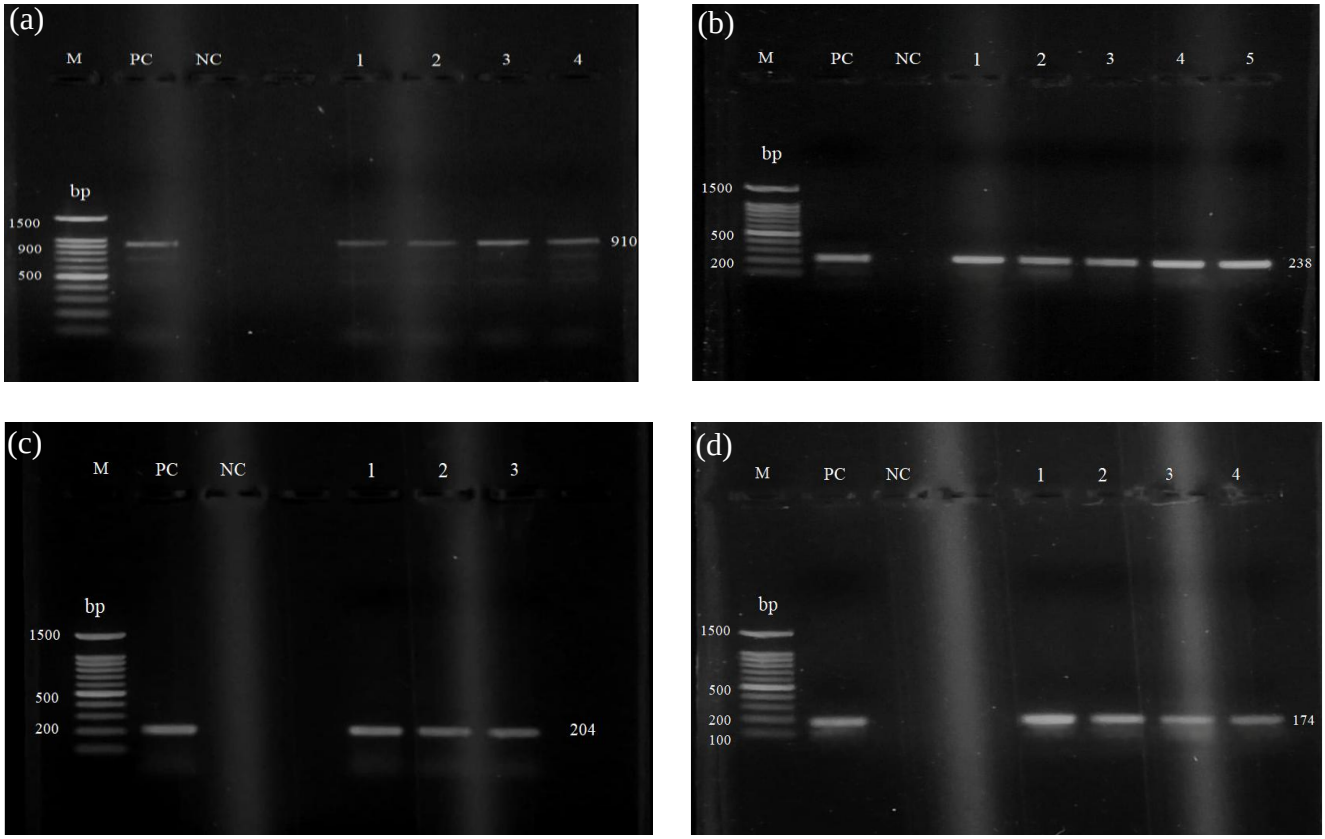

Fig. 2 (a). Electrophoresis pattern of TSHR gene (910bp); **(b).** Electrophoresis pattern of TPO gene (238bp); **(c).** Electrophoresis pattern of TITF1 gene (204bp); **(d).** Electrophoresis pattern of PAX8 gene (174bp)

RATE OF GENETIC IDENTIFICATION OF HYPOTHYROID ASSOCIATED GENES (*TSHR, TPO, TITF1, AND PAX8*) IN HYPOTHYROIDISM PATIENTS

The occurrence of *TSHR, TPO, TITF1, and PAX8* genes was evaluated through polymerase chain reaction, and the results are shown in Table VIII. In this study, the total number of individuals was 120, out of which 25 (20.83%) were positive for hypothyroidism; out of those, 4 (16%) were male and 21 (84%) were female. The rate of existence of those genes in males was 2 (50%) for *TSHR*, 3 (75%) for *TPO*, 0 (0%) for *TITF1*, and 1 (25%) for *PAX8*.

The occurrence of *TSHR, TPO, TITF1, and PAX8* genes was evaluated through polymerase chain reaction, and the results are shown in Table VIII. In this study, the total number of individuals was 120; out of these, 25 (20.83%) were positive for hypothyroidism; out of those, 4 (16%) were male and 21 (84%) were

female. The rate of existence of those genes in females was 12 (57.14%) for *TSHR*, 17 (81%) for *TPO*, 5 (23.8%) for *TITF1*, and 7 (33.33%) for *PAX8*.

Table VIII. Occurrence of hypothyroid associated genes in male and female patients

S. No	Genes	Frequency	Percentage	Frequency	Percentage
		Male patients		Female patients	
1	*TSHR*	2/4	50%	12/21	57.14%
2	*TPO*	3/4	75%	17/21	81%
3	*TITF1*	0/4	0%	5/21	23.80%
4	*PAX8*	1/4	25%	7/21	33.33%

DISCUSSION

The findings of this study provide important insights into the genetic and socio-epidemiological landscape of hypothyroidism in the Quetta district, highlighting the multifactorial nature of the disorder in this population. The detection of thyroid-related genetic variations among patients supports the growing evidence that hereditary predisposition plays a crucial role in the onset of hypothyroidism, particularly in regions with limited healthcare access and delayed diagnosis. Furthermore, socio-epidemiological analysis revealed that low literacy levels, restricted awareness of thyroid disorders, inadequate nutritional intake, and limited access to routine health screening significantly contribute to the prevalence and underdiagnosis of hypothyroidism. Environmental and lifestyle factors, including iodine deficiency, stress, and poor health-seeking behaviors, were also evident among affected individuals, suggesting a strong interaction between genetic susceptibility and socio-economic conditions. Collectively, these results underscore the need for integrated public health strategies that combine genetic screening, community awareness programs, and improved diagnostic facilities to enable early detection and effective management of hypothyroidism in underserved regions like Quetta.

The present study investigated the socio-economic factors and genetics associated with hypothyroidism among the individuals in the Quetta district. The results show that 84% of the affected individuals were female, particularly in the age group of 26-45. Furthermore, most hypothyroid patients belonged to the Pathan ethnicity. At the molecular level, the *TPO* gene was most frequently identified in hypothyroid patients, followed by *TSHR*, *PAX8*, and *TITF1*.

During the research, the findings revealed the highest occurrence of hypothyroidism cases among the females (84%) as compared to the males (16%). Similar results were reported by Vanderpump, 2011, who noticed that hypothyroidism and even other thyroid disorders were 10 times more common in females as compared to males (21). Moreover, Hollowell *et al*., 2002, reported that females, specifically middle-aged women, had the highest risk of thyroid disorders (22).

In this study, it was also noticed that the age group of 26-45 years was more frequently affected by the disease. But according to the report by Unnikrishnan *et al*., 2013, the occurrence of hypothyroidism was more frequent in the age group of 46-54 years (23).

The current study revealed that the majority of the patients with hypothyroidism were housewives, but Sayeed *et al*., 2019, reported the occurrence of hypothyroidism was found to affect all categories (housewives, students, etc.), not just significantly affect housewives (24).

Additionally, the study also shows that most cases of hypothyroidism came from the Pathan ethnic group. Since this ethnicity-specific finding is new and still not discussed or reported in existing literature, that suggests the need for more research regarding this area. Genetically, the study showed varying frequencies of the presence of hypothyroid-associated genes in males and females**.** The *TPO* (thyroid peroxidase) was the most frequently identified gene among the hypothyroid patients: 81% in females and 75% in males. These molecular findings are consistent with previous studies; i.e., Rousset *et al*., 2015, identified the TPO gene as one of the most frequent gene associated with autoimmune thyroid disease (25). Similarly, Balmiki *et al*., 2014, reported the association of the *TPO* gene with hypothyroidism. They revealed that the mutations in the *TPO* (thyroid peroxidase) gene may be the cause of hypothyroidism (26).

In this study, the findings revealed that the *TSHR* gene showed a moderate rate of gene occurrence, 57% in females and 50% in males. Similarly, Hussain *et al*., 2018, reported that the TSHR gene mutations are

linked with hypothyroidism specifically in females (27). But according to De Felice *et al*., 2004, TSHR gene mutation is linked with congenital hypothyroidism in children (28).

The *PAX8* was the less frequently identified gene among hypothyroid patients: 33% in females and 25% in males. Lastly, the *TITF1* gene revealed the least frequency among the hypothyroid patients: 23 % in females and absent in male patients (19). The results of the study revealed the importance of genetic testing for hypothyroidism, while those genes may be used as genetic biomarkers for early disease diagnosis and management.

CONCLUSION

The study revealed the socio-economic factors and genetics contributing to hypothyroidism in the Quetta district. By identifying the genes that were associated with hypothyroidism, including *TSHR, TITF1, PAX8,* and *TPO*, this study indicates the significant association between the development of hypothyroid conditions and those genes, particularly in women. Also, the findings of the study indicate that the female gender, and particularly the age group 26-45 years, had the highest existence rate of hypothyroidism. Most patients were from the Quetta district and belonged to the Pathan ethnicity. On the basis of occupation, most patients were housewives. These findings indicate the importance of genetic testing for hypothyroidism, as those genes may be serving as biomarkers for early diagnosis and treatment of disease.

Recommendation:

The current study had a small sampling size, so for the future research the sample size should expanded and targets the large scale studies in the different districts of Balochistan to better understand the results and see how things may vary region to region. Future research should also use advanced genomic methods like next generation sequencing techniques, to help in discover the rare or new gene mutations linked with hypothyroidism. Future studies should focus on using thyroid related genes as biomarkers to support earlier diagnosis and better management of hypothyroidism. This could help doctors to detect the disease even before symptoms appear, allowing treatment to begin sooner and reducing long term health issues. It's important to raise awareness about thyroid health in the community, especially for women of reproductive age and people who have a family history of thyroid problems. Simple, local campaigns can help people understand the risks and encourage them to get tested early

Conflict of interest:

All authors declare no conflict of interest.

Authors' contribution:

AD Conceived the study idea, sample collection, lab experiments and research methodology; ISS Supervised the overall project, performed data analysis, and critically revised the manuscript; FN Contributed to laboratory experimentation and assisted in molecular analysis; PG Patient recruitment, clinical data collection, and fieldwork supervision; MT Sample processing and contributed to statistical analysis; MD Assisted in drafting the manuscript and validating epidemiological data; IM Contributed to DNA extraction, PCR optimization, and data recording. KA Assisted in literature review, data entry, and manuscript formatting. All authors reviewed and approved the final manuscript.

References:

1. Khan YS, Farhana A. Histology, thyroid gland. 2019.
2. Nilsson M, Fagman H. Development of the thyroid gland. Development. 2017;144(12):2123-40.
3. Williams AT, Chen J, Coley K, Batini C, Izquierdo A, Packer R, Abner E, Kanoni S, Shepherd DJ, Free RC, Hollox EJ. Genome-wide association study of thyroid-stimulating hormone highlights new genes, pathways and associations with thyroid disease. Nature Communications. 2023;14(1):6713.
4. Wilson SA, Stem LA, Bruehlman RD. Hypothyroidism: diagnosis and treatment. American family physician. 2021;103(10):605-13.
5. Lee SY, Pearce EN. Hyperthyroidism: a review. Jama. 2023;330(15):1472-83.

6. Babic Leko M, Gunjaca I, Pleic N, Zemunik T. Environmental factors affecting thyroid-stimulating hormone and thyroid hormone levels. International journal of molecular sciences. 2021 Jun 17;22 (12):6521.
7. Pirahanchi Y, Toro F, Jialal I. Physiology, thyroid stimulating hormone, 2018.
8. Panicker V. Genetics of thyroid function and disease. The Clinical Biochemist Reviews. 2011;32(4):165.
9. Levy MJ, Koulouri O, Gurnell M. How to interpret thyroid function tests. Clinical Medicine. 2013;13(3):282-6.
10. Gaitonde DY, Rowley KD, Sweeney LB. Hypothyroidism: an update. South African Family Practice. 2012;54(5):384-90.
11. Moore D. Hypothyroidism and nursing care: Learn to recognize the symptoms of this commonly overlooked condition. American Nurse Today. 2018;13(2):44-7.
12. Iwahashi-Odano M, Fujisawa Y, Ogata T, Nakashima S, Muramatsu M, Narumi S. Identification and functional characterization of a novel PAX8 mutation (p. His39Pro) causing familial thyroid hypoplasia. clinical pediatric endocrinology. 2020;29(4):173-8.
13. Shen F, Cai W, Gan X, Feng J, Chen Z, Guo M, Wei F, Cao J, Xu B. Prediction of genetic factors of hyperthyroidism based on gene interaction network. Frontiers in Cell and Developmental Biology. 2021;9:700355.
14. Zucker R, Kovalerchik M, Stern A, Kaufman H, Linial M. Revealing the genetic complexity of hypothyroidism: integrating complementary association methods. Frontiers in Genetics. 2024;15:1409226.
15. Wolf K, Gilbert PA. EDTA—Ethylene di-amine tetra-acetic acid. In Detergents. Berlin, Heidelberg: Springer Berlin Heidelberg. 1992:243-259.
16. Hashemipour M, Soheilipour F, Karimizare S, Khanahmad H, Karimipour M, Aminzadeh S, Kokabee L, Amini M, Hovsepian S, Hadian R. Thyroid peroxidase gene mutation in patients with congenital hypothyroidism in Isfahan, Iran. International journal of endocrinology. 2012; 2012 (1):717283.
17. Narumi S, Muroya K, Abe Y, Yasui M, Asakura Y, Adachi M, Hasegawa T. TSHR mutations as a cause of congenital hypothyroidism in Japan: a population-based genetic epidemiology study. The Journal of Clinical Endocrinology & Metabolism. 2009;94(4):1317-23.
18. Guria S, Bankura B, Balmiki N, Pattanayak AK, Das TK, Sinha A, Chakrabarti S, Chowdhury S, Das M. Functional analysis of thyroid peroxidase gene mutations detected in patients with thyroid dyshormonogenesis. International journal of endocrinology. 2014;2014(1):390121.
19. Trueba SS, Augé J, Mattei G, Etchevers H, Martinovic J, Czernichow P, Vekemans M, Polak M, Attié-Bitach T. PAX8, TITF1, and FOXE1 gene expression patterns during human development: new insights into human thyroid development and thyroid dysgenesis-associated malformations. The Journal of Clinical Endocrinology & Metabolism. 2005;90(1):455-62.
20. Esperante SA, Rivolta CM, Miravalle L, Herzovich V, Iorcansky S, Baralle M, Targovnik HM. Identification and characterization of four PAX8 rare sequence variants (p. T225M, p. L233L, p. G336S and p. A439A) in patients with congenital hypothyroidism and dysgenetic thyroid glands. Clinical endocrinology. 2008;68(5):828-35.
21. Vanderpump MP. The epidemiology of thyroid disease. British medical bulletin. 2011;99(1).
22. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). The Journal of Clinical Endocrinology & Metabolism. 2002;87(2):489-99.
23. Unnikrishnan AG, Kalra S, Sahay RK, Bantwal G, John M, Tewari N. Prevalence of hypothyroidism in adults: An epidemiological study in eight cities of India. Indian journal of endocrinology and metabolism. 2013;17(4):647-52.
24. Sayeed MA, Mohsena M, Haq T, Morshed AH, Afroz S, Tomalika N, Momtaz H, Rahaman MM. Prevalence of hypothyroidism in different occupational groups of Bangladeshi population. IMC Journal of Medical Science. 2019;13(2):9-17.
25. Rousset B, Dupuy C, Miot F, Dumont J. Thyroid hormone synthesis and secretion.
26. Balmiki N, Bankura B, Guria S, Das TK, Pattanayak AK, Sinha A, Chakrabarti S, Chowdhury S, Das M. Genetic analysis of thyroid peroxidase (TPO) gene in patients whose hypothyroidism was found in adulthood in West Bengal, India. Endocrine journal. 2014;61(3):289-96.
27. Hussain BI, Hadi MA, AL-Harbi HJ. The association of TSHR gene rs2268458 polymorphism with hypothyroidism in females of Babylon Province-Iraq. Journal of University of Babylon for Pure and Applied Sciences. 2018;26(8):7-18.

28. De Felice M, Di Lauro R. Thyroid development and its disorders: genetics and molecular mechanisms. Endocrine reviews. 2004;25(5):722-46.