



Original Article



Prevalence and Molecular Docking-Based Drug Evaluation of Thyroiditis in Hyderabad, Pakistan

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ARTICLE INFO

Keywords:

Thyroiditis, Molecular Docking, Hypothyroidism, Hyperthyroidism

How to Cite:Khazada, B., Nisar, A., Saleem, H., Hoor, H., Saleem, N., Kaif, M., & Khan, L. (2025). Prevalence and Molecular Docking-Based Drug Evaluation of Thyroiditis in Hyderabad, Pakistan: Prevalence and Molecular Docking-Based Drug Evaluation. *Pakistan BioMedical Journal*, 8(7), 39-44. <https://doi.org/10.54393/pbmj.v8i7.1270>***Corresponding Author:**Beenish Khazada
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ABSTRACT

Thyroiditis, an inflammation of the thyroid gland, is a globally significant health issue, with a prevalence of 5–10% worldwide. In Pakistan, the prevalence of clinical and subclinical hypothyroidism is estimated at 4–5%. **Objectives:** To determine the prevalence, clinical features, and risk factors of thyroiditis in Hyderabad, Pakistan, and to evaluate the drug-protein interactions of commonly used thyroid medications (levothyroxine, methimazole, and propranolol) with thyroid-stimulating hormone (TSH) using molecular docking analysis. **Methods:** This prospective, observational, cross-sectional study included 72 patients aged 18 to 65 years from Hyderabad. Demographic and clinical data were collected through structured questionnaires. Blood samples were analyzed using ELISA to assess biochemical markers (T3, T4, TSH). Molecular docking was performed using the PyRx virtual screening tool to evaluate drug interactions with TSH. Statistical analysis was conducted using SPSS version 26.0. **Results:** The majority of patients (68.05%) were between 21–40 years of age, with a predominance of females (81.9%) and rural residents (54.1%). Hypothyroidism was the most prevalent condition (47.2%). Significant risk factors included smoking and autoimmune disorders ($P=0.045$), whereas family history and iodized salt consumption were not significantly associated ($P>0.05$). Molecular docking revealed that propranolol had the highest binding affinity to TSH ($K_d=-6.3$), followed by levothyroxine ($K_d=-5.3$), while methimazole showed the lowest affinity ($K_d=-3.7$). **Conclusions:** The study concluded a high prevalence of thyroiditis (52.78%; 95% CI: 41.3%–64.3%) in females aged 21–40 from rural areas of Hyderabad. Propranolol exhibited the strongest interaction with TSH, suggesting potential therapeutic implications.

INTRODUCTION

Thyroiditis is defined as the inflammation of the thyroid gland, which can impair its ability to function properly, leading to changes in hormone production. Thyroiditis can result from various causes, including autoimmune disorders, infections, iodine imbalances, or certain medications. This inflammation can lead to either an overproduction (hyperthyroidism) or underproduction (hypothyroidism) of thyroid hormones, affecting the metabolic and hormonal balance of the body. Thyroiditis may cause symptoms such as pain or swelling in the neck, fatigue, weight changes, and changes in heart rate, among others. The most common forms of thyroiditis are Hashimoto's thyroiditis, Graves' disease, subacute

thyroiditis, and postpartum thyroiditis [1]. Hashimoto's thyroiditis is an autoimmune condition that leads to hypothyroidism; women are 5 to 10 times more likely to be affected, with the highest incidence occurring between the ages of 50 and 60 [2]. A family history of autoimmune diseases, iodine excess or deficiency, and environmental factors such as radiation exposure are significant risk factors of Hashimoto's thyroiditis. Graves' disease, another autoimmune disorder, is the leading cause of hyperthyroidism. Women between the ages of 30 and 50 and associated with genetic susceptibility, stress, and environmental triggers such as smoking are linked to higher risk [3]. Subacute thyroiditis (also known as De



Quervain's thyroiditis) is a transient inflammatory condition often triggered by viral infections, such as those following upper respiratory tract infections. It is less common with a typical incidence rate of 1-2 per 100,000 people annually, and is most commonly seen in women aged 20-50 [4]. Postpartum thyroiditis is another autoimmune thyroid disorder that affects approximately 5-10% of women within the first year after childbirth. Women with a family history of thyroid disease or other autoimmune conditions are at higher risk for this condition [5, 6]. Through diet, lack of iodine consumption in developing countries is identified as a major risk factor for hypothyroidism [7]. Experimenters have examined Women in their 60s and older, are more likely to have hypothyroidism [8]. Later, it can be their periods, lactation, and housework [9]. The thyroiditis threat increases with seafood and iodized salt consumption [10]. To treat hypothyroidism, treatment with levothyroxine (LT4) is first-line [11]. Its start occurs from a low dose and increases gradually every 6-8 weeks [12]. To treat hyperthyroidism, antithyroid drugs like methimazole are the first line of treatment. Propylthiuracil (PTU) is often preferred for pregnant females in their first trimester [13]. In Hashimoto's thyroiditis, a person suffers from hypothyroidism, so standard treatment to normalize by levothyroxine is necessary to suppress TSH levels and tumor growth [14]. This study employs a dual approach combining clinical data with *in silico* molecular docking to assess thyroiditis burden and identify potential molecular targets. This integration strengthens the translational relevance by linking epidemiological findings with predicted therapeutic insights.

Although thyroid disorders have been widely studied globally and in Pakistan, limited region-specific data exist regarding the combined clinical prevalence and molecular interaction analysis of thyroiditis in Hyderabad, Sindh. Previous studies have largely focused either on epidemiological patterns or on therapeutic mechanisms independently, with minimal integration of clinical findings and *in silico* molecular docking approaches. Furthermore, comparative binding affinity analysis of commonly prescribed drugs (levothyroxine, methimazole, and propranolol) with thyroid-stimulating hormone remains underexplored in local populations. Therefore, this study aimed to bridge this gap by integrating prevalence assessment with molecular docking evaluation to provide translational insight into therapeutic selection. This study aims to conduct molecular docking of selected drugs (levothyroxine, methimazole, and propranolol) with thyroid-stimulating hormone (TSH) to assess their binding affinity and potential molecular interactions.

METHODS

This prospective, observational, cross-sectional study was conducted at Liaquat University Hospital (LUH), Hyderabad, from August 2024 to January 2025, to determine the prevalence, risk factors, and clinical characteristics of thyroiditis. In addition, an *in silico* molecular docking analysis was performed to evaluate drug interactions with thyroid-related proteins, aiming to identify potential therapeutic targets. Ethical approval was obtained from the Ethical Committee of the Institute of Biochemistry, University of Sindh, Jamshoro (Ref. No: IOB/264/2024). Written and verbal informed consent was obtained from all participants. A total of 72 patients aged 18-65 years with clinical signs of thyroid dysfunction were enrolled, primarily from the outpatient department (85-90%) and some from admitted wards. Exclusion criteria included individuals under 18 years of age, those with prior radiation exposure or thyroid surgery, and patients with severe chronic illnesses (excluding comorbid diabetes, autoimmune, or psychiatric conditions related to thyroid function). Each participant underwent a physical examination for common thyroid-related symptoms, including weight changes, neck swelling, tremors, arrhythmias, and heart rate abnormalities. Demographic and clinical data including menstrual irregularities, postpartum depression, family history of thyroid disorders, iodine intake, smoking habits, and comorbidities—were collected using a structured questionnaire. The sample size was calculated based on the expected prevalence of thyroiditis in the local population, ensuring adequate statistical power. Venous blood samples (5 mL) were collected from each participant to assess serum levels of TSH, FT3, and FT4 using ELISA kits. Based on hormone profiles, patients were categorized as hypothyroid, hyperthyroid, or euthyroid. For molecular docking, thyroid-stimulating hormone (TSH, PDB ID: 7XW5) was retrieved from the Protein Data Bank. Levothyroxine (CID: 5819), Methimazole (CID: 1349907), and Propranolol (CID: 4946) were obtained from PubChem in SDF format. Structures were prepared using Discovery Studio Visualizer by removing water molecules and heteroatoms, followed by conversion to PDBQT format. PyRx with AutoDock Vina was used to perform docking and calculate binding affinities using an empirical scoring function. Results (Kd values) were exported in CSV format, and 2D and 3D interaction images were generated using Discovery Studio. Data were analyzed using SPSS version 26.0. Frequencies and percentages were calculated for demographic and clinical variables. The Chi-square test was used to assess associations between risk factors and thyroid status. A *p*-value < 0.05 was considered statistically significant.

RESULTS

The socio-demographic analysis shows that the majority of patients (68.05%) were aged 21–40 years, and this age group had a significantly higher association with thyroiditis ($p = 0.000$). Based on thyroid function test results, the overall prevalence of thyroiditis among the study participants was 52.78%. The 95% confidence interval (CI) for this prevalence was calculated as 41.3% to 64.3%, indicating a moderately wide interval due to the sample size. Among specific conditions, hypothyroidism was the most prevalent form at 47.2% (95% CI: 35.3%–59.3%), followed by hyperthyroidism at 41.6% (95% CI: 29.9%–54.2%), and euthyroidism at 11.1% (95% CI: 4.9%–20.7%), table 1.

Table 1: Demographic and Clinical Characteristics of Study Participants with Statistical Comparison Based on Thyroiditis Presentation

Demographics	Frequency (%)	p-Value
Age		
≤ 20	3 (4.2%)	<0.001
21 - 40	49 (68.05%)	
41 - 60	16 (22.2%)	
≥ 61	4 (5.5%)	
Gender		
Male	13 (18.05%)	0.999
Female	59 (81.9%)	
Marital Status		
Single	13 (18%)	0.001
Married	59 (81.9%)	
Thyroid Status Distribution		
Hyperthyroidism	30 (41.6%)	0.001
Hypothyroidism	34 (47.2%)	
Euthyroidism	8 (11.11%)	

Subacute thyroiditis typically shows normal or slightly elevated T3/T4 levels with low or normal TSH, often due to viral infections. Postpartum thyroiditis involves fluctuating TSH levels, initially low (hyperthyroid phase) and later elevated (hypothyroid phase), with fluctuating or low T3/T4 during the hypothyroid phase. However, these types were not seen to be more prevalent as compared to hypo and hyper conditions of thyroid disease.

The different types of thyroiditis that compared the mean \pm standard deviation of biochemical parameters TSH, T3, and T4. If over t hyperthyroid, TSH (12.3 ± 4.997) levels were low, indicating the suppression of the pituitary gland. T3 (1.06 ± 0.1682) and T4 (7.53 ± 0.757) levels were relatively stable, showing the mild or controlled form of hyperthyroidism. In subacute hyperthyroid, TSH (5.58 ± 17.97) levels were low, and T3 (6.26 ± 8.938) and T4 (55.7 ± 116.86) levels were elevated, which shows the release of more thyroid hormone due to subacute, (Table 2).

Table 2: Biochemical Parameters of Thyroiditis

Parameters	Over Hyperthyroid (Mean \pm S.D)	Subacute Hyperthyroid (Mean \pm S.D)	Primary Hypothyroid (Mean \pm S.D)	Hashimoto's Hypothyroid (Mean \pm S.D)
TSH	12.3 ± 4.997	5.58 ± 17.97	19.0 ± 15.70	29.5 ± 36.61
T3	1.06 ± 0.1682	6.26 ± 8.938	13.7 ± 27.08	5.057 ± 8.015
T4	7.53 ± 0.757	55.7 ± 116.86	5.011 ± 3.940	54.6 ± 71.33

Smoking has been identified as a significant environmental risk factor (p value=0.045) for autoimmune thyroid diseases (AITDs), particularly Graves' disease, due to its complex effects on immune regulation and thyroid function (Table 3).

Table 3: Risk Factors of Thyroiditis

Risk Factors	Group	Frequency	p-Value
Family History	Yes	9 (12.5%)	0.136
	No	63 (87.5%)	
Smoking Status	Smokers	4 (5.55%)	0.045*
	Non-Smokers	68 (94.4%)	
Iodized Salt	Consumers	29 (40.2%)	0.999
	Non-Consumers	43 (59.7%)	

A total of 72 patients participated in the study. Among hyperthyroid patients, 55.5% patients reported heat intolerance, 22.2% of them had moist skin and 22.3% experienced thyroid neck pain. For the hypothyroid patients, majority experienced dry skin (38%) and hair loss (32%). Among the Euthyroid patients, 62.5% experienced neck or throat discomfort, 12.5% had neck swelling, and 25% showed weight fluctuation (Table 4).

Table 4: Clinical Symptoms Observed Among Patients with Different Thyroid Conditions (n=72)

Thyroid Status	Symptom	No. of Patients (n)
Hyperthyroidism	Heat intolerance	10 (55.5%)
	Moist skin	4 (22.2%)
	Neck pain	4 (22.3%)
Hypothyroidism	Cold skin	20 (29.4%)
	Dry skin	26 (38.2%)
	Hair loss	22 (32.35%)
Euthyroidism	Neck or throat discomfort	5 (62.5%)
	Neck swelling	1 (12.5%)
	Weight fluctuation	2 (25%)

Distribution of therapeutic drugs prescribed to thyroiditis patients in Hyderabad hospitals, highlighting the frequent use of levothyroxine for hypothyroidism, methimazole for hyperthyroidism, and propranolol for symptom management (Figure 1).

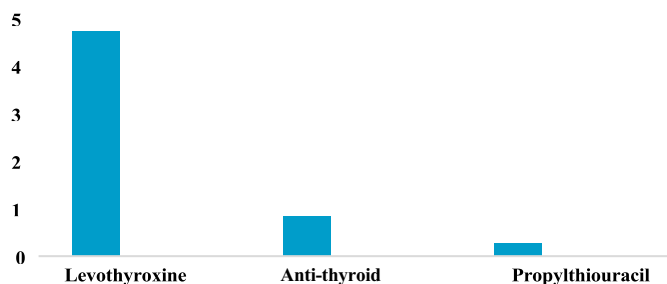


Figure 1: Therapeutic Drug Treatment Against Thyroiditis

As most of the patients in the study suffered from hypothyroidism, so majority received levothyroxine for thyroid treatment. Antithyroid drugs were found to be used as a treatment option for hyperthyroidism patients by decreasing hormone synthesis and secretion from the gland. On the other hand, propylthiouracil was seen to be specifically prescribed to pregnant women. Binding affinities of levothyroxine (CID: 5819), methimazole (CID: 1349907), and propranolol (CID: 4946) docked with Thyroid Stimulating Hormone (TSH, PDBID: 7XW5) (Table 5).

Table 5: Binding Affinities of Ligands (Drug) Interaction with Thyroiditis Protein (TSH)

Drug Used	Binding Affinity with TSH (Kd value)
Levothyroxine	-5.2
Methimazole	-3.7
Propranolol	-6.3

Binding affinities were measured in terms of Kd values, lower the Kd value better would be the binding affinity of target with its ligand. Propranolol showed highest binding affinity with TSH (Kd = -6.3), followed by levothyroxine (-5.2), and methimazole showed the least affinity with Kd value of -3.7, with mode "0" showing the best fit orientation when binding to TSH. "A" shows the representation of the 2D and 3D figure of TSH with levothyroxine showing glutamine at position 26 (GLU A:26) to interact by its amide group with levothyroxine, forming a hydrogen bond with hydrophobic interactions. "B" shows the 2D and 3D diagram of methimazole, which is prescribed to hyperthyroidism patients; it consists of a thiourea group that forms hydrogen bonds with the hydroxyl group of glutamic acid (GLU: Y:26). "C" shows the 2D and 3D diagrams of propranolol. The hydroxyl group of propranolol interacts with the amide side chain of asparagine (ASN R:170), stabilizing its hydrogen bonding position in its beta-adrenergic receptor binding site. The docking results reveal strong binding affinities and key molecular interactions, such as hydrogen bonding with critical active site residues, indicating potential inhibition of thyroid-related enzymes. These *in silico* findings support the clinical data, highlighting the strength of our dual approach. Integrating clinical prevalence analysis with molecular docking offers valuable insight into both disease

burden and therapeutic potential (Figure 2).

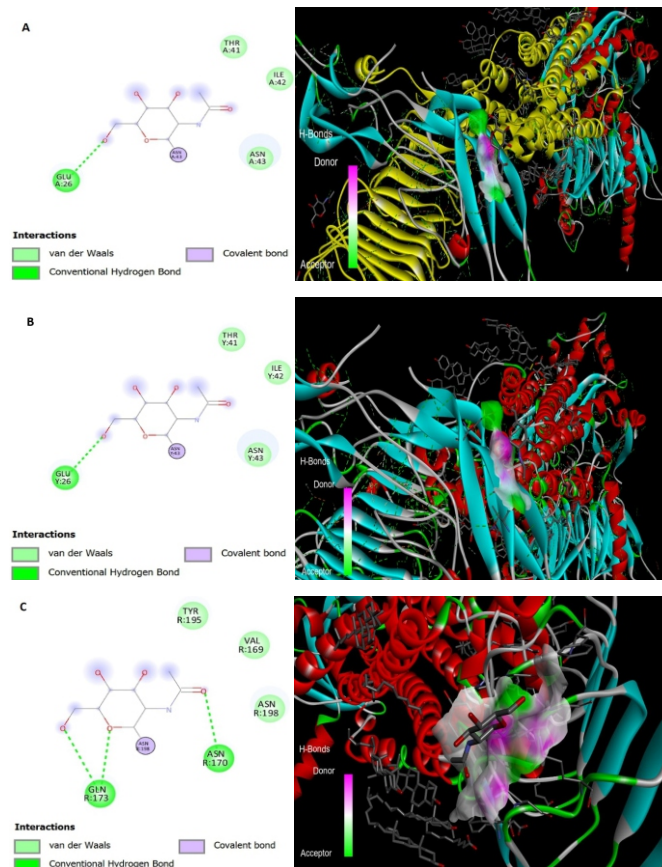


Figure 2: Graphical Representation of Binding modes ligands with Thyroiditis proteins A.TSH with Levothyroxine, B.TSH with Methimazole, C.TSH with Propranolol

DISCUSSIONS

The current study revealed that females (81.9%) were more affected by thyroiditis. It may be due to sex differences in thyroid hormone levels may be influenced by factors such as the menstrual cycle, pregnancy, and menopause, with estrogen playing a crucial role in modulating thyroid function [14,15]. In this study, no significant association was found between gender and thyroid diseases; this is in line with another study [13], which also showed no significant association between gender and thyroid diseases. Moreover, we also found the age group 21-40 years suffering more with thyroid disorders, and these results are in agreement with another study conducted at Hyderabad Sindh in 2021 [16]. In the case of primary hypothyroidism, a high level of TSH shows the thyroid gland is too underactive, and the pituitary gland tries to compensate. T3 and T4 levels were low, which are typical biochemical markers of hypothyroidism. In Hashimoto's hypothyroidism, TSH was high, which shows a severely damaged autoimmune thyroid gland. T3 and T4 levels showed significant variability that represent the chronic inflammation or disease progression [6]. Family history

and iodine intake were not significantly associated with thyroiditis; however, smoking was found to be a major risk factor (p value=0.045) for both hyper and hypo conditions of the thyroid gland. Cigarette smoke contains toxic compounds that can modulate the immune system, promoting the production of thyroid-stimulating immunoglobulins (TSIs) and increasing the risk of hyperthyroidism. This mechanism highlights the detrimental role of smoking in thyroid autoimmunity [17]. Additionally, smoking induces oxidative stress, which damages thyroid cells and triggers autoimmune responses. It is also a major risk factor for exacerbating Graves' ophthalmopathy, likely due to its impact on orbital fibroblasts and cytokine production [18]. Prevalence was 52.78% during the study period in which females were with hypothyroidism due to their postpartum depression, menstrual cycle, and family history. However, mortality was very low as the thyroiditis is controlled state. In a previous Hyderabad based study goiter was observed to be 27%, Hashimotos to be 9%, Thyroid Cancer to be 10%, and Iodine Deficiency to be 88% [16]. Heat intolerance was found to be the most common symptom because BMR increases due to elevated T3 and T4 levels, which causes the release of heat as a byproduct, which results in environmental discomfort to the patient, and due to stimulation of sweat glands, blood flow increases, resulting in perspiration and moist skin [19]. While pain in the thyroid gland during hyperthyroidism is due to the inflammation or pressure of the enlarged thyroid gland, which later results in tenderness and throat discomfort. Dry skin was the most frequently observed symptom in hypothyroid cases. In hypothyroidism, due to the production of fewer T3 and T4 hormones, metabolism slows down, leading to decreased heat generation, causing the intolerant sensitivity to cold, and as sweat gland activity is reduced, blood flow also decreases, which causes the disturbance to the skin, like the skin becoming dehydrated, rough, dry, and scaly. Neck discomfort was the most prevalent sign in this group [20]. Throat pain and neck swelling are the most commonly reported symptoms during euthyroidism because of nodules or goiter formation, which causes throat pain and thyroid hormone imbalance. Levothyroxine is a proven and widely prescribed drug for treating thyroid disease in patients with hypothyroidism. However, higher-than-recommended dosages may lead to adverse effects [21]. Binding affinities were measured in terms of Kd values. The lower the Kd value, the better the binding affinity of the target with its ligand [22]. Through molecular docking, it was predicted that propranolol should be used as a choice of drug (as it showed the highest binding affinity) if a patient is suffering from hyperthyroidism complications like anxiety, stress, and arrhythmia. However, in case of

hypothyroidism, levothyroxine should be prescribed, and if complications aren't severe, patients can take methimazole only.

The study had a small sample size, limiting statistical power. Thyroid antibody testing was not performed, restricting autoimmune confirmation. Lack of follow-up data and experimental validation of docking results also limit the findings' clinical relevance. Future research should include multicenter, large-scale cohorts with comprehensive antibody testing and longitudinal follow-up. Additionally, experimental validation of docking results and exploration of additional molecular targets are recommended to strengthen clinical applicability.

CONCLUSIONS

This study concluded a high prevalence of thyroiditis among adults in Hyderabad, with hypothyroidism being most common, especially in rural areas. Smoking and autoimmune disorders were key risk factors. Propranolol showed the strongest molecular binding with TSH, followed by levothyroxine and methimazole, indicating its potential therapeutic role. These findings highlight the need for early diagnosis and targeted treatment strategies.

Authors' Contribution

Conceptualization: BK

Methodology: AN, MK,

Formal analysis: HS, HH, NS, MK, LK

Writing and Drafting: BK, AN, HS, LK

Review and Editing: BK, AN, MK, HS, HH, NS, MK, LK

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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