ORIGINAL ARTICLE

Evaluation of Thyroid Hormone Profile in Patients with Suspected Thyroid Dysfunction in Southern Punjab

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ABSTRACT

Background Objective: To assess T3, T4 &TSH levels and prevalence of thyroid disorders in the suspected patients received at collection centers of Aga Khan Lab in Southern Punjab districts.
Place and Duration: A retrospective study was conducted from July 2012 to December 2012 at Multan Stat Laboratory of Aga Khan University Hospital.
Methods: 1784 suspected patients were evaluated for thyroid hormonal assay-tri iodo thyronine (T3), tetra iodo thyronine (T4) and thyroid stimulating hormone (TSH) by using cobas kits on Cobas e 411 immunoassay auto analyzer using electrochemiluminescence immunoassay(ECLIA) technique.
Results: Out of total one thousand seven hundred and eighty four cases of suspected individuals,472 (26.45%) were males and 1312(73.54%) were females. Out of total ,518(29.03%)subjects including139 (26.83%) males and (73.16%) 379 females showed thyroid dysfunction with male to female ratio 1:2.73. Of these 1784 cases, 357 (20.01%) were in age group of < 20 years, 1189 (66.65%) were in 21-54 years, and 238 (13.34%) were in age group of > 55 years. . Most common thyroid disorder found was hyperthyroidism (14.18) followed by hypothyroidism (6.73%) which was followed by subclinical hyperthyroidism (4.87%) and subclinical hypothyroidism (3.25%) .
Conclusion: The prevalence of thyroid dysfunction was very high (29.03 %) with hyperthyroidism (14.18%) being most common. Females were more affected.

Keywords: T3, T4, TSH, Hyperthyroidism, Hypothyroidism

INTRODUCTION

Thyroid is an important endocrine gland and elaborates two key metabolic hormones thyroxine (T4) and tri-iodo thyronine (T3). The later is biologically more active and is produced mainly by the coversion of prohormone (T4) to (T3) by enzyme 5-deiodonase in the peripheral tissues mainly in liver and kidney. Both these hormones are under the control of thyroid stimulating hormone (TSH) of anterior pituatory gland which in turn is controlled by thyrotrophin releasing hormone (TRH) from hypothalamus[1]. The thyroid hormones, thyroxine/tetraiodothyronine (T4) and triiodothyronine (T3), act on virtually every tissue in the body. These hormones bind to nuclear receptors, resulting in alterations in gene expression and protein production. They also regulate metabolism of protein, fats, and carbohydrates, and are essential for normal development of the fetus and newborn.[2]

Thyroid dysfunction affects several systems of our body[3]. The disorder manifests in a wide spectrum of clinical and biochemical disease from clinically undiagnosed disease to myxoedema coma[4]. Thyroid disorders are amongst the most common endocrine dysfunctions. The total prevalence of these disorders estimated to be 200 million world wide.[5]

Diseases of the thyroid are among the most prevalent of medical conditions, especially in women, but the symptoms can be relatively nonspecific or mild. For this reason, clinicians have been placing increased reliance on the laboratory for assistance in the diagnosis of thyroid disorders6. Physicians need quality laboratory testing support for the accurate diagnosis and cost-effective management of thyroid disorders.

Thyroid function test panel is commonly used for evaluating and screening thyroid disorders..The American Thyroid Association recommends that adults must be screened for thyroid disorders by measurement of the serum thyroid stimulating hormone (TSH) concentration at the age 35 years and every 5 years thereafter[7]. The spectrum of thyroid disorders range from a condition of hypothyroidism (under active) to hyperthyroidism

(over active.)The biochemical and clinical classification of thyroid disorders include primary disorders due to thyroid gland dysfunction itself such as primary hypothyoidism, primary hyperthyroidism. Secondary disorders due to pituatory gland disorder include secondary hypothyroidism and secondary hyperthyroidism. Tertiary disorders due to hypothalamic diseases include tertiary hypo and hyperthyroidism8.

Subclinical hyperthyroidism is defined as a low serum TSH concentration with normal serum FT4 and FT3 concentrations. Subclinical hypothyroidism is defined as an increased TSH with normal FT4 and FT3 levels9.Those having normal T3, T4 & TSH levels were categorized as euthyroid, those having low T3, T4 & high TSH were hypothyroid and those having normal levels of T3, T4 & low TSH were categorized as hyperthyroid respectively with respect to the reference range.The spectrum and the prevalence of thyroid disorders are known to be influenced by environmental factors, especially by iodine intake 10. The signs and symptoms associated with thyroid disease, both hypo- and hyperthyroidism, are nonspecific; thus, laboratory tests play crucial roles in the diagnosis and management of disease, including monitoring response to therapy[11-14]

There are several studies on thyroid disorder but the prevalence varies because it depends upon the subject group and the diagnostic criteria used. The classification of patients with thyroid dysfunction has undergone dramatic changes with the improvement in the methods of analysis.American Academy of clinical endocrinologist state that a sensitive TSH test should be used as standard criteria for screening[15,16,17]. An abnormal TSH is the first abnormality to appear in thyroid disease, where other thyroid tests can be normal. Using TSH as a single criterion has been shown to accurately classify the thyroid state of a patient in over 95% of cases[18].

A number of international studies can be found on the thyroid disorder, its consequences and precautionary measures[19-21]; However, very few such studies22-25 have been carried out in Pakistan. Hypothyroidism and hyperthyroidism are two widespread thyroid problems, but there are no reports on the incidence and prevalence of these disorders in this part of our country.This study was planned to compare the TSH, T4 and T3 levels in suspected patients for thyroid disorders with respect to different gender and age groups in the samples received at collection centers of Aga Khan Lab in Southern Punjab districts.The study was conducted from July 2012 to December 2012.

MATERIAL & METHODS

This retrospective study was carried out in the Aga Khan Stat laboratory Multan, with samples collected from all collection points in different areas in Southern Punjab from 1st July 2012 to 30th December 2012.The study included blood specimens for routine testing for Thyroid function received from individuals suspected for thyroid dysfunction by doctors at eighteen collection centers of Aga Khan Lab in different districts. One thousand seven hundred and eighty four cases of suspected individuals of both the sex of varying age group were undertaken for study . Suspected cases were divided initially into two categories on the basis of sex and three catagories on the basis of age groups. Samples were collected by using tubes containing separating gel and centrifuged prior to testing. The screening was performed in Aga Khan Multan Stat Lab by using the Elecsys T3 (Triiodothyronine) ,T4(Thyroxine),TSH(Thyrotropin) assay kits on Cobas e 411 immunoassay analyzer. The reference intervals for T3,T4 and TSH for our laboratory were as follows:

T3 – 1.23 – 3.0 nmol/L; T4 – 66 – 181 nmol/L or 5.5-11 µ g/dl F,4.6-10.5 µ g/dl M ; TSH 0.4-4.2 µ IU/ml 0.7-6.4 µIU/ml (21 weeks to 20 years)

The screened subjects taken in the study were grouped into hypothyroid,subclinical hypothyroid, euthyroid , hyperthyroid and subclinical hyperthyroid based on their TSH levels.Subjects were categorized according to measurements of serum TSH and total thyroid hormone concentrations as follows:

Overt hyperthyroidism [serum TSH < 0.4 µ IU/ml with raised total T4 and total T3 or raised total T3 alone

- (T3-toxicosis)
- Subclinical hyperthyroidism (serum TSH < 0.4 µ IU/ml with normal total T4 and total T3)
- Euthyroid (serum TSH 0.4–6.6µ IU/ml with normal T3 and T4)
- Subclinical hypothyroidism (serum TSH >6.6 µ IU/ml with normal total T4 and T3)
- Overt hypothyroidism (serum TSH > 6.6 µ IU/ml with low total T4 and T3).26

PRINCIPLE OF THE TEST

Test principle(T3)

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample and a T3-specific antibody labeled with a ruthenium complex; bound T3 is released from the binding proteins in the sample by ANS.
- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T3, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode

Test principle(T4)

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample and a T4 specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by ANS.
- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T4, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Test principle(TSH)

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Statistical Analysis

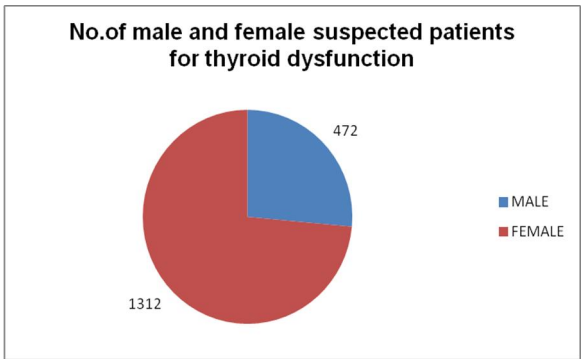
Data were collected from computerized record of Aga Khan Laboratory University Karachi . Permission was obtained from the Head of Stat Laboratories, Aga Khan University hospital before the data collection. Data were represented as percentage, mean and standard deviation. The results obtained and expressed in mean ± SD. . Percentages were calculated directly for T3,T4,TSH in different gender and age . Mean values of the three hormones TSH, T3 and T4 along with Standard Deviations (SD) were calculated for all participants with each diagnosis, and with respect to gender and age groups.

RESULTS

Table 1 showed that out of the total one thousand seven hundred and eighty four cases of suspected individuals , 472 (26.45%) were males and 1312(73.54%) were females: Out of total ,518(29.03%)subjects including139 (26.83%) males and (73.16%) 379 females showed thyroid dysfunction with male to female ratio 1:2.73. The study depicted that 38 male patients (8.06%) out of 472 showed subclinical thyroid disorders, whereas 101 (21.39%) exhibited true thyroid disease. Moreover, the remaining 333 (70.55%) male subjects suspected of an altered thyroid status exhibited normal thyroid hormone and TSH levels and devoid of any sub-clinical or true thyroid disease disorders. Similarly, in female group of

patients, 107 (8.15%) showed subclinical thyroid disorders from a total of 1312, whereas 272 (20.73%) exhibited true thyroid disease. 933 (71.12%) female suspected patients exhibited normal thyroid hormone and TSH levels.

Table 2a showed the age wise distribution of the study population. Of these 1784 cases, 357 (20.01%) were in age group of < 20 years, 1189 (66.65%) were in 21-54 years, and 238 (13.34%) were in age group of > 55 years.The maximum number of patients enrolled(66.65%) belonged to the age bracket of 21- 54 years. In males the highest proportion of 260 (14.57%) cases were reported in 21-54 years and was followed by in <20years age group with 129 cases (7.23%). In the females also, highest proportion of 929 (52.07%) cases was in 21-54 years, followed by <20 years with 228 (12.78%) cases. Table 2b also showed % age distribution of thyroid disorders in present study.Out of total patients 19.06%(340) were HYPERTHYROID, 9.97%(178) were HYPOTHYROID and 70.97%(1266) were EUTHYROID . In the present study overall prevalence among patients with thyroid disorders was with hyperthyroidism to hypothyroidism ratio of 1.9:1.



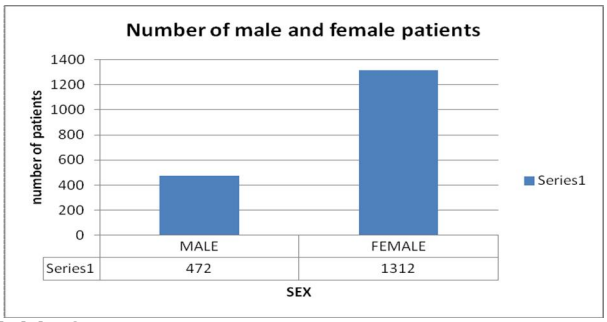


Table 2a

Table 2c showed that out of total hyperthyroid patients,253 (14.18%) were true hyperthyroid and 87(4.87%) were subclinical hyperthyroid. Out of total hypothyroid patients 120(6.73%) were true hypothyroid and 58(3.25%) were subclinical hypothyroid. Most common thyroid disorder found was hyperthyroidism (14.18) followed by hypothyroidism (6.73%) which was followed by subclinical hyperthyroidism (4.87%) and subclinical hypothyroidism (3.25%) .

Age wise distribution of the study population.
AgeGroups(yrs) M/F number %
>20 129/288 357 20.01%
21-54 260/929 1189 66.65%
>55 83/155 238 13.34%

Table 2b: % Age Distribution of Thyroid Disorder In Study Populaton

Thyroid Status	Frequency	% Age
Hyperthyroid	340	19.00%
Hypothyroid	178	9.94%
Euthyroid	1266	70.76%

Table 2c: % Age Distribution of Thyroid Disorder in Study Populaton

Thyroid function	Number	%
Euthyroid	1266	70.97%
Hyperthyroid	253	14.18%
Hypothyroid	120	6.73%
Subclinical hyperthyroid	87	4.87%
Subclinical Hypothyroid	58	3.25%
Total	1784	100%

Comparison was based on age groups(i.e, <21 years, 21-54 years ,and >55 years) of TSH, T4 and T3 Levels in patients with thyroid disorders among male and female . Age group of <21 yrs had 77subjects with thyroid disorders of which 15 were males and 59 were females . Out of 77 patients , 12 were subclinical hypothyroid ,19 were true hypothyroid,11 were subclinical hyperthyroid and 35 were true hyperthyroid.There were 352 patients with thyroid disorders in the age group of 21-54 years, in which 86 were males and 266 were females . Out of 352 patients , 43 were subclinical hypothyroid ,72 were true hypothyroid,60 were subclinical hyperthyroid and 177 were true hyperthyroid. 89 subjects, 35 males and 54 females were in the age group of>55 years. Out of 89 patients , 3 were subclinical hypothyroid ,29

were true hypothyroid,16 were subclinical hyperthyroid and 41 were true hyperthyroid.

Within different age groups, higher prevalence of hyperthyroidism was seen in female patients who are within the age group of 21-54 years (7.11%) and in male patients who are within the age group of 21-54 years (2.80%) and hypothyroidism was also high in female patients who are within the age group of 21-54 years (3.30%) followed by the age group of >55 years (0.89%), and <21 years (0.67%). Hypothyroidism was observed 0.72%in male patients in age group 21-54 years and >55 years.

Table 2: Comparison of True & Sub Clinical Thyroid Disorders Among Different Age Groups Of Males & Females

	Subclinical Thyroid Disorder		True Thyroid Disorder		Euthyroid
No of Subjects	**S.C. Hypothyroid**	**S.C. Hyperthyroid**	**True Hypothyroid**	**True Hyperthyroid**	
Total Males (472)	11	27	33	68	333
<21 Years Males	4	1	7	6	111
21-54 Years Males	5	18	13	50	174
>55 Years Males	2	8	13	12	48
Total Females (1312)	47	60	87	185	933
<21 Years Females	8	10	12	29	169
21-54 Years Females	38	42	59	127	663
>55 Years Females	1	8	16	29	101

Circulating T3, T4 and TSH level of the males & females are shown in Table 3. Comparison of thyroid hormones level among different age groups of males are shown in Table 4. Comparison of thyroid hormones level among different age groups of females are shown in table 5.

Table 3: Comparison Of Thyroid Hormones Level Among Males & Females

	Males		Females	
Thyroid Hormones	**Mean**	**SD**	**Mean**	**SD**
T3 (nmol/L)	2.22	1.31	2.12	1.26
T4 (µg/dl)	9.09	3.87	9.36	3.91
TSH (µIU/ml)	6.98	19.38	7.02	19.22

Table 4: Comparison of Thyroid Hormones Level Among Different Age Groups of Males

	<21 Years Males		21-54 Years Males		>55 Years Males	
Thyroid Hormones	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
T3(nmol/L)	2.26	0.76	2.26	1.48	2.04	1.39
T4(µg/dl)	8.98	3.04	9.35	4.09	8.45	4.2
TSH (µIU/ml)	9.79	23.89	4.74	14.2	9.7	24.49

Table 5: Comparison of Thyroid Hormones Level Among Different Age Groups of Females

	<21 Years Females		21-54 Years Females		>55 Years Females	
Thyroid Hormones	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
T3 (nmol/L)	2.14	0.96	2.12	1.31	2.1	1.35
T4 (µg/dl)	9.25	3.16	9.34	4	9.66	4.34
TSH (µIU/ml)	8.1	21.64	6.67	18.47	7.52	19.75

DISCUSSION

Thyroid disorders constitute the most prevalent endocrine disorder in our country. Abnormal thyroid function has multiple implications for public health. We conducted this study to assess the prevalence of thyroid dysfunction among the suspected patients attending collection centers of Aga khan Hospital. We have evaluated the biochemical profile of 1784 suspected patients who underwent thyroid functional analysis in our laboratory over a period of six months.

Overall, 70.97% (1266/1784 cases) of patients have normal thyroid outcome in this study. This study demonstrated a 29.03% prevalence of thyroid disorders in the suspected patients studied. Another study conducted by Meena Desai et.al.[27] revealed presence of thyroid disease in 26%.

In the present study the prevalence of thyroid disorders was more in females as compared to males (73.16% vs 26.83%). Our results are consistent with study of Afsheen Maqsood *et al.,* in Karachi,[28] reports females are 85.5% and males only in 11.5% . Antony, *et al* in another study29, reported (152) 14% males and (936)86% females. Mahato RV *et al.,* in Nepal[30] reported that thyroid disorders was 83.27% in females compared to 16.73% in males.

Although all age group presented with thyroid dysfunctions in our study but a high number of subjects was observed between age groups of 21-54 yrs of age. Afsheen Maqsood *et al.,*[28] reports that in males, it is higher in above the age of 40 years and in females it is above the age of 30 years. Pradip Kumar *et al.,*[31] in their study on thyroid stimulating hormone measurement in Kolkata, reports high thyroid disorders in 36-45 years age group.The results of our study are in accordance with the studies of Afsheen Maqsood *et al and* Pradip Kumar *et al* who also found high prevalence of thyroid disorder in patients with advancing age. Rosemary Ikem *et al.*[32] reveals high affected group of thyroid disorders are in 40-60 years of age. Antony, *et al* reported 29the highest number of cases in 40-60 years followed by 20-40 years. It is 40.80% in 40-60 years and 38.41% in 20-40 years. In males the most affected in the age group of 40-60 years is 38.2% followed by 31.6% in 20-40 years. In females, this trend is repeating where 41.2% is reported in 40-60 years which is followed by 39.5% in the 20-40 age groups.

In our study Hyperthyroidism is relatively more frequent as compared to hypothyroidism. Among thyroid dysfunction maximum prevalence was found to be of Hyperthyroidism (14.18%) whereas subclinical hypothyroidism (3.25%) was least found. Our study showed 19.05% prevalence of total hyperthyroidism including both overt hyperthyroidism(14.18%) and subclinical hyperthyroidism (4.87%) in suspected subjects for thyroid disorders in southern punjab. The findings of our study are consistent with study of Gupta,et al33 in which 23.09% prevalence of total hyperthyroidism including both overt hyperthyroidism (12.65%) and subclinical hyperthyroidism (10.44%) in population of central nepal was reported. Olurin et al noted that thyrotoxicosis occurred in 53% of cases of thyroid disorders in 874 Nigerians34 .Our study is in accordance with another study by Yadav et al,35 a hospital-based study done in eastern part of Nepal which showed 24.8% hyperthyroidism including 14.9% overt hyperthyroidism and 9.9% subclinical hyperthyroidism.

Our study revealed that women are more vulnerable to thyroid dysfunction.We have found F:M ratio of thyroid disorders 2.73:1. The finding of our study is consistent with study of ---------- where F:M ratio of thyroid disorders was 2.9:1.

CONCLUSION

In conclusion, it was observed that hyperthyroidism constituted the greatest proportion of the thyroid disorders seen between July 2012 and December 2012 and that most patients with thyroid disorders tended to present late. Hence, from our study, we can conclude that the people residing in southern Punjab have higher risk for hyperthyroid disorders, with higher prevalence being in females as compared to males. The reason(s) for such a high prevalence of hyperthyroidism in southern punjab needs to be studied further. Detailed population based studies are required to establish the accurate prevalence.Since the present study was a laboratory based study, it may not appropriately represent the entire population. But this study has identified the burden of thyroid dysfunction in the community and can serve as a baseline for future studies.

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DECLARATION OF INTERESTS
None.

LIMITATIONS OF STUDY
Clinical history on thyroid dysfunction and medications of patients were not considered in the study

AUTHORS' CONTRIBUTIONS
JA and AS contribute equally to the skillful editing of the manuscript and interpretation the results.

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