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Research Articles

Editorial

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Original Article

Comparison of Thyroid Function Tests by RIA / IRMA Based Commercial Kit and in House Method in Hypothyroid Patients

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Abstract

The diagnosis of hypothyroidism is based on the combination of clinical presentation and laboratory tests. The current study focuses on the strategy for the differential diagnosis of hypothyroidism and a comparison of thyroid function tests (TFTs) by commercial kit based RIA / IRMA (radioimmunoassay / immunoradiometric assay) procedures with those by in – house method. The study encompasses 75 hypothyroid patients whose clinical history were recorded and TFTs were performed on their serum samples by commercial kit based RIA / IRMA. Moreover, 40 samples were assayed again for TFTs by in – house RIA / IRMA procedures to check whether the results by both methods were comparable. It has been found that in most cases, hypothyroidism is a clinical diagnosis and

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estimation of TFTs is required for its confirmation but to reach the differential diagnosis of hypothyroidism, additional relevant investigations are required. Furthermore, when TFTs results by commercial kit based RIA / IRMA were compared with those by in – house RIA / IRMA procedures, no statistically significant difference was found between the two methods. This implies that in – house RIA / IRMA procedures may be applied in place of commercial kit based methods to perform TFTs provided the criteria for quality control are fulfilled.
Keywords: Hypothyroidism, RIA, IRMA.

Introduction

The thyroid is an endocrine gland located in the front of neck. The gland is made up of epithelial cells or thyrocytes formed into millions of sac like follicles which produce and secrete thyroid hormones.[1] The iodine – containing hormones of the thyroid include triiodothyronine (T_3) and thyroxine (T_4). The physiological actions of T_3 and T_4 in the regulation of diverse cellular activities, including normal growth and general metabolism, are well defined.[2] Only 20% of all circulating T_3 is directly secreted by the thyroid and the remainder is formed by the peripheral deiodination of T_4 by monodeiodinases.[3] Normal thyroid hormone secretion depends on the intact feedback loop of the hypothalamic – pituitary-thyroid axis. Thyrotropin-releasing hormone (TRH), produced by the hypothalamus, stimulates thyrotroph cells in the anterior pitui-

tary to secrete thyrotropin or thyroid stimulating hormone (TSH) which, in turn, stimulates thyroid follicular cells to release thyroxine (T_4) and tri-iodothyronine (T_3). Release of TRH and TSH is suppressed by the increased levels of T_4 and T_3 completing the feedback loop.[4]

Hypothyroidism is a clinical state resulting from an insufficient amount of circulating thyroid hormones to support normal body function.[5] It is a common endocrine disorder, arising more often in women than men and increasing in incidence with age, especially after the onset of middle life. The National Health and Nutrition Examination Survey (NHANES 1999 – 2002) of 4,392 individuals reflecting the US population reported hypothyroidism (defined as TSH levels > 4.5 mIU/L) in 3.7% of the population.[6] The female to male ratio in hypothyroidism ranges from 2:1 to 8:1 in various epidemiological surveys.[7,8] In a survey of 2,779 persons carried out in County Durham, England, hypothyroidism was detected in 1.9% of women whereas the prevalence in men was less than 0.1%.[8] Hypothyroidism is more prevalent in elderly population, with 2% to as much as 20% of older age groups having some form of hypothyroidism.[9] In women and men aged older than 74 years screened at a Colorado health fair, the prevalence of hypothyroidism (defined as TSH levels > 10 mIU/L) was found to be 21% and 16%, respectively.[10] In the Rosses' survey, the prevalence of primary hypothyroidism has been stated as 8.6% in the women above the age of fifty years as compared to only 0.9% in younger females.[11]

Hypothyroidism can be classified on the basis of its time of onset (congenital or acquired), its severity (overt clinical, mild or subclinical), and the level of endocrine dysfunction responsible (primary, central secondary or tertiary).[12] Hypothyroidism can result from a defect anywhere in the hypothalamic-pituitary-thyroid axis, either insufficient TSH from the pituitary (secondary hypothyroidism) or insufficient TRH from the hypothalamus (tertiary hypothyroidism). In the vast majority of cases, it is primary hypothyroidism, which is decreased secretion of T_4 and T_3 by the gland itself, which results in a compensatory increase in TSH secretion.[13] The diagnosis of hypothyroidism is based on the combination of clinical presentation and laboratory tests. A clinical suspicion of hypothyroidism should lead to the measurement of serum TSH, total or free T_4, and total or free T_3 depending upon the situation. Radioimmunoassay (RIA) and immunoradiometric assay (IRMA) are commonly employed methods for the laboratory measurement of thyroid hormones (T_4 and T_3) and TSH respectively.

The current study focuses on the strategy to reach the differential diagnosis of hypothyroidism. Moreover, TFTs results by commercial kit based RIA / IRMA has been compared with those by in – house RIA / IRMA procedures.

Materials and Methods

Seventy – five patients, who were suspected for hypothyroid state clinically at Institute of Nuclear Medicine and Oncology Lahore (INMOL), were included in this study. After documenting clinical history of each corresponding patient, blood samples from these patients were drawn and serum was isolated for thyroid function tests (TFTs). Local research ethics committee's permission and individual informed consent were obtained.

Serum TSH and T_4 or FT_4 levels were determined for all patients whereas T_3 levels were determined for limited number of cases. TSH levels were determined by IRMA whereas T_4 or FT_4 and T_3 levels were determined by RIA. Reference values for T_3, T_4, FT_4 and TSH used at INMOL were considered to diagnose hypothyroidism in this study. These values are shown in Table 1.

Additional tests were administered for cases where needed. Based on this information differential diagnosis of hypothyroidism was made for each patient. During this study, commercial kits were used to perform the thyroid function tests for all serum samples whereas 40 serum samples were assayed again by in – house method that required preparation of some reagents.

Table 1: The reference values of T_3, T_4, FT_4 and TSH concentration.

Sr. No	Analyte	Normal Range	Hypothyroid
1.	Serum T_3	0.8 – 3.0 nmol/L	< 0.8 nmol/L
2.	Serum T_4	60 – 160 nmol/L	< 60 nmol/L
3.	Serum FT_4	11.5 – 23 pmol/L	< 11.5 pmol/L
4.	Serum TSH	0.3 – 6.0 mIU/L	> 6.0 mIU/L

Stock phosphate buffer (0.5 M, pH 7.4) was prepared by dissolving 57.1 g Na_2HPO_4 and 15.3 g $NaH_2PO_4.2H_2O$ in distilled water up to final volume of 1L. **Stock saline (3M)** was prepared by dissolving 175.3 g of NaCl in distilled water up to final volume of 1L. **Triton X-100 (10%)** was prepared by dissolving 10 g of Triton X–100 and 0.1 g of sodium azide in distilled H_2O making final volume up to 100 ml. **Buffer PBSX (25 mM phosphate buffer saline containing Triton X – 100 and sodium azide)** was prepared by adding 5 ml of stock phosphate, 5 ml stock saline, 1 ml of 10% Triton solution and 0.1 g sodium azide to distilled water with a final volume of 100 ml.

Wash buffer was prepared by adding 1 ml of 10% Triton solution to 1 g of azide making final volume up to 1L by the addition of distilled H_2O. **IRMA assay buffer** was prepared by adding 90 ml of PBSX to 10 ml stock protein solution with final volume of 100 ml. **RIA tracer buffer for T_4** was prepared by adding 45 mg of ANS and 50 mg of BSA to 50 ml of PBSX. **RIA tracer buffer for T_3**was prepared by the addition of 30 mg of ANS and 50 mg of BSA to 50 ml of PBSX. **RIA tracer buffer for FT_4** was prepared by adding 100 mg of 0.1% hydrolyzed gelatin to 100 ml of PBSX. **Tracer solutions:** Tracer solution of ^{125}I monoclonal anti-TSH was reconstituted by adding 12.5 ml IRMA assay buffer to give counts within the range of 60,000 – 70,000 cpm / 50 μL. Similarly tracer solutions of ^{125}I-T_4 and ^{125}I–T_3 were reconstituted by adding 20 ml of respective RIA tracer buffers to give radioactivity counts within the range of 20,000 – 25,000 cpm / 50 μL for T_4 and 14,000 – 18,000 cpm / 50 μL for T_3. **Lyophilized standards** for TSH, T_3, T_4 and FT_4 and IQC (internal quality control) pools were reconstituted with distilled water.

In – House Protocol

Assay tubes were first labeled in series of duplicates for standards, QCs and samples. Two tubes were labeled as T containing only tracer for the determination of total counts. A fixed amount of standards, quality controls and samples were taken in their respective assay tubes. Then an excess amount of ^{125}I anti-monoclonal–tracer was added to all tubes. The assay tubes were then vortexed. Antibody – coated bead was loaded in all the tubes except in T tubes, and all the other tubes were placed on rotator mixer overnight. After that 2 ml of wash buffer was added in tubes. The supernatant in each tube was then decanted. Washing and decantation step was performed twice. Then the radioactivity of all tubes was determined for 1 minute on gamma counter (Multi Crystal LB 2111, Berthold Technologies).

Data Processing

During this study International Atomic Energy Agency (IAEA) immunoassay data reduction analysis software program was used which was based upon 4 – 5 – parameter curve fit procedures. These parameters also evaluate imprecision in the standard curve, patient and IQC samples and provides overall information about the assay batch performance, its acceptability and rejection criteria. The program also analyzes IQC data. The response (Bound counts) and concentrations of line data processing provided the required information.

Statistical Analysis

Numerical values were reported as mean ± standard error of means and categorical variables as proportions. Independent sample t-test was used to compare the two groups for significant difference. P-value ≤ 0.05 was considered statistically significant. All calculations were carried out with the SPSS version 18 (SPSS, Inc. Chicago, IL, USA).

Results

During the current study, among 75 clinically suspected hypothyroid patients, 57 (76.00%) were females and 18 (24.00%) were males. The mean age of the cases was approximately 33.44 years with minimum age of 1 month and maximum age of 70 years. Based on the clinical history and TFTs results (additional tests in some cases) the patients were classified into 15 different subtypes of hypothyroidism. These subtypes are shown in Table 2.

The values of TSH, T_3, T_4 or FT_4 concentrations determined in hypothyroid patients' serum by comercial kit based RIA / IRMA are shown in Table 3.

Repeat Assays

Forty samples (at least one from each subtype of hypothyroidism) were analyzed again for TFTs by in-house method of RIA and IRMA (Table 4).

The results of these repeat assays by in – house method were comparable with those obtained by using commercial kits. There was no statistically significant

Table 2: Sub-types of hypothyroidism patients diagnosed during study.

Sr. No.	Subtypes	No. of Cases	Percentage
1.	Congenital hypothyroidism	12	16%
2.	Drug–induced hypothyroidism	9	12%
3.	Senile hypothyroidism	9	12%
4.	Sub-clinical hypothyroidism	8	10.66%
5.	Post-thyroidectomy hypothyroidism	8	10.66%
6.	Iodine deficiency induced goiter	6	8%
7.	Post-radioiodine therapy induced hypothyroidism	4	5.33%
8.	Postpartum thyroiditis hypothyroidism	4	5.33%
9.	Hashimoto's thyroiditis	3	4%
10.	Thyroid cancer hypothyroidism	3	4%
11.	Post-subacute thyroiditis hypothyroidism	3	4%
12.	Secondary hypothyroidism	2	2.66%
13.	Idiopathic hypothyroidism	2	2.66%
14.	Juvenile hypothyroidism	1	1.33%
15.	Non-thyroidal illness	1	1.33%

Table 3: Data providing information about the patient's age and TFTs (T_3, T_4, and TSH test results) by commercial methods of RIA and IRMA (*indicates FT_4 levels).

Patient ID	Age Years	Gender	TSH mIU/L	TT_3 nmol/L	TT_4 or FT4 (nmol/L or pmol/L)
P1	8	F	10.3	2.57	125
P2	1 month	M	7.48	-	42
P3	5	M	6.67	3.20	23
P4	12	F	38.54	-	10*
P5	22	F	14.11	-	15.1
P6	7	M	> 50	0.72	3.20*
P7	13	F	> 50	1.58	4.90*
P8	13	M	> 50	1.20	1.10*
P9	6	M	15.0	-	122
P10	13	F	> 50	2.29	5.20*
P11	22	F	> 50	0.53	14
P12	31	F	12.14	1.04	13.70*
P13	32	M	30.17	2.5	9.3*
P14	33	F	9.28	1.79	7.5*
P15	40	F	10.58	1.94	9.30*
P16	36	M	49.81	< 0.2	10*

Patient ID	Age Years	Gender	TSH mIU/L	TT_3 nmol/L	TT_4 or FT4 (nmol/L or pmol/L)
P17	20	M	12.63	1.90	9.3*
P18	45	F	44.01	1.60	2.50*
P19	40	F	>50	-	2.0*
P20	30	F	> 50	1.20	17.00
P21	20	F	> 50	2.54	9.00*
P22	34	M	8.34	2.11	16.00*
P23	70	M	6.06	1.51	176
P24	40	F	12.02	-	94
P25	42	F	10.04	1.75	99
P26	60	M	10.19	1.08	73
P27	20	F	< 60	-	< 10
P28	40	M	8.26	1.12	91
P29	48	F	10.28	-	97
P30	22	F	> 50	-	8.0*
P31	60	F	12.57	-	12.2*
P32	51	M	20.02	1.21	6.9*
P33	50	F	12.68	1.43	9.7*
P34	62	F	14.40	1.70	12.70*
P35	67	F	32.00	-	110
P36	60	M	44.28	-	< 10
P37	70	F	> 50	-	8.90*
P38	61	F	> 50	0.25	10
P39	60	F	48.13	-	44
P40	65	F	12.33	1.44	11.20*
P41	60	F	> 50	-	6.2*
P42	60	F	9.39	1.0	87
P43	30	F	8.38	1.92	8.10*
P44	20	F	7.65	1.80	93
P45	30	F	10.04	2.00	9.7*
P46	17	F	10.53	1.71	37
P47	43	F	12.09	0.98	39
P48	50	F	8.06	0.67	85
P49	38	F	11	1.65	10.0*
P50	29	F	> 50	0.78	12*
P51	32	F	> 50	-	-

Patient ID	Age Years	Gender	TSH mIU/L	TT_3 nmol/L	TT_4 or FT4 (nmol/L or pmol/L)
P52	18	F	23.43	2.25	10.0*
P53	12	F	28.84	-	27
P54	16	M	31.85	2.21	9.00
P55	14	M	13.20	1.96	1.60*
P56	35	F	> 50	0.91	27
P57	30	F	> 50	0.42	8
P58	32	F	> 50	-	1.1*
P59	30	F	> 50	2.03	120
P60	35	F	3.24	-	17
P61	42	F	0.51	1.27	11.20*
P62	25	F	55.14	0.25	26
P63	30	F	13.40	2.24	10.90*
P64	57	M	> 50	1.46	4.70*
P65	22	M	> 50	-	10.0*
P66	22	F	13.27	1.76	47
P67	43	F	9.38	1.54	77
P68	48	F	> 50	-	10*
P69	15	F	> 50	1.65	9.40*
P70	30	F	10.47	1.20	14.10*
P71	45	F	14.12	1.70	15.10*
P72	40	F	5.01	1.30	38
P73	33	F	> 50	0.24	9.00*
P74	40	F	> 50	0.27	10
P75	30	F	36.58	-	10

Table 4: Comparison of TSH, T_4, FT_4 and T_3 assay values by in – house and commercial IRMA / RIA methods (*indicates FT_4 levels).

Sr. No.	Patients ID	TSH Concentration (mIU/L)		TT_3 Concentration (nmol/L)		TT_4 or FT_4 Concentration (nmol/L or pmol/L)	
		In – House Method	Commercial Method	In – House Method	Commercial Method	In – House Method	Commercial Method
1.	P3	6.88	6.67	3.17	3.20	25.00	23.00
2.	P6	> 50	> 50	0.91	0.72	2.91*	3.20*
3.	P7	> 50	> 50	1.44	1.58	5.95*	4.90*
4.	P8	> 50	> 50	1.70	1.20	1.50*	1.10*
5.	P12	9.90	10.58	1.81	1.94	8.65*	9.30*

Sr. No.	Patients ID	TSH Concentration (mIU/L)		TT_3 Concentration (nmol/L)		TT_4 or FT_4 Concentration (nmol/L or pmol/L)	
		In – House Method	Commercial Method	In – House Method	Commercial Method	In – House Method	Commercial Method
6	P15	44.25	44.01	1.59	1.60	2.40*	2.50*
7	P17	> 50	> 50	0.98	1.20	16.26	17.00
8	P18	48.47	> 50	2.01	2.54	9.50*	9.00*
9	P19	9.01	8.34	1.91	2.19	19.01*	16.00*
10	P20	6.86	6.06	1.38	1.51	173.00	176.00
11	P23	9.50	10.19	1.24	1.08	68.00	73.00
12	P27	17.83	20.02	1.78	1.21	6.15*	6.90*
13	P28	11.07	12.68	1.28	1.43	8.75*	9.70*
14	P29	18.10	14.4	1.64	1.70	8.70*	12.70*
15	P33	> 50	> 50	0.55	0.25	10.80	10.00
16	P35	11.17	12.33	1.44	1.44	11.36*	11.20*
17	P37	7.23	8.38	1.60	1.92	8.48*	8.10*
18	P38	8.78	7.65	1.59	1.80	70.74	93.00
19	P40	9.29	10.53	1.68	1.71	41.00	37.00
20	P42	10.05	8.06	0.59	0.67	79.33	85.00
21	P45	23.49	23.43	2.04	0.25	6.98*	10.00*
22	P47	28.80	31.85	1.90	2.21	11.23	9.00
23	P48	12.42	13.20	1.58	1.96	2.73*	1.60*
24	P49	> 50	> 50	0.71	0.42	9.20	8.00
25	P53	14.07	13.4	2.17	2.24	11.29*	10.90*
26	P54	> 50	> 50	1.47	1.46	6.00*	4.70*
27	P56	12.78	13.27	1.58	1.76	40.52	47.00
28	P57	12.79	9.38	1.43	1.54	74.31	77.00
29	P59	> 50	> 50	1.55	1.65	10.00*	9.40*
30	P60	> 50	> 50	2.18	2.03	145.00	120.00
31	P63	11.01	8.26	1.00	1.12	113.00	91.00
32	P65	49.28	> 50	1.97	2.29	5.53*	5.20*
33	P66	> 50	> 50	0.59	0.53	18.00	14.00
34	P67	> 50	> 50	0.44	0.27	14.32	10.00
35	P69	> 50	> 50	1.20	0.78	11.69*	12.00*
36	P70	15.81	10.47	1.03	1.20	16.00*	14.10*
37	P71	15.90	12.14	0.92	1.04	12.00*	13.70*
38	P72	> 50	> 50	0.61	0.91	32.00	27.00
39	P73	13.47	14.12	1.68	1.70	16.32*	15.10*
40	P75	> 50	> 50	0.32	0.24	9.50*	9.00*

Table 5: Mean ± SEM of TSH, FT_4, TT_4 and TT3 by In – house Method and commercial method.

Thyroid Hormones	In – House Method Mean ± SEM	Commercial Method Mean ± SEM	P-Value
TSH	16.85 ± 2.41	13.72 ± 1.76	0.307
FT_4	8.76 ± 0.94	8.71 ± 0.90	0.971
TT_4	28.5 ± 6.24	27.93 ± 6.06	0.941
TT_3	1.41 ± 0.09	1.41 ± 0.10	0.976

difference (p value > 0.05) in TFTs values obtained by in – house RIA/IRMA and commercial kit – based RIA / IRMA (Table 5).

Discussion

Thyroid disorders are very prevalent these days. Hypothyroidism covers a wide spectrum of clinical and biochemical features which ranges from clinically unapparent disease to myxedema coma. Physician can make the diagnosis of hypothyroidism after completing clinical history, physical examination and performing relevant sensitive laboratory investigations. Advances in diagnostic methods now make it possible to detect hypothyroidism in almost all cases before severe symptoms develop. The present study was aimed to understand the strategy for the differential diagnosis of hypothyroidism through clinical manifestation of the patients and correlating it to thyroid function tests. Furthermore, TFTs values obtained by commercial kit based methods were compared with TFTs values obtained by in-house RIA / IRMA procedures.

For the hypothyroidism, the incidence increases with age, and females were affected 2 – 8 times more than males across the age range.[14] The current study also shows predominance of hypothyroidism in females as 76% of total hypothyroid cases were found to be females; however, only 17.33% of the total cases were 50+ in age.

Most patients showed classical signs and symptoms of hypothyroidism like weight gain, cold intolerance, pallor, fatigue, lethargy, generalized body aches and pains, constipation, sleep and menstrual disturbances.

Patients 1 – 12 were diagnosed as congenital hypothyroidism (CH). All patients except P_2 were previously diagnosed in infancy and were on thyroxine. P_2 showed signs and symptoms of pallor, poor feeding, constipation, enlarged tongue etc. TFTs showed high TSH and low T_4 thus confirming the diagnosis. CH is one of the most common diseases in the pediatric endocrinology and avoidable cause of severe mental retardation.[15] Moderate to severe mental retardation have also been observed in some of the congenital hypothyroid cases of the current study. The important causes of CH include developmental defects, dyshormonogenesis and hypothalamic – pituitary dysfunction. Thyroid hemiagenesis is believed to be one of the rare developmental anomalies of the thyroid gland. Thyroid agenesis may be complete, unilateral or isthmic.[16] The thyroid scan of P_{12} showed absence of left lobe of thyroid gland thus diagnosed as a case of hemiagenesis. P_{10} and P_{11} were cases of dyshormonogenesis. Radioactive iodine uptake and per chlorate discharge test were performed to determine the presence of an iodide uptake or organification defect in both patients. Dyshormonogenesis is an uncommon cause of congenital hypothyroidism. The most common abnormality is absent or insufficient thyroid peroxidase enzyme.[17]

Patients 13 – 21 were classified as drug induced hypothyroidism. All except P_{16} were previously diagnosed as hyperthyroid and were taking anti-thyroid drugs (Neomercazole). P_{16} was previously suffering from mania and was taking Lithium – a mood stabilizer. High TSH and low TT_4 were suggestive of hypothyroid state. These patients showed typical features of hypothyroidism and diagnosis was easy as there was clear history of chronic drug intake that causes hypothyroidism. Drug induced hypothyroidism commonly occurs after anti-thyroid drugs, lithium and iodine containing medications. Some newer drugs like interferon and tyrosine kinase inhibitors (sunitinib) also cause hypothyroidism.[18,19]

Patients 22 – 29 were classified as post-thyroidectomy hypothyroidism. These patients had clinical history of sub-total thyroidectomy as a treatment for hyperthyroidism. Serum TSH was high. Although TT_4 found to be normal in these patients, still these patients were in hypothyroid state based on the clinical features and high serum TSH levels. History was clear and dia-

gnosis was certain. Hypothyroidism following thyroid surgery is common.Factors such as increased age, operation type, histopathologic type, underlying disease, lymphocytic infiltration and use of levothyroxine before surgery were associated with the increased incidence of hypothyroidism.[20]

Patients 30 – 33 were diagnosed as post radioactive iodine induced hypothyroidism. These all patients were hyperthyroid and were given radioactive iodine as a therapeutic agent. Clinical symptoms indicated hypothyroidism in these patients that was also supported by high TSH. The use of I^{-131} for the treatment of hyperthyroidism is a very simple and cheap form of therapy and highly effective and is now the predominant definitive therapy both as primary treatment and following relapse after anti-thyroid drugs. Hypothyroidism that occurs in the first year following radioactive iodine therapy is related to dose.[21]

Patients 34 – 42 were included in the subtype of senile hypothyroidism. The mean age was 62.77 years. Clinical features and high TSH value support the diagnosis. An increased prevalence of hypothyroidism has been demonstrated in the elderly population. Aging has been proposed to represent a trigger for the development of autoimmune phenomena resulting in the production of both organ and non-organ specific antibodies. Signs and symptoms of hypothyroidism in younger patients e.g. cold intolerance, weight gain, dry skin, constipation, mental and physical slowing can easily be mistaken for normal aging in elderly population.[22,23] So careful clinical history, physical examination should be done for making the diagnosis of senile hypothyroidism. This must be confirmed with relevant laboratory investigations.

Patients 43 – 50 classified as subclinical hypothyroidism. These patients did not show clear signs and symptoms of hypothyroidism. TSH was minimally elevated and T_3 and T_4 were normal. Subclinical hypothyroidism is usually an asymptomatic condition, diagnosed when there are no specific symptoms or signs of thyroid dysfunction but the patient has an elevated serum TSH in the face of normal circulating thyroid hormone levels. The diagnosis is therefore biochemical, based almost entirely on a raised TSH concentration.[24] It is more common in women than men, and its prevalence increases with age.[25] All patients in this category were found to be females in this study.

Patients 51 – 56 identified as iodine deficiency goiter. These patients showed typical signs and symptoms of hypothyroidism. TSH values were high and T_3 and T_4 were low. These patients showed increased size of the thyroid gland. These patients came from areas that belong to the goiter belts and iodine deficiency regions of Pakistan. Iodine deficiency is a common nutritional problem affecting a large number of people especially living in the mountainous regions of Pakistan, although it is also seen in the plains. The higher prevalence may partly be attributed to the poor economic conditions of the people, low dietary iodine intake and adverse climatic conditions of the studied area.[26] The patients included in this subtype were residents of Gilgit, Skardu, Baltistan, Khunjurab, Chitral and Swat.

Patients 57 – 60 were cases of post-partum thyroiditis hypothyroidism. These subjects developed hyperthyroidism within 1 month after delivery and after 6 months they showed signs and symptoms of hypothyroidism. High TSH and low T_4 / FT_4 also supported the diagnosis. These patients showed biphasic pattern of the disease. Postpartum thyroiditis is an autoimmune disease. The immune system changes significantly during pregnancy. Post-partum autoimmune thyroiditis (PPAT) is a common endocrinological disorder that uniquely manifests itself within one year after delivery.[27]

Patients 61 – 63 grouped under Hashimoto's thyroiditis. All patients in this group were females. Only P61 presented with goiter but showed normal TFTs as she was on thyroxine. All patients showed anti-peroxidase antibodies. Biopsy of the thyroid gland showed diffuse lymphocytes infiltrates thus confirming the diagnosis in these patients. It is an organ – specific autoimmune disorder characterized by infiltration of the thyroid gland by inflammatory cells, often followed by hypothyroidism due to destruction and fibrosis of the gland.[28] It mainly affects females and it has been demonstrated that there is increased risk of papillary thyroid cancer especially in females affected with Hashimoto thyroiditis.[29]

P_{64} – P_{66} included in the group of thyroid cancer. These patients had painless palpable thyroid nodule. Thyroid scan showed cold nodule and suspicion of malignancy was raised. FNAC showed thyroid cancer (papillary carcinoma). All patients were hypothyroid at presentation. Thyroid nodules represent a common clinical problem. The clinical importance of thyroid nodules rests with the need to exclude thyroid cancer that occurs in 5 – 15% depending on age, sex, radiation exposure history, family history, and other factors.[30] Papillary carcinoma is the most common malignant tumor of the thyroid.[31]

$P_{67 - 69}$ got the diagnosis of post sub-acute thyroiditis hypothyroidism. These patients were previously

had thyrotoxicosis for 1 – 2 months and then hypothyroid phase was observed. An important finding includes throat infection with painful goiter. The disease has tri-phasic clinical course of hyperthyroidism, hypothyroidism, and return to normal thyroid function. Subacute thyroiditis (SAT) is a self – limiting inflammatory disease of the thyroid. Although a viral infection has most often been implicated as the cause of SAT, the evidence is generally indirect, and includes a preceding upper respiratory tract infection, the presence of elevated titers of several viral antibodies in the sera, and seasonal and geographic case clusters.[32]

P_{70} was found to be a case of juvenile hypothyroidism. Juvenile hypothyroidism is a common endocrine disorder that manifests as short stature and delayed puberty. However, there are anecdotal reports of precocious puberty, isolated menarche, multicystic ovaries and galactorrheain these patients.[33] P_{70} in this study also showed short stature, delayed puberty, isolated menarche and galactorrhea so diagnosed for juvenile hypothyroidism.

P_{71} and P_{72} were diagnosed as secondary hypothyroid cases. This is also called pituitary hypothyroidism. P_{71} was having pituitary macroadenoma whereas in P_{72} TSH levels were low with low T_4 and T_3 and anterior pituitary gland was absent with empty sella. Both patients had MRI of brain that showed suspected lesion. So diagnosis was confirmed for pituitary hypothyroidism. The pituitary CH (central hypothyroidism) implies a reduction in the number of functioning thyrotropes, mainly accounting for the quantitative impairment of TSH secretion.[34] The main causes of central hypothyroidism include pituitary adenomas, pituitary tumor apoplexy, craniopharyngioma, empty sella syndrome, Sheehan syndrome and radiation therapy.[35]

P_{73} was classified as having non-thyroid illness. This is also called as euthyroid sick syndrome. The patient showed decreased T_3, low normal FT_4 and high TSH. Nonthyroidal illness syndrome is generally characterized by low serum T_3, normal free T_4 and TSH, and elevated rT_3 values. Unique changes in thyroid function parameters are observed in various clinical states, including starvation and fasting, cardiac disease, renal disease, hepatic disease, and infection.[36] The cause of nonthyroidal illness in P_{73} was infection. P_{74} and$_{75}$ were included in idiopathic hypothyroidism as no cause was found for hypothyroidism.

We performed TFTs for all patients by commercial kit method of RIA / IRMA. We also compared these results with the in – house method by selecting patient samples from each category of hypothyroidism mentioned above. So 40 repeat assays of TFTs were performed by in – house method of RIA / IRMA and compared with the commercial kit based methods. There was no statistical difference observed. So results obtained by both commercial kit and in – house RIA / IRMA method were equally comparable.

Commercial kit for the estimation of TFTs is very expensive as compared to in – house method. In developing countries like Pakistan where low socio-economics status directly effects the health, replacing commercial kit methods with the in house method for the estimation of TFTs can be offered. In this way cost-effective confirmation by relevant investigation may be helpful. Thus, in – house methods can be brought into practice for performing TFTs provided criteria for quality control is fulfilled.

Conclusion

Hypothyroidism is a clinical diagnosis and it should be confirmed with estimation of TFTs. Depending on the additional clinical symptoms other relevant investigations including thyroid scan, fine – needle aspiration cytology (FNAC), radioactive iodine uptake test, perchlorate discharge test, anti-thyroid antibodies test, thyroid biopsy must be done for the differential diagnosis of hypothyroidism. Cost effective in – house methods of RIA / IRMA for the measurement of TFTs may be done in place of costly commercial kit methods.

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