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Original Research Paper

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STUDY OF THYROID PROFILE ESTIMATIONS BY IMMUNODIAGNOSIS IN RURAL POPULATION

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ABSTRACT Thyroid profile involves testing of Thyroid Stimulating Hormone (TSH), Thyroxine (T4) and Triiodothyronine (T3). It enables the diagnosis and prognosis of thyroid patients by clinicians. Hypothyroidism and hyperthyroidisms have distinct clinical symptoms. The present study is undertaken in rural population involving the recent and newer technique of thyroid profile estimation by immunodiagnosis; which utilizes enhanced chemiluminesence for better detection of thyroid diseases. Both out and in-patients of both genders recommended for thyroid profile are used for this present study. The detection technique has good sensitivity. The integrated dual read output mode gives greater accuracy, sensitivity and precision. In the present study frequency of euthyroids are greater than prevalence of thyroid disease in the rural population. Prevalence of thyroid disease is greater amongst the females and out-patients as compared to males and in-patients in the rural population. Factors causing variations in the results should be considered while interpreting the thyroid profile.

KEYWORDS : Thyroid Profile, Euthyroid, Immunodiagnosis, Chemiluminesence

Introduction

Worldwide endocrine disorders are the major threat to public health [1] [Bose et al ,2015].The commonest endocrinopathy across the globe are thyroid dysfunction [2] [Unnikrishnan and Menon ,2011].Recent research estimates, approximately 300 million people are affected by thyroid disorders globally. In India, 42 million people approximately have thyroid diseases [3] [Nimmy et al, 2012].Thyroid gland is an important endocrine organ that produces hormones that regulate the bodily metabolism [4] [5] [Policeni et al, 2012] [Skarulis and Stack, 2015]. Thyrotropin or thyroid stimulating hormone (TSH) secreted by the pituitary gland stimulates and enables the thyroid gland to synthesize, store and secrete the thyroid hormones – tetraiodothyronine (thyroxine T_4) and triiodothyronine (T_3) [6], [7] [Arora et al , 2016) (Gónzalez et al ,2014].

Thyroid diseases result due to the auto-antigens inherent in the thyroid gland which may trigger an abnormal immune response in certain conditions [8] [Przybylik-Mazurek et al, 2007].Thyroid dysfunction clinically causes a variety of signs and symptoms. In laboratory diagnostic testing, thyroid function tests (TFT) are the most common endocrine tests required by clinicians to diagnose, assess and monitor thyroid patients. However, prior to the treatment of the patients, the clinical signs and symptoms serve as vital factors for clinicians [9], [10] [Kapoor, 2015] [Begum, 2015]. Based on the results of Thyroid Function Tests (TFT), patients can be categorized as hypothyroid, hyperthyroid and euthyroid. Euthyroid population depicts their results of thyroid profile within the normal ranges of biochemical estimations done. Hypothyroidism means deficiency in thyroid hormone secretion; whereas hyperthyroidism shows elevation T_3 and T_4 hormones. Secretions of the thyroid glands are affected and since the thyroid gland is the origin of these hormones, the disorder is classified as 'primary. The defect is 'central' provided the hypophysis or hypothalamus is affected. The condition maybe 'subclinical' hypo or hyperthyroidism when the concentrations of TSH is altered and there are no clinical symptoms [6] [11] [Arora et al, 2016] [Mayayo et al, 2002].

Hypothyroidism results in clinical symptoms such as cold intolerance, sleep distrubances, depression, constipation, alopecia, dry rough pale skin, weight gain , fatigue, muscular aches, memory losses, irregular menstrual cycles, goiters (enlargement of thyroid gland) and thyroid cancer [12], [13][Ismail and Jatwa ,2014][Jonklaas et al , 2014].Hyperthyroidism manifests as heat sensitivity, palpitations, hyperactivity, excessive appetite ,diarrhea and /or frequent bowel movements, eyes being light sensitive, high blood pressure, moist skin, excessive sweating, tremors, nervousness and exophthalmic goiter (bulging eyes) [12], [14] [Ismail and Jatwa, 2014][Intidhar et al ,2006].The laboratory of TSH and thyroid hormone levels assists in evaluation of thyroid dysfunction and prognosis in thyroid disorders [15], [16] [Greenspan ,2004] [Gietka-Czernel ,2008].

In the laboratory practice of Thyroid Function Test (TFT) several combinations of thyroid profile are routinely analyzed .Thyroid stimulating hormone (TSH) is the single test of thyroid function, since TSH is central to the negative-feedback system by the thyroid hormones. Small deviations in levels of thyroid hormones result in logarithmic amplifications in TSH secretion. With the most advanced and recent (third generation chemiluminescent) TSH assays detection of both elevation and decrease in TSH levels is possible reliably. This assists in the diagnosis of subclinical thyrotoxicosis. TSH detection is suggested and estimated in patients with suspected goiters, screening for congenital hypothyroidism, atrial fibrillations, dyslipidaemic patients, osteoporosis and infertility. Inappropriately detected or increased serum TSH concentration in addition to increased FT_4 and or FT_3 defines the biochemical diagnosis of Inappropriate TSH caused due to TSH secreting pituitary tumor (TSH oma) or a syndrome of thyroid hormone resistance. The diagnosis is confirmed by pituitary imaging. The analytical laboratory errors and assay artifacts may also be considered. If on repeated analysis the condition persists the reasons for elevation of FT_4 may be due to binding protein abnormalities such as familial dysalbuminaemia and hyperthyroxinaemia or assay dependent antibody interferences in the measurement of FT_4, FT_3 or TSH. To differentiate between TSH omas and thyroid hormone resistance, sex hormone binding globulin (SHBG), circulating α subunit and other anterior pituitary hormones maybe estimated [17] [Joshi,2011].

In thyroid testing, total T_4 and total T_3 detected since the circulating hormones in inactive forms are bound to carrier proteins such as thyroid binding globulin (TBG), transthyretin and albumin. In congenital abnormalities of binding protein, drug induced alteration and presence of antibodies to thyroid hormones may result in elevated total T_3 and TotalT_4 whereas free T_3 and T_4 levels maybe normal. Free T_3/Total T_3 measurements are estimated in patients suspected of T_3 thyrotoxicosis, consumption of drugs (e.g. dexamethasone, propranolol, propylthiouracil, amiodarone and iodine containing contrasting media). When pituitary-thyroid axis is affected or unstable the measurement of testing both serum TSH and FT_4 arises. These conditions include regulating thyroxine (T_4) therapy in newly diagnosed hypothyroid patients, in diagnosis and monitoring of thyroid status during pregnancy, monitoring of thyroid states during pregnancy, monitoring hyperthyroidism during early months after treatment, diagnosis and monitoring of drugs for central hyperthyroidism, end organ thyroid hormone

resistance, sick euthyroid state, TSH –secreting pituitary adenomas , women with type I diabetes, subclinical hypothyroidism, overtly hypothyroid patients. In patients with high serum TSH levels and normal FT_4 (subclinical hypothyroidism),the TSH and FT_4 measurements should be repeated after 3-6 months to rule out non –thyroid diseases and drug interferences. Thus combination of TSH and FT_4 helps in confirmation rather than testing TSH alone [17] [Joshi,2011].

In intensive care unit (ICU) admitted patients in the absence of abnormal thyroid gland on physical examination. The first line serum TSH should be carried out followed by FT_4 and FT_3 estimations later when indicated. The other clinical conditions which alter and affect the thyroid profile include estrogen treatment contraceptive pills and pregnancy which cause protein abnormalities causing total T_3 to increase in absence of hyperthyroidism. When hypothyroidism is suspected free-T_3 testing is suggested since total T_3 and free-T_3 have inadequate sensitivity and specificity in such condition. In T_3-toxicosis there is selective elevation in serum T_3 concentration or hyperthyroidism when suspected a combination of free -T_4 estimate or a total and free T_3 estimate provides the severity of hyperthyroidism [17] [Joshi,2011].

In the past many methodologies /techniques have been utilized to estimate TSH and thyroid hormones such as chromatography [Wang et al ,2003] [18], ultrafiltration (UF) [Christofides and Midgley, 2009] [19], liquid chromatography-tandem mass spectrometry (LC/MS) [Kunisue et al,2011] [20], radioimmunoassay (RIA) [Hemmati and Pishwa , 2009] [21], bioluminescent immunoassay (BLIA) [Frank et al , 2004] [22], equilibrium dialysis (ED) [Yue et al , 2008] [23] , time-resolved fluorometry (TRFIA) [Zhou et al , 2012] [24], fluoroimmunoassay (FIA) [Hertzberg et al , 2010] [25] ,enzyme linked immunoassay (ELISA) [Islam et al , 2011] [26], chemiluminescent assay (CLA) [Huang et al , 2010] [27], electrochemiluminescent immunoassay (ECLA) [Zhang et al , 2012] [28].

Although, the RIA method is an accurate, reliable, fast and sensitive technique it requires prolonged incubation periods, trained personnels to operate specialized instruments and radioactivity. The exposure to radioactive biohazards, disposal of radioactive wastes and short half – life of radioactive labels are the disadvantages of RIA .Recent and safer methods such as CLA and ELISA and preferred nowadays over RIA and are routinely utilized in medical laboratories for diagnosis and research purposes [Eshratkheh et al , 2010,2011 a] [29] [30], [Jin et al , 2009] [31], [Lin et al , 2008] [32] , [Nayak and Nayak , 2007] [33] [Xiao et al , 2010] [34].

The present study deals with the recent and newer technique of thyroid profile estimations by immunodiagnosis which utilizes enhanced chemiluminesence for better detection in the rural population studied. The availability of state-of-art automated instruments, facilities and resources are the important factors which motivated to undertake such a study on thyroid profile estimations in the rural population.

Ethical Clearance: Prior to the commencement of the study the ethical permission was sought from the Institutional Ethical Committee (IEC) of Pravara Institute of Medical Sciences (Deemed to be University). The study was approved by the IEC and was registered as a research study with registration No: PIMS/ RMC/ DR/2017/224 on April 2018.

Study Design: Cross-sectional –Analytical

Aims and Objectives:

The present study intended the following

1) To establish abnormal thyroid function as well as trend in rural population
2) State the salient features of Immunodiagnostic technique utilized

Materials and Methods:

The blood samples that were directed to Biochemistry Section of Central Clinical Laboratory (CCL) for Thyroid Profile by the clinicians of Pravara Rural Hospital (PRH) were utilized for the study.

Inclusion criteria:

1) Both sexes that is females and males recommended for Thyroid Profile
2) Out patients (OPD) and in-patient (IPD) samples sent and received by Central Clinical Laboratory (CCL), Biochemistry

Exclusion criteria:

1) Hemolysed samples were excluded from the study

Variables studied and measured are thyroid stimulating hormone (TSH) , thyroxine (T_4) and triiodothyronine (T_3),demographic details e.g. age, sex, IPD /OPD patients and clinical condition based on thyroid profile.

Sample Size: The total sample size was deduced to be 1000 samples based on the daily, monthly and yearly estimations of Thyroid Profile conducted at Central Clinical Laboratory, Biochemistry for the last three years.

Statistical Analysis : Aid of SPSS Statistical package 21 for statistical analysis of data.

Estimation of Thyroid Stimulating Hormone (TSH) : The TSH test is performed utilizing VITROS TSH Reagent Pack consisting of 100 coated wells of streptavidin, horseradhish peroxidase-mouse monoclonal anti-TSH as conjugate reagent and biotinylated antibody reagent and VITROS TSH Calibrators which consists of 1,2 and 3 (recombinant TSH in bovine serum and antimicrobial agent) with nominal values 0,0.092 and 15.9 µIU/ml. The system used for detection is VITROS ECi Immunodiagnostic Systems developed by Ortho Clinical Diagnostics. It is an immunometric immunoassay technique utilizing enhanced chemiluminesence for detection purpose. TSH present in the serum sample reacts with biotinylated antibody (mouse monoclonal anti-whole TSH) and horseradhish. peroxidase (HRP) –labeled antibody conjugate (mouse monoclonal anti-TSH –β- subunit).The streptavidin coated microwells capture the antigen –antibody complex. The subsequent wash removes the unbound materials. The bound HRP conjugate is detected by luminescent reaction. The wells have added reagents containing luminogenic substances (a luminol derivative and a peracid salt) and an electron transfer agent. The oxidation of the luminol derivative by the HRP in the bound conjugate produces the light. The intensity of light produced and increase in its emission (a substituted acetanilanide).The light signals are read by the instrument. The amount of HRP conjugate bound is directly proportional to the TSH concentration present in the serum sample. The incubation period for the test is approximately 29 minutes occurring at test temperature of 37°C and the amount of serum sample required is approximately 80 µl (microliters).

Estimation of Total T_3 (Tt_3): The VITROS Total T_3 is performed using VITROS Total T_3 Reagent Pack consisting of 100 coated wells with donkey ant-sheep), horseradhish peroxidase conjugate reagent and an assay reagent consisting of sheep –anti- T3 which is capable of binding to the triiodothyronine (T_3) and VITROS Total T_3 Calibrators consisting of 1,2 and 3 (T_3 in human serum and antimicrobial agent) with nominal values 0, 0,98 and 3.91 ng/ml. This biochemical test is performed on the VITROS ECi Immunodiagnostic System which detects by enhanced chemiluminesence developed by Ortho Clinical Diagnostics. It is a competitive immunoassay technique in which T3 present in the serum sample competes with horseradhish peroxidase (HRP) –labeled T_3 conjugate foe limited number of binding sites on sheep anti-T_3 antibody present in liquid phase. Binding protein effects are removed by usage of appropriate buffer and blocking agent. The antigen –antibody complex captured by a donkey anti-sheep second antibody coated on the wells. Subsequent washing removes the unbound materials. The bound HRP conjugate is estimated by the same luminescent reaction utilized and described in TSH estimation. The incubation period in 29 minutes , results are obtained within 37 minutes at temperature of 37°C and serum sample required is approximately 20 µl (microliters).

Estimation of Total Thyroxine (Tt_4): Is performed by utilizing VITROS T_4 Reagent Pack consisting of 100 coated wells of donkey anti-sheep. HRP conjugate reagent and assay reagent consisting of sheep anti-T_4 and VITROS Total T_4 Calibrators performed on the VITROS ECi Immunodiagnostic System developed by Ortho Clinical Diagnostics. VITROS Total T_4 Calibrators 1,2,3 (consists of T_4 in human serum with antimicrobial agent). It is a competitive immunoassay technique which is dependent on the competition between T_4 present in the serum sample and the horseradish peroxidase (HRP) –labeled T_4 conjugate for the limiting binding sites on sheep anti-T_4 antibody present in the liquid phase. Appropriate buffer containing displacement agent removes the effects of binding proteins. The antigen –antibody complex are captured by donkey anti-sheep second antibody coated on the wells. Subsequent washing removes the unbound materials. The bound HRP conjugate is measured by luminescent reaction as described in the TSH assay. The incubation time required is 16 minutes, the results are obtained in 24 minutes at temperature of 37°C and the amount of serum sample required is 10 µl (microliters) [35]

Table 1: Units of Variables studied in present study

Parameter	Reporting Units in the present study
TSH	µIU/ml (mU/L x1)
T3	ng/ml (nmol/L X 0.651)
T4	µg/dl (nmol/L X 0.0777

Table 2: Normal Ranges of the Biochemical Parameters studied

Parameter	Normal Range
TSH	0.465-4.68 µIU/ml
T3	0.970-1.69 ng/ml
T4	5.53-11.0 µg/dl

Figure 1: Diagrammatic representation of estimation of Thyroid stimulating hormone (TSH) by Immunodiagnosis

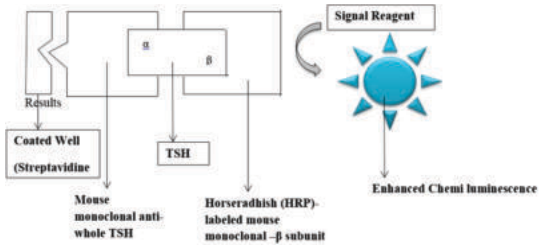

Figure 2: Diagrammatic representation of Competitive immunoassay of Total triiodothyronine (TT3) and Total thyroxine (Tt4)

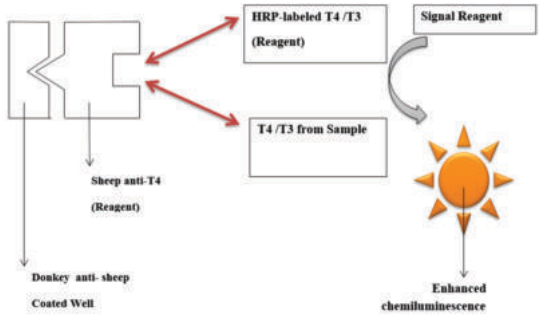

Figure 3: Prevalence of thyroid disease in the rural population of present study

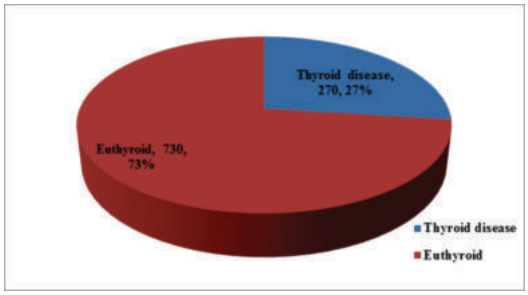

Figure 4 : Sex -wise distribution of thyroid disease in rural population

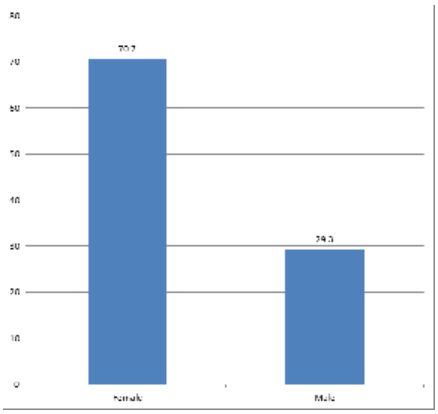

Figure 5: Regisration of patients in present study

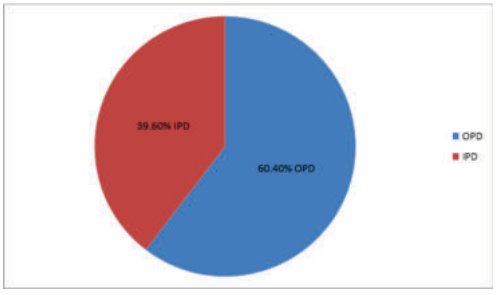

Figure 6 : Thyroid Stimulating Hormone (TSH) evaluation in the present study

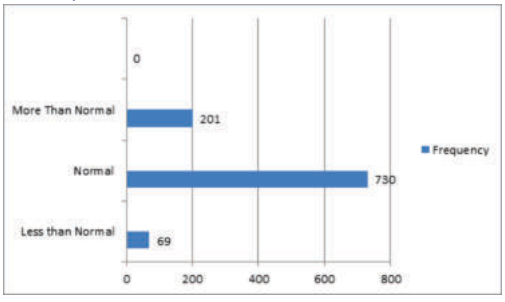

Table 3: Distribution of thyroid patients according to age in the present study

		Disease present	Disease absent	Total
	Infant	32	52	84
	1-20 yrs	66	182	248
	21-44 yrs	100	310	410
	45-64 yrs	54	130	184
	65 and above	18	56	74
Total		270	730	1000

Chi-square: 7.465, Degrees of Freedom :4, P value= 0.1132, Variables are not significantly associated

Table 4: Descriptive Statistics of Thyroid Profile in present study

Thyroid Profile	No	Minimum	Maximum	Mean	Std. Deviation
T_3	1000	0.071	8.680	1.28499	.630401
T_4	1000	0.405	24.900	9.15462	3.596069
TSH	1000	0.013	315.000	6.33100	18.908585

Table 5: Classification of Thyroid Diseases Based on Thyroid Profile

THYROID DISEASE	NO.	%
Subclinical hypothyroidism	86	31.85

Primary hypothyroidism	29	10.74
Central hypothyroidism	07	2.59
Subclinical hyperthyroidism	14	5.18
Primary hyperthyroidism	13	4.81
Central hyperthyroidism	09	3.33
Thyroid dysfunction	112	41.48

Discussion

In the present study of sample size (n=1000); 270 (27%) patients suffer from thyroid diseases whereas 730 (73%) are detected euthyroid as depicted in Figure No 1.The results of the present study are in consensus with a similar study of thyroid disorders in rural population done by Arora et al in 2016 [6] wherein 992 (25.17%) had thyroid disorders and 2948 (74.82%) patients were euthyroid.

Amongst the patients with thyroid dysfunction the prevalence are higher in females 191 (70.2%) as compared to males 79 (29.3%) as represented by Figure 2.Sex- wise distribution of thyroid diseases were found to be greater in females than males in a hospital based study by Begum in 2015 [10].The out –patient registered are 163 (60.4%) in the present study as compared in –patients 107 (39.6%) as represented in Figure 3.

As per data analysis on the basis estimations of Thyroid Stimulating Hormone (TSH); 69 (6.9%) show low TSH levels, whereas 201 (20.2%) denote high TSH and 730 (73%) patients represent normal levels of TSH amongst the sample size of 1000 shown in Figure 4. In a study reported by Begum in 2015 (10); patients with elevated TSH were 2569 (14.83%), low TSH were 1079(6.22%) and normal levels of TSH in 13677 (78.95%) which is in consensus with results of the present study.

As per the data of the present study (Table 3) the age group 21-44 years are mainly affected mainly with thyroid dysfunction the prevalence is lower in elderly group of 65 years and above. On applying the chi square test the variables are not significantly associated in the present study.

According to the present study considering the results in terms of Mean ± SD of T3 is 1.28± 0.63ng/ml, T4 9.15 ±3.59 µg/dl and TSH 6.33± 18.90 µIU/ml as per descriptive statistics depicted in Table 4.As per classification of thyroid profile based on biological reference interval (BRI) published in the Laboratory Support for the diagnosis and monitoring of Thyroid disease [36] [Deners and Spencer ,2002] [7] [Gónzalez et al ,2014], [11] [Mayayo et al 2002] [37] [Unanua et al ,2008] ; the thyroid patients are classified as primary hypothyroidism 29(10.74%),primary hyperthyroidism 13 (4.81%), subclinical hypothyroidism 86 (31.85%), subclinical hyperthyroidism 14(5.18%), central hypothyroidism 7(2.59%) and central hyperthyroidism 9 (3.33%) ; however &112 (41.48%) of the patients did not aptly and stringently fit in the criteria of classification used in the present study as depicted in Table 5.

The measurement of serum TSH along with T3 and T$ forms the basis of laboratory diagnosis and monitoring of thyroid dysfunction [38][Sanchez-Carbayo et al. 1994].The Vitros ECi (Ortho Clinical Diagnostics) fully automated instrument utilizes a recent enhanced chemiluminescent technology. The microwells and assay reagent are supplied together in combined packs with calibration information stored on magnetic calibration cards which are bar-coded. The technical performance with ease, good operation and rapid turnaround time are characteristic features of thyroid evaluation by the Vitros ECi [39][Sanu et al ,1999].

The enhanced chemiluminesence technology utilizes dual read mode which automatically performs multiple readings adjusting to low or high light output. This enables better detection, even in case of low analytes concentration. Hence proves a greater advantage in making better clinical decisions based on accuracy, sensitivity and precision of the method adopted [40][Thorpe et al 1985][35][Summers et al ,1995].

It is recommended that preanalytical steps should be standardized to reduce the preanalytical variations in thyroid profile detection. Prolonged venipressure and posture maintained during phlebotomy [41][Esther et al, 2004], may alter serum concentrations of T_3 and T_4 slightly since they are protein bound. The levels of TSH and thyroid hormones in serum maybe influenced by drugs [42][Surks and Sievert,1995] [43] [Meier and Burger], smoking [44],[45],[46'a'] [Vestergoad,2002][Brix et al , 2000] [Knudsen et al , 2002] and pregnancy [47] [46'b'] [Glisner ,1999] [Knudsen et al ,2002] but their effect of physiology of thyroid functioning is less known.

Analytical variation causes imprecision due to biological variation .However, if biologic variation is high then analytical variation gains less importance and requirement of precision of analysis diminishes [Fraser and Harris ,1989] [48] [Fraser,1983] [49].The goals set are that due to analytical error the variance should not exceed 20% [49] [Fraser ,1983]. The circadian and seasonal variations affect the biological variations in thyroid function tests [50] [Andersen et al, 2003].Low levels of TSH are observed during the day with characteristic elevation during the night, more than 100% peak after midnight. But such increase in serum T_3 and T_4 is seen due to nocturnal rise in TSH [51] [Fisher, 1996] [52] [Persani et al, 1995] [53] [Greenspan et al 1976] [54] [Azukizawa et al, 1976]. However, these fluctuations does not exceed the normal ranges and are contributory to the width of the normal ranges [55] [Lucke et al ,1977].

The environmental factors govern the circadian rhythm of TSH. Sleep decreased the pulse amplitude but not pulse frequency of TSH [56] [Brabant et al,1990] [57] [Rossman et al,1979] [58] [Parker et al ,1976] , similarly fasting [59] [Romijn et al ,1990] and T_3 and T_4 infusion [60] [Brabant et al ,1987].The circadian pulsatile secretion was found to be remarkably reproducible in the individuals but showed considerable and unaltered differences between individuals [56] [Brabant et al ,1990][Greenspan et al , 1976] [53]. Seasonal variations such as serum T_3 being elevated during winter while T_4 and TSH were less consistent to seasonal changes [50] [Andersen et al,2003].Ambient temperature and luminosity [61] [Leppaluoto et al , 1998] [Levine et al ,1995] [62] cause seasonal variations; with decrease in temperature T_3 levels are elevated [63] [Reed , 1995] [64] [Hassi et al ,2001].

Conclusion

In the present study the prevalence of thyroid diseases is greater in females as compared to males in the rural population. The patients suffering from thyroid diseases are less as compared to euthyroid patients. But gender and age are not statistically significant in the entire rural population studied It may be due to the accuracy in biochemical testing wherein even low concentration of analyte is measured by immunodiagnosis technique for thyroid profile estimation. However , the possible variations in the levels of thyroid hormones should also be considered when interpreting the results of thyroid profile.

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