ORIGINAL ARTICLE - CORELATION BETWEEN FINE NEEDLE ASPIRATION CYTOLOGY AND THYROID FUNCTION TEST IN CASES OF BENIGN THYROID LESION

Vidya Viswanathan^{1*}, Shruti Vimal², Khushali Parikh³, Arpana Dharwadkar⁴, Banyameen Iqbal⁵, Rupali Kulkarni⁶

^{1*}Professor, Department Of Pathology, Dr D.Y.Patil Medical College Hospital And Research Centre, Dr D Y Patil Vidyapeeth, Pimpri Pune, Maharashtra. Ph No: 9822046668 Email ID – Docvidya11@Gmail.Com

²Professor, Department Of Pathology Symbiosis Medical College For Women, Symbiosis International University Lavale, Pune, Maharashtra. Ph No: 9823611291 Mail ID – Shruti.Vimal@Smcw.Siu.Edu.In ORCID ID: 0000-0003-4800-5954

³Post-Graduate Student Dr D.Y.Patil Medical College Hospital And Research Centre, Dr D Y Patil Vidyapeeth, Pimpri Pune, Maharashtra. Ph No: 9909395860 E-Mail ID – Khushali Prkh@Yahoo.Com, Khushali1693@Gmail.Com

⁴Professor, Department Of Pathology Dr D.Y.Patil Medical College Hospital And Research Centre, Dr D Y Patil Vidyapeeth, Pimpri Pune, Maharashtra. Ph No: 9423222540 Email ID – Arpana.Dharwadkar@Dpu.Edu.In ORCID ID: 0000-0001-8126-0303

⁵Professor, Department Of Pathology Dr D.Y.Patil Medical College Hospital And Research Centre, Dr D Y Patil Vidyapeeth, Pimpri Pune, Maharashtra. Ph No: 9730388872 Email ID – Mb.Iqbal@Dpu.Edu.In ORCID ID: 0000-0002-1117-8379

⁶Professor, Department Of Pathology Dr D.Y.Patil Medical College Hospital And Research Centre, Dr D Y Patil Vidyapeeth, Pimpri Pune, Maharashtra. Ph No: 8149367249 Email ID – Rupali.Bavikar@Dpu.Edu.In ORCID ID: 0000-0002-1414-5273

*Corresponding Author: Vidya Viswanathan

*Professor, Department Of Pathology, Dr D.Y.Patil Medical College Hospital And Research Centre, Dr D Y Patil Vidyapeeth, Pimpri Pune, Maharashtra. Ph No: 9822046668Email ID – Docvidya11@Gmail.Com

Most thyroid lesions present as benign swellings which require fine needle aspiration for diagnosis. Fine needle aspiration cytology (FNAC) and serological methods can both be used to identify the alterations occurring at the cellular level. The recent epidemiological trends of benign thyroid lesions seen at a tertiary care centre in Pune, India were identified. FNAC (fine needle aspiration cytology) and serological tests were used to diagnose the thyroid lesion and the results were correlated. Patients with probable benign thyroid illness who presented to the outpatient department were sent for FNAC (fine needle aspiration cytology) and the smears were stained with hematoxylin and eosin. 2 to 5 cc of blood was aspirated into a red-capped vacutainer and analysed using chemiluminescence principle and ELISA (enzyme linked immuno sorbent assay)for thyroid function test. There were 76 patients enrolled in the study, with 17% men and 83% women. The age range of 45 to 60 years saw the highest number of instances. The thyroid profile revealed 37 euthyroid patients, 19 hypothyroid cases, and 20 hyperthyroid cases. 12 out of 14 subjects with thyroid swelling diagnosed as single nodular goitre was having euthyoid status while 7 out of 9 diagnosed as multinodular goitre were having hyperthyroid status. Eight of the cases of Hashimoto's thyroiditis 5 cases were hypothyroid, while 1 instances was euthyroid, 2 cases were hyperthyroid. As opposed to lymphocytic thyroiditis, which had 5 euthyroid, 4 hypothyroid, and 1 hyperthyroid patients. There was no discernible relationship between thyroid hormone levels and FNAC (fine needle aspiration cytology) results when they were compared. In light of this situation, separate and individual assessments of thyroid hormone levels and FNAC (fine needle aspiration cytology) are required for accurate evaluation.

Key Words: Benign thyroid lesions, FNAC, Fine Needle Aspiration Cytology, TFT, Thyroid Function Test, colloid goitre, Hashimoto's thyroiditis

INTRODUCTION

Thyroid is an endocrine gland situated in the lower part of fount and sides of the neck. It functions by regulating the basal metabolic rate, stimulating somatic and psychic growth and plays an important role in calcium metabolism. Goiter is defined as any enlargement of the thyroid gland. On clinical examination a nodule that appears to be single in one lobe of the thyroid with no palpable abnormality elsewhere in the gland is known as a solitary nodule of the thyroid. The incidence of which in the neck in India is 9% ^[1, 2].

While mentioning about the homeostasis and normal physiology of the body one generally ignores the role of thyroid gland. The gland produces the hormone T3 (tri-iodo-Thyronine) and T4 (thyroxine) required for maintaining the basal metabolic rate, protein synthesis and as a synergist to growth hormone. The gland is located in the highly vascular region of the neck with two lobes left and right and an isthmus connecting them. The hormones are formed within the colloid follicles of the gland ^[1, 2].

Warren H Cole in 1949 concluded higher incidence of malignancy in solitary nodule as compared to Multi-nodular goiter (MNG) raising interest in the study of these palpable nodules of thyroid ^[3]. Thyroid nodule is very common and the prevalence ranges from 4% by palpation to 67% by ultrasonography. Autopsy studies reveal that 50% of adults had nodules, the majority of which are impalpable. Thyroid nodules are 4 times less common in males than in females ^[46]. A palpable nodule is a clinical diagnosis and not a pathological diagnosis. Benign nodules are classified as thyroiditis, adenomas, hyperplastic nodules, colloid nodules, infectious nodules, cysts, lymphocytic or granulomatous, and congenital anomalies. Rarely it may be the cause of inflammatory thyroid lesions and developmental abnormalities such as dermoid cyst, teratoma etc. In a clinically suspected case of malignancy, the nodule is usually firm in consistency, whereas a mobile nodule has a higher probability of being benign but it still carries a small but significant risk of being malignant ^[7].

Of all the disorders of the thyroid gland, multinodular goiter is the most commonest condition encountered. Multinodular goiter is thought to be due to genetic heterogeneity of follicular cells which is related to function (i.e. thyroid hormone synthesis) and growth and due to acquisition of new qualities that were not present in thyroid cells primarily and become inheritable during further replication ^[8].

Mutations occurring in follicular cells form activated adenomas cause hyperthyroidism. This leads to further loss of anatomical and functional integrity of the follicles and the gland. These These process ultimately lead to goiter formation and are accelerated by stimulatory factors such as elevated serum TSH (thyroid stimulating hormone) which are brought about by events such as iodine deficiency, inborn errors of thyroid hormone synthesis, goitrogens or local tissue growth regulating factors ^[8].

Iodine has a critical role in the functioning of the thyroid gland. It is important for hormonogenesis and for the normal functioning of the thyroid gland. A number of studies show the relationship between iodine intake and hormone production. The U-shaped curve indicates the double-edged nature of iodine i.e. deficiency or excess of iodine in the diet can result in the increased prevalence of thyroid disorders ^[9].

The preoperative evaluation of thyroid nodules is very important as it helps to distinguish between benign and malignant conditions which further helps the clinician to decide the course of treatment. Unnecessary surgery and surgery related adverse effects such as hypocalcemia, hypothyroidism and recurrent laryngeal nerve injury can be prevented ^[10].

A fast, efficient, precise and inexpensive pre-operative investigation method for diagnosis of thyroid lesions is fine needle aspiration cytology which is considered to be the gold standard preoperatively.

Any solitary or dominant thyroid nodule larger than 1 cm is fit for FNAC examination as lesions smaller may be accompanied with an increased risk of injury^[11].

Thyroid function tests are used in assessing the levels of the hormones namely T3 (triiodothyronine), T4 (thyroxine) and TSH (thyroid stimulating hormone). Generally, conditions with multinodular goitre show values ranging from normal, subclinical or overt hypo or hyperthyroidism.

Currently, cytological evaluation of routine MGG, also known as May Grunwald Giemsa stain or PAP, also known as Papanicolaou or commonly with Hematotoxylin and eosin, stained slides for FNAC (fine needle aspiration cytology), the results of which are correlated with thyroid function tests is the method employed to established the diagnosis. The present study was conducted at our tertiary care centre at DR. D. Y. Patil medical college and reseach centre, Pimpri, Maharashtra, India to evaluate the clinical and biochemical parameters and to correlate the values with the FNAC (fine needle aspiration cytology) findings.

MATERIALS AND METHODS

Our current study is a prospective and retrospective investigation carried out at the Dr. D.Y. Patil Medical College hospital and research Centre Pimpri, Pune, Maharashtra, India. The trial ran for duration of 2 years. 76 cases were included in the study. Patients presenting with possible benign thyroid lesions were subjected to fine needle aspiration cytology in the department of pathology. There were no ethical or patient related issues during the collection of data. A verbal and written consent were taken from the patient to perform the Fine needle aspiration cytology procedure after explaining them focus of the study and then performed. 2cc to 5cc of blood sample was collected and stored in plain red capped vacutainers for thyroid hormone evaluation after separating the serum with the help of centrifugation, and analysed using chemiluminescence principle and ELISA (enzyme linked immuno sorbent assay) for thyroid function test. Cases above 18 years of age with a suspicion of benign thyroid lesion were included whereas cases less than 18 years and pregnant and lactating women were excluded.

FNAC (Fine Needle Aspiration Cytology) was performed on all the patients along with evaluation of thyroid function test. Smears were air-dried and then stained with giemsa and with wet ethanol fixed smears stained with hematoxylin and eosin or with Papanicolaou The stained slides were viewed and reported under the microscope. Smears showing adequate cellularity with thick or thin colloid admixed with sheets of evenly spaced follicular cells which suggest a diagnosis of benign thyroid lesion were considered. Bethesda system for Reporting Thyroid Cytopathology category I (Non-diagnostic or Unsatisfactory) or II (benign) are considered positive for Benign Thyroid Lesion depending on the colloid fluid quantity aspirated on FNAC (Fine Needle Aspiration Cytology).

Blood samples of all the patients undergoing FNAC (Fine Needle Aspiration Cytology) procedure was collected and thyroid function test was performed. The samples was separated for serum by centrifuging the same at 2000 rpm for 10 minutes and then analysed under Beckman Coulter access using the Chemiluminescence principle or with ELISA (Enzyme Linked Immuno-Sorbent Assay) The normal acceptable levels of the thyroid profile, in our study, is mentioned in the TABLE 1 below

Table 1: Normal acceptable levels of Thyrioid function test or Thyroid Profile, ι	used in o	our
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Thyroid profile	Normal range
Free T3 (Thyronine)	0.8-2 ng/dL
Free T4 (Thyroxine)	1.4-4.2 pg/dL
TSH	0.4-4.2 mIU/L

Any value within the normal range of the free T3 (thyronine), free T4 (thyroxine) and TSH (thyroid stimulating hormone) is considered to be euthyroid stage.

A high value of TSH (thyroid stimulating hormone) and free T4 (thyroxine) is considered to be hyperthyroid. A hyperthyroid state of thyroid is considered even when the value of TSH (thyroid stimulating hormone) is lower whereas that of T3 (thyronine) and T4 (thyroxine) is higher.

For values of lower T4 (thyroxine) with increased TSH (thyroid stimulating hormone) is considered to be in hypothyroid state. Statistical calculations were done using the ANOVA (Analysis of Variance) methods using the SPSS (Statistical Package for Social Sciences) version 16 software and results were calculated.

RESULTS

In the current study sample of 76 cases, mean age was 44.57 years (standard deviation - 16.902 years), with the highest 90 years and lowest 21 years. There were 63 (83%) females and 13 (17%) males in the study. Maximum number of cases 22 (29%) were from 31-40 years age group followed by 16 (21.33%) subjects were from 41-50 years age group

(Figure 1). Of the 76 cases, 37 (49%) subjects were euthyroid, while 20 (26%) were hyperthyroid and 19 (25%) hypothyroid (Figure 2), with maximum duration of symptoms belonged to 7-12 months with 25 cases (33%) and least cases, 4(5%), having symptoms with duration over 24 months, (Figure 3) and 34 (45%) subjects were having diffuse thyroid enlargement 32 (42%) subjects were showing nodular thyroid enlargement and 10 (13%) were showing cystic enlargement (figure 4) out of the 42 nodular and cystic swellings, 33 (79%) of the swellings were single while in remaining 9 (21%) multiple nodules were palpated.

Mean FT4 (Free thyroxine) in nodular enlargement thyroid swelling (5.71+3.11 ng/dl) was higher than cystic (3.98+2.45 ng/dl) & diffuse (3.57+2.57 ng/dl) swelling lesion and difference was statistically significant (p value - 0.009) while there was no significant difference in FT3 (free thyronine) between them (p value 0.891) (figure 5). Mean FT4 (free thyroxine) among study sample was 4.52 pg/dl (SD - 2.95) and it ranges from 0.80 to 11.70 pg/dl (Range - 10.90 pg/dl) and Mean TSH (thyroid stimulating hormone) in diffuse enlargement thyroid swelling (3.74+2.76 mIU/L) was higher than cystic (2.56+1.50 mIU/L) & nodular (2.29+2.15 mIU/L) swelling lesion and difference was statistically significant (p value - 0.047) (figure 6). Mean TSH (thyroid stimulating hormone) among study sample was 2.97 mIU/L (SD - 2.46) and it ranges from 0.09 to 9.78 mIU/L (Range - 9.69 mIU/L).

FNAC (Free needle Aspiration Cytology) findings with 18 smears showing lymphocytic infiltration, 8 smears showing Hurthle cell changes and 3 smears showing adenomatoid changes figure 7 63 (83%) of the smears were showing adequate follicular cells, 10(13%) of the smears were pauci-cellular whereas only 3(4%) of the smears were hypercellular. 47 (62%) smears were having thick colloid 27 (35%) smears were having sparse colloid and 2 (3%) smears didn't show any colloid (Table 2). Out of the 76 cases, single/solitary nodule goiter has the maximum occurrence with 20 cases, followed by 12 cases of diffuse goiter, 10 cases each of benign cystic lesion and lymphocytic thyroiditis, 9 cases had multi-

nodular goiter. Hashimoto's thyroiditis occurred in 8 cases. Grave's disease and adenomatoid nodule had the least number of cases 4 and 3 respectively. 11 out of 63 females were having single nodular goitre followed by 9 with lymphatic thyroiditis and benign cystic lesion each while 4 out of 13 men were having diffuse goitre, 6 out of 22 subjects with thyroid swellings were from 31-40 years were having single nodular goiter. 4 subjects of lymphocytic thyroiditis were from 41-50 years age group while 4 subjects of diffuse goitre were from 21-30 years. 12 out of 14 subjects with thyroid swelling diagnosed as single nodular goitre was having euthyoid status while 7 out of 9 diagnosed as multinodular goitre were having hyperthyroid status



Figure 1 - Bar diagram showing age and gender wise distribution of study sample - In the current study sample of 76 cases, mean age was 44.57 years (standard deviation – 16.902 years), with the highest 90 years and lowest 21 years. There were 63(83%) females and 13 (17%) males in the study. Maximum number of cases 22(29%) were from 31-40 years age group followed by 16 (21.33%) subjects were from 41-50 years age group.



Figure 2 – shows that in the current study sample of 76 cases, (49%) 37 subjects were euthyroid, 20 (26%) were hyperthyroid and 19 (25%) hypothyroid.



Figure 3 - Distribution of 76 cases as per duration of symptoms - The current study shows that out of the 76 cases with maximum duration of symptoms belonged to 7-12 months with 25 cases (33%). 4 cases (5%) showed symptoms with duration over 24 months.



Figure 4 - Distribution of cases as per type of thyroid enlargement – out of the 76 cases taken part in the current study 34 (45%) subjects were having diffuse thyroid enlargement, 32 (42%) subjects were showing nodular thyroid enlargement and 10 (13%) were showing cystic enlargement



Figure 5 - Mean FT4 (free thyroxine) in nodular enlargement thyroid swelling (5.71+3.11 ng/dl) was higher than cystic (3.98+2.45 ng/dl) & diffuse (3.57+2.57 ng/dl) swelling lesion and difference was statistically significant (p value - 0.009) while there was no significant difference in FT3 (free thyronine) between them (p value 0.891).



Figure 6 - Mean TSH (thyroid stimulating hormone) in diffuse enlargement thyroid swelling (3.74+2.76 mIU/L) was higher than cystic (2.56+1.50 mIU/L) & nodular (2.29+2.15 mIU/L) swelling lesion and difference was statistically significant (p value - 0.047).



Figure 7 - Above bar diagram shows FNAC (fine needle aspiration cytology) findings of the 76 cases that have taken part in the current study with 18 smears showing lymphocytic infiltration, 8 smears showing Hurthle cell changes and 3 smears showing adenomatoid changes

Diagnosis	Frequency	Percentage
Adenomatoid nodule	3	3.9
Benign cystic lesion	10	13.2
Diffuse goiter	12	15.8
Grave's disease	4	5.3
Hashimotos thyroiditis	8	10.5
Lymphocytic thyroiditis	10	13.2
Multinodular goiter	9	11.8
Single nodular goiter	20	26.3
Total	76	100.0

Table 2 - Diagnosis among study subjects. Out of the 76 cases, single/solitary nodule goiter has the maximum occurrence with 20 cases, followed by 12 cases of diffuse goiter, 10 cases each of benign cystic lesion and lymphocytic thyroiditis, 9 cases had multi-nodular goiter.

Hashimoto's thyroiditis occurred in 8 cases. Grave's disease and adenomatoid nodule had the least number of cases 4 and 3 respectively.

DISCUSSION

FNAC (fine needle aspiration cytology) is a minimally invasive technique. Any palpable thyroid nodules measuring >1 cm in size or a nodule noticed on ultrasonography can be subjected to fine needle aspiration cytology.

FNAC (fine needle aspiration cytology) results should be correlated with other investigations like thyroid function tests, thyroid scan and ultrasonography findings for a better assessment of the thyroid condition. This in turn helps to decide the next stage of treatment.

Thyroid function tests namely free T3 (thyronine), free T4 (thyroxine) and TSH (thyroid stimulating hormone) are extremely useful in assessing the functioning condition of the thyroid. The correlation between FNAC (fine needle aspiration cytology) and biochemical parameters is necessary and can help differentiate the thyroid lesion into benign or malignant.

In the 76 patients in the current investigation, a total of 63 (83%) females and 13 (17%) males are present. The male: female ratio was 1:5. This shows a female predominance as shown and studied by Poudel et al, Ranabhat S. et al and Sood et al. ^[12,14,18]. The mean age of patients is 44.57 years with a minimum age being 21 years whereas the maximum age is 90 years with a mean age of 69 years. Maximum number of cases are encountered between 31-40 years range that is 22 cases (29%), followed by 41-50 age group which has 16 cases (21.33%). The study conducted by Poudel et al. has similar results with maximum cases in the age range of 31-40 years ^[12]. Minimum cases were from the age group of 81-90 years and were 2 in number. In all age groups, there is a female preponderance, except in the last age group.

Our study shows maximum number of cases with euthyroid state of the gland with 37 cases

(49%), followed by 20 (26%) cases being hyperthyroid cases and 19 (25%) cases being hypothyroid. This is similar to the findings encountered by Junu et al., Bashir et al., Ranabhat S et al., Suman P et al. which shows a predominance of euthyroid cases. Junu et al. also shows predominance of euthyroid cases followed by hyperthyroid and then hypothyroid cases, which is similar to our study ^[14-17]

Poudel et al. in his study shows a predominance of nodular swelling over the diffuse swelling. Our study showed a higher percentage of diffuse swelling with 34 cases (45%) while the nodular swelling was 32 cases (42%) and 10 (13%) cases of cystic enlargement. Clinical evidence suggests that these cases are benign rather than malignant since the swelling is more cystic or widespread than a nodule, as is typical of carcinomas. ^[12]. Of the 42 cases having nodular swellings or cystic swelling, 33 (79%) is solitary whereas the remaining 9 cases (21%) are multiple. The number of these swellings helps in assessing the benign status of the patient clinically.

After staining the smears, the FNAC (fine needle aspiration cytology) performed on these individuals is examined, and the slides with the best material and cellularity were utilised to reach the final diagnosis. Cellularity in each case is assessed and classified accordingly. The adequate cellularity to make a slide fit for opinion is six groups of follicular cells with at least 10 cells, 10 groups each with 20 or more cells, 6 group of cells on at least 2 aspirates. The adequacy of the cells on slides is also important to differentiate benign lesions from malignant lesions, where malignant lesions will have a higher number of cells as compared to the benign lesions. In our current study, of the 76 smears studied none are inadequate for opinion. Maximum number of smears 63 (83%) are adequate for reporting and diagnosis is made. 4% of the smears shows hypercellularity, but these smears are deemed benign based on the nuclear characteristics. There are only 10 pauci-cellular smears (13%) overall. In our current study of 76 smears, 33(39.1%) cases of goiter are noted, out of which 20 (26.3%) were solitary or single nodular goiter, followed by 12(15.8%) cases diagnosed as diffuse goiter and the remaining 9 (11.8%) cases of multinodular goiter. The next common diagnosis encountered in our study was of lymphocytic thyroiditis with 10 cases (13.2%) and 10 cases (13.2%) of benign cystic lesion. Followed by Hashimoto's thyroiditis with 8(10.5%) cases and 4 (5.3%) cases

of Grave's diseases. Least number of cases are of adenomatoid nodule with just 3 cases (3.9%). Poudel et al., Karki S et al., Dimple Mehrotra et al., Junu et al., Ranabhat S. et al. carried out studies in which the maximum number of diagnosis are colloid goiter which is similar to the one we found in our study. The high number of goiter cases in the study is attributed to the dietary consumption of iodine and the level of iodine in the body. The endemicity of goiter is seen in the iodine deficient belt of Konkan region where the study was undertaken.

In the studies conducted by Ranabhat S et al. and Karki S et al., goiter is the most common diagnosis followed by lymphocytic thyroiditis and Hashimoto's thyroiditis which is similar to our study

In the current study, female predominance is noted in all the benign thyroid lesions. Out of the 63 females in our study, 17 cases were found to be single nodular goiter followed by 9 cases of lymphocytic thyroiditis. While in males, out of the 13 cases, 4 cases were reported as diffuse goiter followed by single nodular goiter.

Our study focuses on the correlations of the confirmed cases of benign thyroid lesions with the thyroid hormone status with the help of thyroid function test. As various benign lesions have different thyroid states we will discuss them individually.

The maximum cases in our study belonged to single nodular goiter with 20 cases. Of these 20 cases 14 cases are euthyroid, followed by 3 cases of hypothyroid and 1 case having hyperthyroid state. This is attributed to the iodine deficiencies in the diet, which is required to for the production, formation and proper functioning of the thyroid hormones, which in turn is reflected in the thyroid function test.

The next major diagnosis was diffuse goiter which has 12 cases of which 7 are euthyroid, 4 cases are hypothyroid and 1 case was hyperthyroid.

In our study we encountered 9 multinodular goitre. 7 cases has hyperthyroid state, while the other 2 are euthyroid. 10 cases of lymphocytic thyroiditis of which 5 cases are euthyroid, 4 are hypothyroid and 1 case is in hyperthyroid state. Lymphocytic thyroiditis is microscopically diagnosed by lymphocytic infiltration in the follicles along with plasma cells, the colloid is sparse and elevated levels of thyroid microsomal antibodies and anti-thyroglobulin antibodies is seen along with this there is an increase in the level of TSH (thyroid stimulating hormone) which is confirmed in our study by the level of TSH (thyroid stimulating hormone) rise seen in the thyroid function test.

Hashimoto's thyroiditis shows follicular cells infiltrated by lymphocytes along with hurtle cell change. In our study we confirmed the same with a total of 8 cases of which majority are hypothyroid with 5 cases, 1 was euthyroid and 2 were hyperthyroid in state.

Our study noted a maximum of 33 cases out of the 76 cases, falling in the diagnosis of goiter may it be nodular or diffuse, this is similar to the previous studies conducted by Poudel et al., Junu et al., Suman P. et al., Ranabhat S. et al. and Karki S. et al. where maximum cases of goiter were noted [12,13,14,16,17].

Benign cystic lesion of the thyroid comprises 10 cases of the 76 cases, they are microscopically diagnosed by scanty to absent colloid in the follicles with benign follicular cells and cyst macrophages in the background. Out of these 10 cases 8 cases are euthyroid, 1 case is hypothyroid and 1 is hyperthyroid ^[16,17].

4 cases of Grave's disease were noted in our study which is microscopically similar to any goitrous lesion with scanty watery colloid and fire flares appearance of the follicular cells but have higher quantities of thyroid hormones making the thyroid state mainly hyperthyroid which is similar to the 4 cases of hyperthyroid we found in our study ^[17,18].

Lastly 3 cases of adenomatoid nodule were found with scanty to sparse colloid, it showed a microfollicular arrangement of the follicular cells and had a hypercellular smear. Due to its hypercellular nature it may present with a rise in the thyroid hormones giving it a hyperthyroid state which is similar to the one we encountered in the present study ^[19].

Out of the 34 diffuse swelling cases we encountered here 12 are diagnosed as diffuse goiter on smears and 10 with lymphocytic thyroiditis, 8 are diagnosed as Hashimoto's thyroiditis whereas 4 were

diagnosed as Grave's disease. Out of the 32 nodular lesions 20 are diagnosed as single nodular goiter while out of the rest 9 smears are diagnosed multinodular goiter and 3 as adenomatoid nodule. All the 10 cystic lesions are diagnosed as benign cyst lesions of the

thyroid [16-19].

Although a successful one yet certain limitations relating the study did we encounter regarding the decrease in the sample size due to the COVID-19 crisis. yet we managed the same with the reopening of the boundaries and lockdown

CONCLUSION

A study was undertaken to correlate the FNAC (fine needle aspiration cytology) findings with the thyroid function test in benign thyroid lesions in Pathology department of Dr. D. Y. Patil medical college. A total of 76 cases were studied Cases which were clinically diagnosed to be benign lesions were subjected to FNAC (fine needle aspiration cytology) and thyroid function test. The findings of FNAC (fine needle aspiration cytology) diagnosis and thyroid function tests were correlated in our study most of the cases were females belonging to the 31-40 years age group. Microscopically most lesions showed adequate cellularity, with thick colloid and follicular cells. The thyroid state of majority of the cases in our study was euthyroid. Hence, we found no significant correlation between the thyroid hormone levels and FNAC (fine needle aspiration cytology) findings. Considering this scenario, both thyroid hormone levels and FNAC (fine needle aspiration cytology) need to be undertaken separately and individually for proper evaluation. This in turn can help us in deciding the course of medical or surgical therapy. This is in accordance with the previous studies that have been conducted on the similar focus.

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