MALIGNANCY RATES OF ATYPIA OF UNDETERMINED SIGNIFICANCE/FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE (AUS/FLUS) CASES: A TERTIARY CENTER STUDY

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Abstract

Aim. To evaluate the malignancy rates of Atypia of undetermined significance /follicular lesion of undetermined significance (AUS/FLUS) cases in the light of clinical and sonographic features.

Material and Methods. The percentage of AUS/FLUS cases, second fine needle aspiration cytology (FNAC) results, cyto-histopathological correlations and risk of malignancy were analyzed.

Results. 113 out of 1461 thyroid FNAC samples (7.7%) were diagnosed as AUS/FLUS and included in the study. Seventy three out of 113 cases (64.6 %) underwent repeat biopsies or surgery. From 45 cases repeat biopsies were taken and 28 had thyroidectomy or lobectomy. There was a significant relation between nodule size and underwent surgery or repeat FNAC ($p=0.036$). Malignancy rate was 24.6% for cases which had any managements. The malignancy rates were higher in AUS/FLUS cases with cytological atypia (28.8%). After surgery the most common malignancy was papillary thyroid carcinoma, followed by follicular carcinoma.

Conclusion. The risk of malignancy of AUS/FLUS cases is quite high because of the heterogeneity of the group. The sub-classification of this category according to cytological or/and architecture atypia may be more useful in predicting malignancy risk. Further larger studies with ancillary techniques including molecular analysis may be more useful in determining the malignancy risk and appropriate management of this heterogeneous category.

Keywords: thyroid nodules, malignancy, AUS/FLUS, Bethesda system.

INTRODUCTION

Fine needle aspiration cytology (FNAC) is an important process for diagnosing thyroid nodules and managing their treatments (1). FNAC results are categorized in six groups according to the Bethesda system for reporting thyroid cytopathology (TBSRTC)

which resulted from a conference in 2007 and the first edition was published in 2010 (2,3). However, since 2010, there have been a number of important advances in the management of patients with nodular thyroid disease and in the understanding of the biology of thyroid cancer. So a new revised edition was published in 2018 (TBSRTC II) (4,5). Clinical management of the indeterminate “gray zone” categories, especially category III or atypia of undetermined significance / follicular lesion of undetermined significance (AUS/FLUS) is still difficult (1,3-5).

There were malignancy rates and proper managements for each category in TBSRTC and these rates were updated in TBSRTC II (2,5). Although the expected risk of malignancy (ROM) in this category is 5-15% in TBSRTC I and updated to 10-30% in TBSRTC II, variable risks of malignancy ranging from 25% to 50% were reported in different studies (1,2,4-7). For these reasons the biggest challenge for AUS /FLUS category is waiting for repeat biopsies, molecular testing or doing a diagnostic surgery for definitive diagnosis (3,5,8).

The aim of this study to evaluate the rate of malignancy in AUS\FLUS category with clinical and sonographic features and to discuss our results with recently published TBSRTC II.

MATERIALS AND METHODS

The study was found appropriate ethically according to the ethical committee of the Erzurum Region Training and Research Hospital with its decision number 2017/07-42 and date 7th June 2017.

For 3 years (between January 2014-January 2017) 1461 thyroid FNACs examined by 4 pathologists were reviewed, retrospectively. The study included 113 out of 1461 cases (7.7%) diagnosed as AUS/FLUS.

All FNAC specimens of cases were prepared with liquid based cytology technique. BD SurePath

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pap test kit (BD Diagnostics-tripath, Burlington, NC, USA®) was used in liquid based cytology examination techniques. Samples in ethanol-based fixative were centrifuged 2 times (1st 10 min, 2nd 5 min). Then cellular sample was mixed with a vortex homogeneously; 40 µl sample pulled out with micropipette and sample spread as a thin layer in a circular area of microscope slide. The criteria for diagnosing AUS/FLUS based on TBSRTC II. While nuclear enlargement, pale chromatin, inclusion-like appearance and irregular nuclear contours in different degrees were accepted as cytological atypia (Fig. 1), follicular cells in microfollicular pattern or crowded three-dimensional groups and with scant colloid were considered as architectural atypia (Fig. 2). The ROMs were calculated for total and for subgroups such as cytological and architectural atypia.

The demographic data, such as age, gender, were obtained from pathology reports. The ultrasonographic features of nodules (the size, the number of nodules (solitary or multiple) and the

presence of microcalcifications) were also observed. In follow-up of cases repeat FNAC and surgery results were evaluated. The decision of surgery or repeat FNAC is based on imaging and clinical predictions (such as big nodule, solitary nodule, microcalcifications).

Data analysis was performed using SPSS 20.0 program. In the evaluation of results descriptive statistics was shown in the form of mean $\pm$ standard deviation, nominal variables were shown as number of cases and the percentage (%). Pearson Chi-Square, Fisher Exact tests were used for verification, Spearman and Pearson's correlation tests were used for numerical data.

RESULTS

The study included 113 out of 1461 thyroid FNAC (7.7%) cases which were diagnosed as AUS/FLUS. The patients were between 20 and 74 years, and the mean age was 47.35 ± 12.45 ; 86.7% of the patients (n = 98) were female.

Figure 1. a) Inclusion-like appearance in a hypocellular aspirate diagnosed as AUS/FLUS (LBS, PAPx400), b) Tissue section of the same patient, diagnosed as follicular variant papillary thyroid carcinoma (H&Ex400), c) Membrane irregularities and molding in a part of follicular cells. (LBP, PAPx200), d) Tissue section of the same patient, diagnosed as classical papillary thyroid carcinoma (H&Ex200).

The radiological features of nodules that FNAC applied were evaluated. Diameters of nodules were between 0.6 - 3 cm (mean 1.6 ± 0.27 cm). Eighty seven out of 113 patients had multiple nodules. Four patients had microcalcifications.

Table 1. Management of the cases after diagnosis of AUS/FLUS

Management	Number of cases	Number of malignancies	Malignancy rate
Repeat FNAC	45	7	15.5%
Surgery	28	11	39.3%
Total	73	18	24.6%

Table 2. Histopathological diagnosis of AUS/FLUS

Histopathological diagnosis	Number of cases	Percentage
Follicular variant of papillary thyroid carcinoma	7	63.6%
Classical papillary thyroid carcinoma	3	27.3%
Follicular carcinoma	1	9.1%

Seventy three out of 113 cases (64.6%) underwent repeat biopsies or surgery. From 45 cases repeat biopsies were taken and 28 had diagnostic thyroidectomy or lobectomy.

Repeat FNAC which was done within 1- 3 months and 75.6% cases were benign (34/45), 8.8% malignant (4/45), and 7 cases were again categorized as AUS/FLUS. Three cases turned out to be malignant after third FNAC and thus total malignancy rate after repeat FNAC was 15.5% (7/45) (Table 1).

Out of the 28 cases which underwent surgery 17 cases (60.7%) were benign and 11 cases (39.3%) were malignant (Table 1). After surgery the most common malignancy was papillary thyroid carcinoma (PTC) (10/11), followed by follicular carcinoma (FC) (Table 2).

According to these findings ROM of cases was 24.6%. If the cases were divided subgroups such as cytological atypia and architectural atypia the ROMs were 28.8% and 7.1%, respectively (Table 3).

Figure 2. a) An aspirate had a few oncocyctic cells and lymphocytes in background diagnosed as AUS/FLUS (LBS, PAPx100), b) Tissue section of same patients, diagnosed as follicular adenoma (H&Ex100), c) A few microfollicles seen in a hypocellular aspirate with benign follicular cells. (LBS, PAPx200), d) Tissue section of same patient, diagnosed as adenomatous nodule (H&Ex100).

Table 3. AUS/FLUS sub-classification and malignancy rate

Sub-classification	Number of cases	Number of malignancies	Malignancy rate
Cytological atypia	59	17	28.8%
Architectural atypia	14	1	7.1%

As the diameters of nodules increased, the percentage of diagnosis was significantly increased in repeat FNAC ($p=0.034$). Similarly, the diameters of the nodules were significantly higher in cases which underwent surgery or repeat FNAC ($p=0.036$). Most of the nodules that diagnosed as PTC (after surgery or repeat FNAC) were ≥ 1 cm (92.8%). Only in one patient nodule size was < 1 cm (0.9 cm).

Most of the patients that have solitary nodules (65.3%) underwent surgery or repeat FNAC. Out of 4 patients 3 cases had microcalcification diagnosed as malignant (2 of them by repeat FNAC, 1 of them by surgery). Lobectomy procedure was preferred only for cases with architectural atypia which were called FLUS according to previous TBSRTC (2).

DISCUSSION

The FNAC specimens diagnosed as AUS/FLUS category are insufficient to be classified as suspicious for a follicular neoplasm, suspicious for malignancy, or malignant (1,5,7,9). A provisional goal of limiting AUS/FLUS interpretations to approximately 7% of all thyroid FNAC specimens was proposed in the first TBSRTC. But in practice, many laboratories struggle to achieve this figure. So in TBSRTC II an upper limit of 10% has been shown as more realistic goal for this category (2,4,5). In our study the 7.7% of 1461 FNAC specimens were diagnosed as AUS/FLUS. However, different studies have reported variable rates of AUS/FLUS ranging from 3% to 27.2% (3,10,11).

The ROM of AUS/FLUS category was estimated as 5% to 15% in first TBSRTC and the usual recommended management is repeat FNAC (1,2,11,12). However, studies have observed higher malignancy rate in different clinical settings and recommend reconsideration of malignancy rate and management guidelines in this category (12-14). In a study which determined the rate of malignancy in 81 thyroid nodules reported as AUS/FLUS, ROM was found 33.3% (15). On the other hand, a study from India ROM of AUS/FLUS cases was 66.7% (16). The ROM of this category was revised to 10-30% in TBSRTC II and new recommended managements are added to repeat FNAC, such as molecular testing and lobectomy (4,5). The malignancy rate of the present

study was 24.6% and it is appropriate for the estimated risk in this category in TBSRTC II.

The authors suggest that sub-classification of the AUS/FLUS category into subgroups may be useful in reducing the heterogeneity of AUS/FLUS category and more appropriate prediction of malignancy. AUS/FLUS aspirates with cytological (nuclear) atypia have an approximately twofold higher ROM compared with AUS/FLUS cases with architectural atypia (4,17-19). In our study if we divided cases according to cytological atypia and architectural atypia the ROMs were 28.8% and 7.1%, respectively (Table 3).

The introduction of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) terminology in 2016 will likely alter these figures further, with early data suggesting that the ROM for AUS/FLUS may be reduced by as much as 45% (20,21). In our study there were no NIFTP cases diagnosed after the NIFTP entity was introduced in 2016 or in former cases which evaluated respectively. If AUS/FLUS cases underwent surgery the most common malignancy was PTC followed by FC in histopathological examination (15,16). Similarly, in our study 90.9% (10/11) of tumors were PTC.

The 2015 American Thyroid Association guidelines recommend conservative managements such as repeat FNAC or molecular testing (22). A repeat FNAC usually results in a more definitive cytologic interpretation; only about 10–30% of initially AUS/FLUS nodules are reported again as AUS/FLUS in second FNAC specimens (23). In our study 15.5% of AUS/FLUS cases were reported again as AUS/FLUS in repeat FNAC. Mutational testing for AUS/FLUS exhibits low sensitivity for the BRAF V600E mutation alone (24). Expanded mutation panels exhibit higher sensitivity, but specificity is decreased by the increased prevalence of RAS mutations. In a blind, multi-institutional study of the Afirma gene expression classifier (GEC), a negative result with AUS/FLUS aspirates reduced the ROM from 24% to 5%. A negative GEC result is more likely in AUS/FLUS with isolated architectural atypia than in AUS/FLUS with cytologic atypia (25).

BRAF V600E point mutation accounts for more than 90% of BRAF mutations and it is most common genetic alteration in PTC (26,27). Aggressive behavior in PTC, such as lymph node metastasis, distant

metastasis, extrathyroidal extension, higher tumor staging, is associated with BRAF V600E mutation (26,28). Although molecular testing has been accepted as the gold standard for the detection of BRAF V600E mutation, molecular workup needs experience, time, financial and other resources. And this is not always possible. So, for detection of BRAF V600E mutation immunohistochemistry (IHC) has had increased practical utility (26,29). In studies there was a high degree of concordance between the IHC and molecular testing in detection of BRAF V600E mutation (26).

However, there are studies that specified BRAF V600E mutation analysis has limited utility in FNA specimen in improving the preoperative malignancy diagnosis (30).

The limitations of the present study were that a small number of cases were included into the study (only 64.6% of 113 AUS/FLUS cases underwent surgery or have repeat FNAC in their follow-up). So the malignancy rate was determined only for these cases. All resection materials of PTC cases (n:10) were evaluated respectively for NIFTP entity. There were no cases that met the criteria of NIFTP in our study group.

In conclusion, in the present study ROM of AUS/FLUS cases is 24.6%. It is higher than previous TBSRTC (5–15%) but it is similar for the second one (10-30%). In addition, sub-classification of this category according to cytological and architecture atypia may be more useful in predicting malignancy risk. Further larger studies with ancillary techniques including molecular analysis may be more useful in determining the malignancy risk and appropriate management of this heterogeneous category.

Conflict of interest

The authors declare that they have no conflict of interest.

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