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RESEARCH

Significance of AgNORs and Ki-67 Proliferative Markers in Differential Diagnosis of Thyroid Lesions

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Abstract We aimed to assess the utility of quantitative analysis of AgNORs and Ki67 labeling index (LI) in the differential diagnosis of different thyroid lesions. This study included: 25 papillary carcinomas, 7 follicular carcinomas, 21 follicular adenomas and 27 nodular goiters. Using a semiautomatic image analysis system, Ag NORs parameters were measured and calculated including: total area of AgNORs, mean Ag NOR number in nuclei, nuclear area, mean area of AgNOR dots per each nucleus, number of central and marginal AgNOR dots, and the relative ratio of total area of AgNOR dots/total area of nucleus. Ki67 immunostaining was performed and the LI was determined. There was a significant difference between groups of thyroid lesions regarding total area of AgNORs, Ag NOR number and number of marginal Ag NOR dots. According to receiver operating characteristic curve, Ag NORs number =2.91 and marginal Ag NORs=2.67 were useful cut off values above which follicular carcinoma can be diagnosed with 100 % sensitivity, 79 % specificity, 76 % PPV, 100 % NPV and 85 % diagnostic accuracy for both parameters. Mean Ki67 LI in our study was 14.12±2.29, 61.42±3.77, 34.90±3.49 and 18.60±1.96 for papillary carcinoma, follicular carcinoma, follicular adenoma and nodular goiter respectively. Ki67 LI showed statistically significant difference between follicular carcinoma and follicular adenoma (p=0.026) and between papillary carcinoma and follicular adenoma (p=0.007). Quantification of Ag NORs and Ki67 LI could be used as helpful ancillary methods in the differentiation between different thyroid lesions.

Keywords Ag NORs · Ki 67 labeling index · Follicular carcinoma · Follicular adenoma · Papillary carcinoma

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Introduction

Although most thyroid nodules can be diagnosed easily based on histological and cytological features, some cases still impose diagnostic difficulty. The most common problem is the nodule with predominant follicular pattern [1]. The presence of questionable nuclear features of papillary thyroid carcinoma (PTC) or questionable capsular invasion in follicular lesion can raise the possibility of malignancy [2]. Therefore, many immunohistochemical markers have been suggested to aid in the differentiation between benign and malignant lesions such as Galectin-3, HBME-1 and beta catenin [3–5].

Mitotic activity is not an indicator of malignancy in thyroid lesions, however, it was reported that increased mitotic figures is a worrisome feature in the follicular neoplasms. The term atypical adenoma was proposed to define the noninvasive, nonpapillary encapsulated follicular lesions with at least 5 mitoses/HPF and/or necrosis [6]. Furthermore, Volante et al., (2007) found that the presence of ≥3 mitoses/HPF in addition to solid pattern, absence of nuclear features of PTC and tumor necrosis support the diagnosis of poorly differentiated thyroid carcinoma [7].

Various proliferation markers such as Ki67 [8], proliferating cell nuclear antigen (PCNA) [9] and argyrophilic nucleolar organizer regions (AgNORs) [10] have been examined in thyroid tumors.

The nucleolar organizer regions (NORs) are DNA segments, encoding for ribosomal RNA [11]. Associated with NORs, there are some nucleolar proteins, which are stained black with silver methods (Ag NOR proteins or Ag NORs) [12, 13]. It has been reported that Ag NORs are related to the rate of cell proliferative activity [14] and therefore could be used to distinguish between benign and malignant lesions [9, 15–17].

Ki-67 is a well known nuclear antigen expressed in all the phases of the cellular cycle, except in G0 [18]. In thyroid specimens, many studies have reported that Ki 67 LI or MIB-1 may be a reliable prognostic indicator especially in PTC [8, 19, 20]. However, there is a considerable controversy regarding the utility of Ki 67 labiling indix (Ki67 LI) in differentiation between benign and malignant thyroid lesions. Some reports showed that Ki67 LI has no additive diagnostic value [21–23]. Some authors reported that Ki67 is useful in the differentiation between follicular thyroid carcinoma (FTC) and follicular adenoma (FTA) [24, 25].

Aim of the Work We aimed to assess the diagnostic utility of quantitative analysis of AgNORs and Ki67 immunostaining in the differential diagnosis of different thyroid lesions

Material and Methods

This study included a total of 80 cases diagnosed according to WHO guidelines [26]: 25 PTC (19 classic variant, 6 follicular variant), 7 FTC (5 minimally and 2 widely invasive), 21 FTA (17 macrofollicular, 4 microfollicular), 27 nodular goiters (NG). All the cases were retrieved from archives of Pathology Department, Faculty of Medicine, Menoufyia University during the period between January 2006 to January 2009. The specimens were routinely fixed and processed in paraffin wax. The cases with oncocytic (oxyphilic) metaplasia (Hürthle cells) which showed obvious nuclear anomalies and poorly differentiated carcinoma cases were excluded from the current study, to avoid errors of statistical analysis.

Ag NORs Quantitative Analysis

Ag NORs staining was performed using the one-step silver colloid method [12] and the recommendation of Aubele et al., (1994) [27]. Variable quantitative parameters describing Ag NORs, were assessed by a system formed of Tri-nocular microscope (Olympus Corporation, Japan), a digital video camera (Panasonic, Japan) and a personal computer (Dell, inspiron 6400).

Morphometric measurements were performed with the help of Digimizer program version 2. Measurements were calibrated using Nikon micrometer slide before performing any measurements. An image to the slide stage micrometer (at magnification 1000 x) was captured and saved on the computer in a JPG file format. The image was used for system calibration by opening in Digimizer program window. A straight line measuring 10 μm was copied and pasted to the image to be measured for calibration. From the selected areas, an average of 5–10 microscopic fields, at magnification x1000 were captured from each case (Fig. 1). At least 80 nuclei were analyzed in each case, whole nuclei representing the actual lesion. Overlapped and fragmented nuclei were discarded. A total of seven parameters were estimated. The measured parameters included: total area of AgNOR dots (Tag) expressed in μm^2, mean Ag NOR number of nuclei, nuclear area (NA) expressed in μm^2, mean area of AgNOR dots per each nucleus (Mag) expressed in μm^2, number of central AgNOR dots and marginal AgNOR dots. While the calculated parameters included: the relative ratio of total area of AgNOR dots/total area of nucleus (Tag/NA) [9, 28].

Ki-67 Immunohistochemistry and Determination of the Labeling Index (LI)

Four μm-thick paraffin sections were mounted on positively charged glass slides, then deparaffinized and rehydrated. Sections were incubated in hydrogen peroxide (3 % H2O2 in absolute methyl alcohol) for 10–15 min in humidity chamber. For antigen retrieval, tissue sections were boiled in 10 mM citrate buffer, pH 6.0, for 10 min. Sections were incubated overnight with monoclonal mouse, clone MIB-1 at the dilution 1:50 (DakoCytomation, Copenhagen, Denmark). The detection kit was Dako LSAB2, (HRP) horseradish peroxides kit (DakoCytomation, Copenhagen, Denmark). Biotinylated

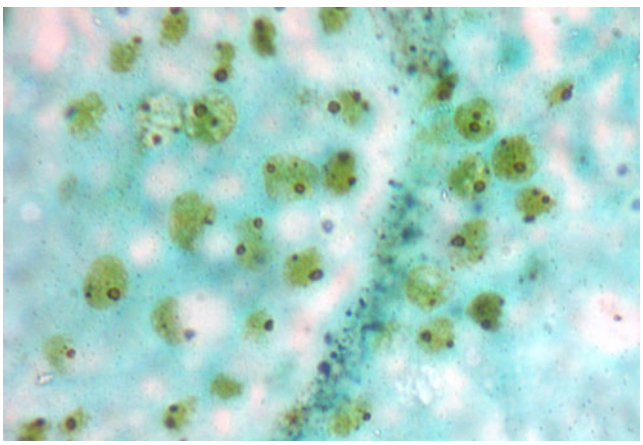

Fig. 1 Ag NOR dots in nuclei of follicular thyroid carcinoma (silver staining ×1000)

Goat Anti-polyvalent secondary antibody was applied for 10 min at room temperature. Sections were incubated in streptavidin -HRP for 10 min at room temperature. Reaction was developed using diaminobenzidine (DAB) as chromogenic substrates. After counter-staining using Mayer's hematoxylin, tissue sections were dehydrated and the cover slips were mounted by using Canada balsam mounting media.

Positive control slides were breast carcinoma known to express MIB-1. Negative control was performed by omitting the primary antibody step. Evaluation of specimens was performed independently by two authors (HA and AA), without knowledge of clinical or surgical information on the patients. Ki-67 labeling index was analyzed in 5 different high-power microscopic fields (x400) representing hot spots in the primary lesions. Generally, Ki-67 LI was higher in the peripheral portion than in the central portion of the lesion. Values were expressed as percentages of the highest Ki-67 expression measured in hot spots [20]. Inter-observer strong agreement was observed (kappa value 0.85). The discrepant cases were revised on multi-head microscope and consensus was reached on each case.

A statistical analysis was performed with the use of Package for Social Science program (SPSS) version 16 for Windows (SPSS Inc., Chicago, Illinois, USA). For comparison between groups Kruskal-Wallis test was used. For multiple comparisons, post hoc Bonferroni test was used to detect the exact significance between groups. The P value was assigned significant when ≤0.05. ROC curve was performed to define a cut off value that helps in differentiation between groups.

Results

The current study included a total of 80 cases: 25 (31 %) PTC, 6 of them were follicular variant, 7 (9 %) FTC, 21 (26 %) FTA, 27 (34 %) NG. The age of studied patients ranged between 12 and 68 years with a mean of 44.17± 16.61. Most of the patients were female (81 %). The size of nodules ranged between 0.5 and 8 cm with a mean of 3.5± 2.0 in maximal diameter.

Ag NOR Quantification

We measured Ag NORs in the nuclei of normal thyrocytes from the normal thyroid tissue adjacent to hyperplastic nodules in the cases of nodular goiter. The mean values of measured parameters in normal thyroid tissues were as following: Tag; 1.24±0.42, Ag NORs number; 1.52±0.32, NA; 11.674±4.53, Tag/NA; 0.106±0.042, Mag; 0.816± 0.217, central Ag NORs number; 0.31±0.13, marginal Ag NORs number; 1.23±0.53.

After meticulous selection for proper sections, Ag NORs silver staining was performed for 56 cases: 13 PTC, 6 FTC, 14 FTA and 23 NG. The mean values and standard deviation of all Ag NOR parameters are shown in Table 1. There was a significant difference between groups regarding total area of Ag NORs dots (Tag), Ag NOR number and number of marginal Ag NOR dots (Table 2).

The observed ranges of Tag in different studied groups were 1.3–3.1 μm^2 for PTC, 1.7–3.5 for FTC, 1.2–3.6 for FTA, and 0.5–2.7 for NG. Tag could differentiate significantly all groups from nodular goiter but failed in the remaining comparisons due to marked overlap.

As regards Ag NOR number the observed ranges were 1.1–4.0 for PTC, 2.9–4.8 for FTC, 1.2–3.1 for FTA and 1.1–2.6 for NG cases. Ag NORs number of dots within nuclei significantly differentiated between all groups except between PTC and FTA (Table 3).

The number of marginal Ag NOR dots showed range of 0.8–3.6 for PTC, 2.7–4.6 for FTC, 1.0–3.0 for FA and 0.7–2.3 for NG cases. As a result also marginal Ag NORs could significantly differentiate between all groups except between PTC and FTA (Table 3).

Nodular goiter group could be significantly discriminated from other groups using the three Ag NORs parameters

Table 1 Mean values of Ag NORs parameters in different studied groups

Ag NORs parametrs	Papillary carcinoma (No.13)	Follicular carcinoma (No. 6)	Follicular adenoma (No. 14)	Nodular goiter (No. 23)
Tag	2.282±0.598	2.527±0.643	2.263±0.868	1.56±0.697
Ag NORs number	2.726±0.889	4.046±0.697	2.525±0.532	1.8178±0.449
NA	17.841±5.393	24.859±12.863	20.038±11.739	14.797±5.089
Tag/Tn	0.144±0.0273	0.132±0.050	0.130±0.048	0.109±0.032
Mag	0.952±0.278	0.649±0.097	1.0277±0.424	0.889 ±0.306
Cent. Ag NORs	0.370±0.164	0.346±0.143	0.340±0.231	0.317±0.125
Marg.AG NORs	2.314±0.892	3.684±0.702	2.189±0.514	1.462±0.509

Tag total area of AgNORs, *NA* nuclear area, *Tag/NA* total area of AgNORs/total area of nucleus, *Mag* mean area of AgNORs, *Cent. Ag NORs* number of central AgNORs dots and *Marg. AG NORs* number of marginal AgNORs dots

Table 2 Comparison between AgNORs mean values in different groups

	Tag	Ag NORs number	NA	Tag/NA	Mag	Cent. Ag NORs	Marg Ag NORs
Kruskal Wallis test	12.731	25.631	4.554	9.993	6.524	1.679	25.762
P value	0.005	0.000	0.208	0.052	0.089	0.642	0.000

Tag total area of AgNORs, *NA* nuclear area, *Tag/NA* total area of AgNORs/total area of nucleus, *Mag* mean area of AgNORs, *Cent. Ag NORs* number of central AgNORs dots and *Marg. AG NORs* number of marginal AgNORs dots

(Table 3) on the other hand there was no one Ag NOR parameter that could discriminate PTC from FTA.

To differentiate between FTC and FTA, this was impossible by Tag but was significantly achieved by Ag NORs number and marginal Ag NORs number with a little overlap. According to receiver operating characteristic (ROC) curve Ag NORs number equal 2.91 and marginal Ag NORs equal 2.67 were useful cut off values above which FTC can

Table 3 Multiple comparison using post hoc Bonferroni test

	Histologic types		P value
Tag	Papillary carcinoma	Follicluar ca.	1.000
		Adenoma	1.000
		Nodular goiter	0.034
	Follicluar carcinoma	Pap. Ca	1.000
		Adenoma	1.000
		Nodular goiter	0.030
	Adenoma	Papillary carcinoma	1.000
		Follicluar carcinoma	1.000
		Nodular goiter	0.034
	Nodular goiter	Papillary carcinoma	0.034
		Follicluar ca.	0.030
		Adenoma	0.034
Ag NORs number	Papillary carcinoma	Follicluar carcinoma.	0.000
		Adenoma	1.000
		Nodular goiter	0.001
	Follicluar carcinoma	Papillary carcinoma	0.000
		Adenoma	0.000
		Nodular goiter	0.000
	Adenoma	Papillary carcinoma	1.000
		Follicluar carcinoma.	0.000
		Nodular goiter	0.008
	Nodular goiter	Papillary carcinoma	0.001
		Follicluar ca.	0.000
		Adenoma	0.008
Number of Marginal Ag NORs dots	Papillary carcinoma	Follicluar ca.	0.000
		Adenoma	1.000
		Nodular goiter	0.002
	Follicluar ca.	Papillary carcinoma	0.000
		Adenoma	0.000
		Nodular goiter	0.000
	Adenoma	Papillary carcinoma	1.000
		Follicluar ca.	0.000
		Nodular goiter	0.008
	Nodular goiter	Papillary carcinoma	0.002
		Follicluar ca.	0.000
		Adenoma	0.008

Tag total area of AgNORs

be diagnosed with 100 % sensitivity,79 % specificity, 67 % PPV, 100 % NPV and 85 % diagnostic accuracy for both parameters. Area under the curve (AUC) was 0.96 for Ag NORs number and 0.98 for marginally located Ag NORs (Fig. 2a, b). Both Ag NORs number and marginal Ag NORs correctly categorized all cases of FTC (100 %) and most of FTA except 3 (21 %) which were above the cut off values.

To differentiate between PTC and FTC, Ag NORs number equal 3.62 was a useful cut off value above it FTC can be diagnosed with 83 % sensitivity, 85 % specificity, 92 % PPV, 71 % NPV and 84 % diagnostic accuracy and AUC was 0.88 (Fig. 2c). The cut off value 3.62 could correctly categorize all but one FTC cases (83 %) and most of PTC cases except 2 (85 %).

Ki67 Labeling Index

Examples for immunohistochemical staining of Ki67 are shown in Fig. 3. The mean value, median and range of Ki67 LI in different groups are shown in Table 4. Ki67 LI in FTC was significantly higher than FTA cases (p=0.0260) (Table 5). Interestingly, PTC showed significantly lower Ki67 LI than those of FTC (p=0.000) and FTA (p=0.007). However, there was no significant difference between PTC and NG as regards Ki67 LI (p=1.000) (Table 5).

Discussion

The current study showed that Ag NORs quantification and Ki67 LI could be used as helpful methods in the differentiation between different thyroid lesions. Review the literature revealed that Ag NORs quantification was employed in the differential diagnosis of benign and malignant thyroid lesions on cytological [10, 29–31] and histological preparations [9, 16, 32].

In the current study Ag NORs number showed a significant statistical difference between FTC and FTA (p< 0.0001). According to receiver operating characteristic (ROC) curve Ag NORs number equal 2.91 was a useful cut off value above which FTC can be diagnosed with best sensitivity and specificity. Ag NORs number correctly categorized all cases of FTC (100 %) and most of FTA except 3 (21 %) which were above the cut off values. Similarly, Mehrotra et al., (2002) showed that mean Ag NORs count could differentiate between benign and malignant lesions with an overlap of 1.83 % at the cut off point of 4.0 [10]. Studying Hurthle cell lesions, AgNORs number/nucleus gradually increased from metaplasia to carcinoma passing through adenoma with significant difference between benign and malignant Hurthle cell lesions [16, 32]. Cornianu et al., (2006) found 1–2 NORs/nucleus in normal thyroid

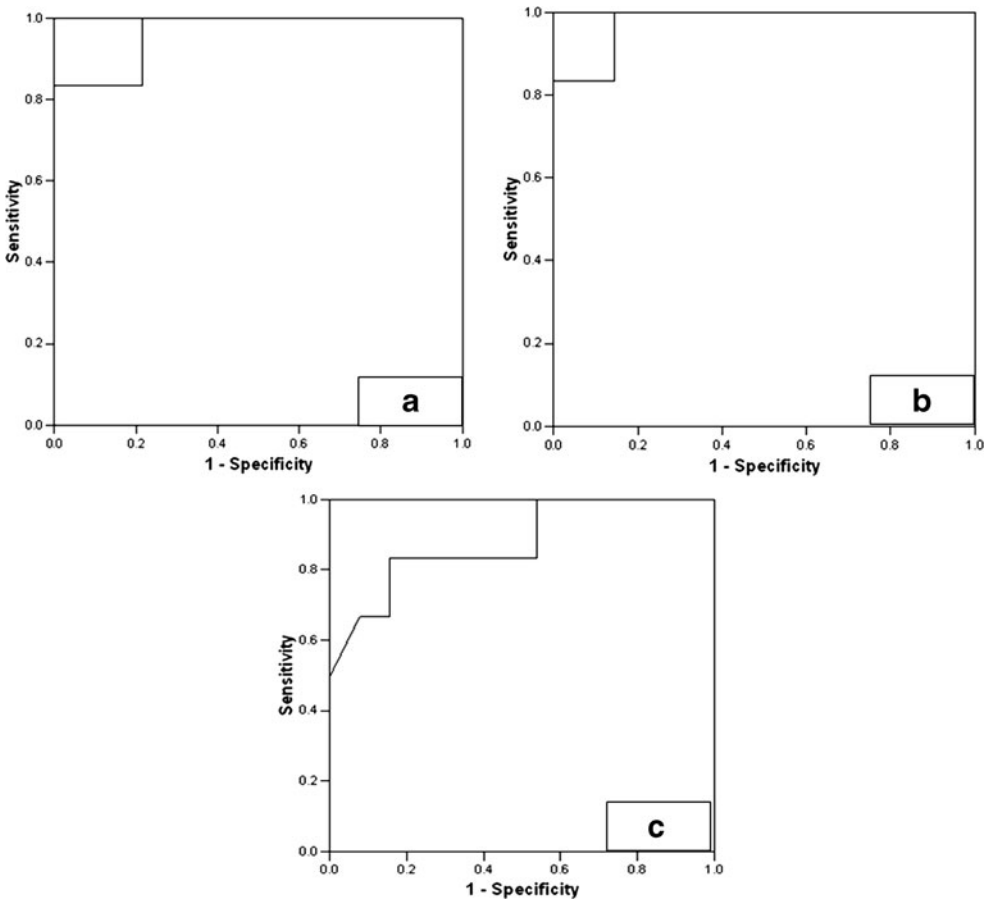

Fig. 2 **a** ROC curve for Ag NORs number in the differentiation between FTC and FTA using 2.91 as cut off value, AUC=0.96. **b** ROC curve for marginal Ag NORs dots in the differentiation between FTC and FTA using 2.67 as cut off value, AUC= 0.98. **c** ROC curve for Ag NORs number in the differentiation between FTC and PTC using 3.62 as cut off value, AUC=0.88

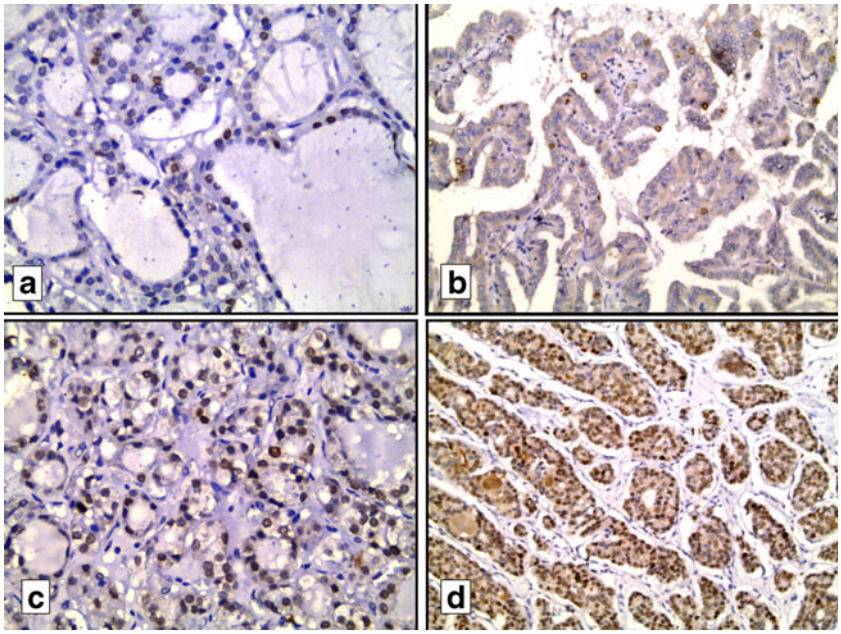

Fig. 3 Immunohistochemical staining showing lower Ki67 LI in NG (**a**) and PTC (**b**) compared to higher Ki67 LI in FTA (**c**) and FTC (**d**) (Immunoperoxidase a, **b** & **c** ×200 and d ×100)

tissue and an increase of NORs number from 2.3 NOR/nucleus in Hurthle cell metaplasia (HCM), to 3.1 NOR/nucleus in Hurthle cell adenoma (HCA) and 5.0 NOR/nucleus in Hurthle cell carcinoma (HCC) [32]. Similarly, Augustynowicz et al., (2004) found that Ag NORs number in HCM=2.5, HCA=3.4, and in HCC=5.1 [16].

In contrast, Ag NORs number was of little diagnostic value in other studies. Although, Solymosi et al., (2006) found that the mean AgNOR count per cell was significantly higher in malignant than in benign lesions, they observed a considerable overlap [30]. Ag NORs count has only helped in the differentiation between FTC and non-neoplastic lesions in other studies [29, 33, 34]. Counting of the dots was considered as a subjective method depending not only on the preservation of tissue samples and on the method of staining, but also on the evaluation [35]. In our opinion, following the strict rules of selecting the viable and non-overlapping nuclei we found that Ag NORs number is an easy and objective method that can successfully differentiate between FTC and FTA.

PTC is usually easily diagnosed based on detection of papillary architecture and the characteristic nuclear features. The follicular variant of PTC may be confused with FTC because it is entirely formed of follicles. To differentiate between PTC and FTC, Ag NORs number equal 3.62 was a useful cut off value above it FTC can be diagnosed with 83 % sensitivity, 85 % specificity, 92 % PPV, 71 % NPV and 84 % and 0.88 AUC. The cut off value 3.62 could correctly categorize all but one FTC cases (83 %) and most of PTC cases except 2 (85 %). From our results, in difficult cases where the nuclear features of PTC are questionable Ag NORs number >3.62 may be an adjuvant method that supports the diagnosis of FTC.

In our study total area of Ag NORs (Tag) was able to correctly identify the nodular goiter cases from all studied groups but did not improve the diagnosis of malignancy. In contrast to our finding, total area of Ag NORs was better than other Ag NOR parameters in this consideration and cutoff values for Tag in the nucleus 3.00 μm^2 and 4.9 $\mu m2$ succeeded to limit FTC from other lesions [9, 29].

The mean area of Ag NORs (Mag), nuclear area (NA) and ratio between NA and Tag (Tag/NA) failed to achieve significant statistical difference between benign and malignant groups in the current study. In the contrary, Solymosi et al., (1996) showed that the differentiation of thyroid benign and malignant lesions are allowed by using Mag with cut off 5 μm^2 and by correlation of NA to Ag NORs area [30]. The discrepancy observed between our findings and others may be due to different image analysis systems used. Most of the studies used automatic image analysis system while in our

Table 4 Ki67 labeling index in the different studied groups

Ki67 LI	Papillary carcinoma No. 25	Follicular carcinoma No. 7	Follicular Adenoma No. 21	Nodular Goiter No. 27	Kruskal Wallis test
Mean±SD	14.12±2.298	61.42±3.779	34.90±3.490	18.60±1.961	K=24.446
Median	10.0000	60.00	40.0	15.0	P=0.000
Range	0–60	60–70	0–60	1–60	

Table 5 Multiple comparison post hoc Bonferroni test

	Histologic types		P value
Ki67 LI	Papillary carcinoma	Follicluar carcinoma	0.000
		Follicular Adenoma	0.007
		Nodular goiter	1.000
	Follicluar carcinoma	Papillary carcinoma	0.000
		Follicular Adenoma	0.026
		Nodular goiter	0.000
	Follicluar Adenoma	Papillary carcinoma	0.007
		Follicluar carcinoma	0.026
		Nodular goiter	0.083
	Nodular goiter	Papillary carcinoma	1.000
		Follicluar carcinoma	0.000
		Follicluar Adenoma	0.083

study we used a semiautomatic system including Digimizer program by which we can properly select the viable non-overlapping Ag NORs dots. This action is difficult to be applied in the automatic systems. Another cause for discrepancy may be because we employed our staining on histological sections and most of the studies were applied on cytological preparations [10, 29, 30].

Ki-67 as a marker of cellular proliferation is associated with biological and prognostic aggressiveness in different tumors [36]. The limited use of Ki67 in differential diagnosis of thyroid nodules is because Ki-67 LI is generally low in thyroid carcinoma, except anaplastic and poorly differentiated carcinoma. Mean Ki67 LI in our study was 14.12± 2.29, 61.42±3.77 and 34.90±3.49 and 18.60±1.96 for PTC, FTC, FTA and NG respectively. Although we did not include anaplastic or poorly differentiated carcinomas in our study, we observed an elevated Ki67 LI compared to that observed in the literature in benign and malignant tumors. For example Mar et al., (2006) showed that the mean Ki67 index was 2.07±1.65 for FTC and 1.78±0.92 for FTA [21]. Similarly, Sofiadis et al., (2009) showed that mean Ki67 index was 2.9 for PTC, 3.5 for FTC and 1.6 for FTA [8]. The elevated proliferative activity of our cases determined by Ki67 LI may be related to epidemiologic or genetic factors specific to Egyptian patients. Our results go with those reported about the unfavorable behavior of thyroid cancer in Egypt [37]. The anaplastic carcinoma accounted for 14 % compared to less than 3 % in other Arab countries and USA. Also differentiated carcinoma accounted for 73 % which is a much less incidence compared to the same countries (90 %–94 %) [37].

Despite the elevated Ki67 LI in our cases, it was successful in the differentiation between FTC and FTA (p=0.026). In accordance with our results, Ki-67 antigen was reported to be a useful marker for differentiation between FTC and FTA [38] and between Hurthle cell adenoma and carcinoma [39]. Similarly, Using flow cytometric analysis Ki67 fraction was significantly higher in malignant compared to benign thyroid tumors [40]. In contrast, other research groups found that the ranges of Ki-67 LI in malignant and benign lesions, such as FTC and FTA showed considerable overlaps [8, 21, 22, 41].

In the present study, there was no significant difference between PTC and nodular goiter as regards Ki67 LI. Similarly, Erickson et al. (2000) established that Ki67 could not distinguish papillary hyperplasia in Graves' disease from PTC [42]. Such a differential diagnosis may be difficult and could be resolved by another immunohistochemical marker such as galectin -3 which was proven useful in that regard [5].

In the present study we put classic and follicular variants of PTC in one group because the follicular variant of PTC shows a behavior analogous to that of classic PTC [43]. The encapsulated follicular variant of PTC has become the most common source of consultation material and controversy in thyroid pathology as it is confused with FTA. In the current study, there was no one Ag NOR parameter succeeded to discriminate PTC from FTA. Although the Ki67 LI was able to discriminate between both lesions (p=0.007), ROC curve failed to achieve a cutoff value that reach an acceptable diagnostic validity (>75 %). Our results confirm the importance of going back to histomorphological changes to resolve this diagnostic problem.

Mean AgNORs counting in fine needle aspiration smears was reported to be more sensitive, simple and cost effective as compared to Ki-67 LI for differentiating between benign and malignant thyroid follicular neoplasms [10]. In the present histological study, Ag NORs number equal 2.91 and percentage of marginally located Ag NORs equal 2.67 were found to be useful cut off values with best diagnostic validity for differentiation between FTC and FTA. In the meantime, Ki67 LI was significantly higher in FTC than FTA (0.026) in addition it was easier in evaluation than Ag NORs quantification. Therefore we suggest the use of both proliferation markers in difficult situations to differentiate between these two categories.

Conclusion

Ag NORs number and Ki67 labeling index are found to be useful in the differentiation between FTC and FTA and between FTC and PTC. Ki67 LI was also able to discriminate between PTC from FTA. Therefore both markers are suggested as adjuvant tools in cases with questionable capsular and/or vascular invasion and in follicular lesions with questionable nuclear features. Further larger study is needed to confirm the suggested cut off values in the current study to be used in the routine pathological practice.

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