Morphometric study in thyroid tumors

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Abstract. Various morphonuclear studies using digital image analysis have been taken into account in order to establish the malignancy of thyroid lesions based on their size and on the chromatographic characteristics of tumor cell nuclei. Nuclear morphometry involves the measurement of nuclear parameters to obtain diagnostically important information in an objective and reproducible manner. The aim of the present study was to evaluate the detailed morphometric analysis of histopathological preparations with lesions of the thyroid gland and to investigate its role in differentiating between benign and malignant thyroid lesions. The present study included 10 benign and 26 malignant thyroid cases with different selected thyroid lesions. Using a microscope connected to a computerized video system, nuclear morphometric parameters including the nuclear area, perimeter, average intensity, red average, width and roundness, were measured and analyzed. The main parameters used in the statistical calculation were significant in distinguishing between benign and malignant thyroid lesions. The association of morphometry in cytological smears for suspected malignant follicular lesions led to increased accuracy in establishing a suspicious malignant diagnosis for follicular lesions. Nuclear morphometry provides an unbiased point of view that increases diagnosis accuracy. Computerized morphometry can positively influence diagnostic accuracy, allowing for a better correlation with clinical and imaging data.

Introduction

Thyroid disorders have a wide range of presentations and can affect multiple organ systems. The thyroid gland is the most commonly affected endocrine gland. Such disorders can be incidentally detected through autopsy as endocrine-related mortality can be anatomically subtle and may require histological examination to identify and document the underlying pathologies. It is important to understand the functioning of the thyroid gland and its effects on vital organs when determining the cause of mortality and the contributing factors. Therefore, a thorough examination of the thyroid gland is recommended, particularly in cases where there is no apparent anatomical cause of mortality, which can often reveal the occurrence of occult pathologies (1).

The microscopic morphometric analysis of thyroid lesions is considered to be an important factor in the diagnosis of thyroid tumors. Computer-aided image analysis and processing applications aim to design, validate and implement additional or complementary tools that can automatically distinguish and classify malignant thyroid nodules. However, the existing literature on the applications of computerized morphometry for the histological study of thyroid lesions is relatively limited compared with the publications focused on microscopic thyroid cytological morphology. This could be due to the complexity of the computerized investigation approach, which requires the availability of specialized software technology based on artificial intelligence (2-5).

The anatomopathological evaluation of thyroid gland investigations typically begins with a cytological examination, which serves as a preoperative elective method for establishing a definitive diagnosis of benignity or malignancy (6).

Fine needle aspiration is a widely used, an efficient, reliable and cost-efficient method that is considered the gold standard of thyroid investigations. Huang *et al* (7) report a sensitivity of 83% and specificity of 92%, although diagnostic discrepancies can occur in 1-21% of cases. However, this technique has limitations in differentiating malignant cells found in lesions that contain a significant number of apparently benign cells, as is the case with the cytodiagnosis of follicular neoplasms. Therefore, these smears require further, more complex cytochemical and computerized morphometric investigations (7-12).

Additionally, the differential histopathological diagnosis of thyroid lesions with follicular architecture can be challenging to interpret, particularly in the case of minimally

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invasive follicular carcinomas, which can be easily confused with follicular adenomas. Capsular or vascular invasion also represent controversial topics when establishing a definitive diagnosis (13).

Since it is difficult to diagnose malignancy based on fine needle aspiration cytology in indeterminate thyroid nodules, surgery is often recommended for all of these cases. However, the cancer rate upon the final histology examination is <30%. To increase the accuracy of diagnosis in such cases, several other test methods have been proposed, including Galectin-3-ICC, BRAF mutation analysis, Gene Expression Classifier (GEC) alone and GEC+BRAF, mutation/fusion (M/F) panel alone, M/F panel + miRNA GEC and M/F panel by next-generation sequencing, FDG-PET/CT, MIBI-Scan and TSHR mRNA blood assay (14).

The computer image analysis system has shown considerable potential for diagnostic application in diverse histological situations. Microscopic morphometric analysis of thyroid lesions is considered to be an adjunct in the diagnosis of thyroid tumors.

Computerized nuclear morphometry can be an effective and cost-efficient tool for assessing histological features. By analyzing the size and shape of nuclei, important parameters, such as nuclear area and nuclear perimeter, can be evaluated, which play a crucial role in facilitating the diagnosis of various neoplasms. Despite the potential benefits, there are only a limited number of research studies on morphometric analyses in thyroid pathologies and they are not yet extensively used in routine histopathological diagnosis. However, these studies have shown that nuclear morphometric parameters, such as perimeter, nuclear area and coefficients of variability, can differentiate between benign and malignant thyroid lesions (2,3,15).

The present study applied a semi-automatic system that can be easily reproduced in any pathology laboratory and aimed to draw attention to the use of nuclear morphometry in diagnosing difficult thyroid lesions. Specifically, the goal was to study the correlations of morphometry in the differential diagnosis of benign and malignant thyroid lesions, with a focus on forms with highly aggressive potential.

Materials and methods

Study cases. The present study presented a comprehensive morphometric analysis of histological specimens from the thyroid gland, obtained from autopsies conducted within the Brăila Forensic Medicine Department (Brăila, Romania) and thyroidectomy specimens analyzed within the Brăila Pathological Anatomy Department (Brăila, Romania). The present study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Braila Emergency County Hospital (approval no. 37948/08.10.2020).

The study sample consisted of 36 cases with various thyroid lesions diagnosed within the Pathology Department of the Brăila County Hospital (Brăila, Romania) over a period of five years (2017-2021). Based on the diagnostic reports, the cases were classified as either benign (follicular adenomas; 10 cases) or malignant lesions, including follicular carcinomas (10 cases), the follicular variant of papillary thyroid carcinomas (10 cases), the diffuse sclerosing variant of papillary thyroid carcinomas (4 cases) and undifferentiated carcinomas (2 cases).

The sections were reviewed by at least two pathologists to ensure the accuracy of the diagnosis and classified according to the WHO criteria (16).

The control group employed in the present study comprised normal thyroid cells that were located in the immediate vicinity of the lesion tissue, thereby minimizing the possibility of processing artifacts and increasing the accuracy of the measurements. To ensure the accuracy of the measurements conducted in this study, normal thyroid cells identified on the same slide as the thyroid lesions were utilized.

Oncocytic (Hürthle cell) cases with nuclear abnormalities were excluded from the cytological study in order to avoid significant errors in statistical analysis.

Each case underwent a detailed analysis of its morphological characteristics, including the presence of cellularity in the smear, the colloid, architectural arrangement, as well as its nuclear features. The morphometric parameters that characterized benign and malignant lesions were compared in this analysis.

Histopathological technique. The 36 histological slide preparations were processed using standard techniques in the pathological anatomy laboratory, including fixation, paraffin embedding, sectioning and staining with hematoxylin and eosin. The chosen tissue samples were immersed in a 10% neutral-buffered formalin solution with a pH of 7.0 for a duration of 24 to 48 h to achieve optimal fixation. The processing of the specimens was performed using the Myr STP 120 Carousel Tissue Processor (Especilidades Medicas MYR, S.L), utilizing 100, 96 and 70˚ ethyl alcohol baths as dehydration agents, while toluene was used as the clearing agent. The processing period lasted for 17 h. Paraffin embedding was performed using the EC 500 Paraffin Embedding Station (Especilidades Medicas MYR, S.L),.

The embedded tissue blocks were then sectioned at a thickness of 5 μm to enable detailed examination. These sections were subjected to the conventional hematoxylin and eosin staining method (at room temperature, for 70 min), a widely used technique in histopathology, to facilitate microscopic evaluation and visualization of cellular structures.

To standardize the morphometry procedure, two new sections of the paraffin blocks were made by the same technician using the same microtome. Morphometric tests were performed blindly without knowledge of the final diagnosis. Images were captured using a Nikon Eclipse Ci trinocular microscope system (Nikon Corporation) equipped with an Mshot MS60 digital camera (Guangzhou Micro-shot Technology Co., Ltd.) and a personal Lenovo computer with a 2.2 GHz i5 CPU, 8 GB RAM and 1 TB SSD.

The Mshot Image Analysis System 1.0 software (Guangzhou Micro-shot Technology Co., Ltd.) was used for image capture.

Data collection. Data collection was achieved by using a manual method for selecting representative cells and morphometric features of interest. The morphometric analysis was performed without knowledge of the final diagnosis, eliminating any

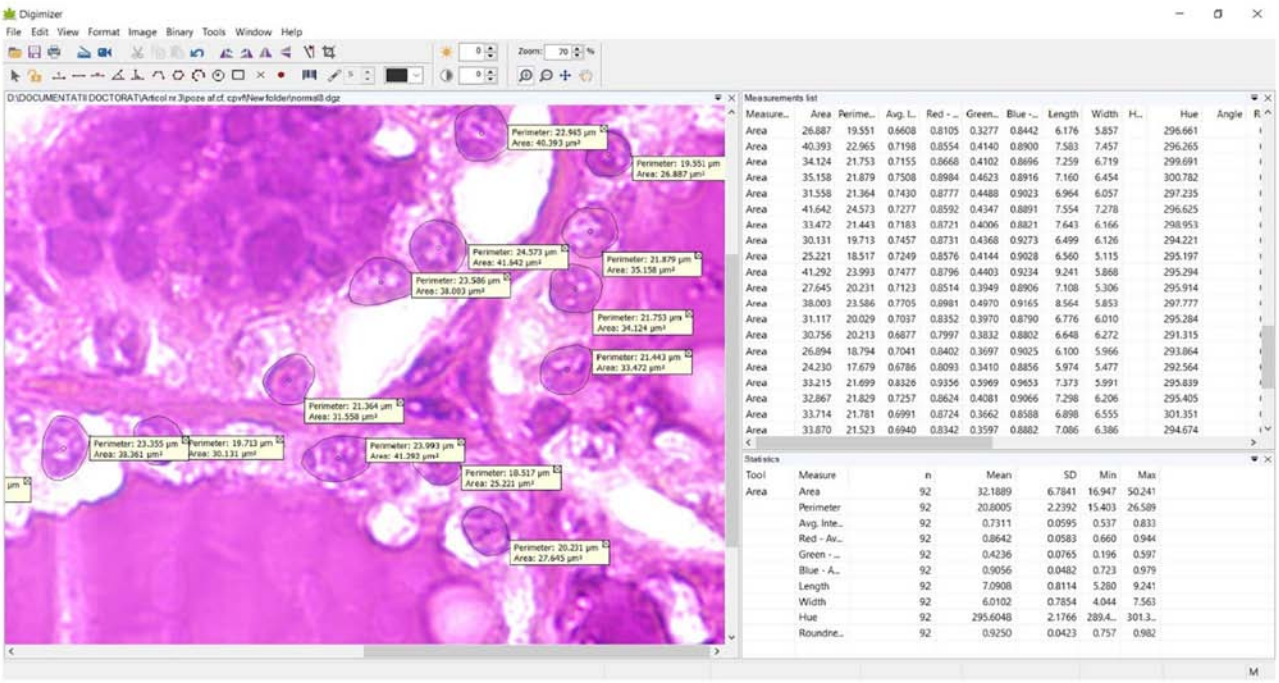

Figure 1. Follicular variant of the papillary thyroid carcinoma, (hematoxylin and eosin staining; x40 magnification), an example of an image in the Digimizer software, showing the measured parameters on a microscopic field.

potential bias. Microscopic fields were digitized using a X40 high-power field (HPF) objective, providing an appropriate degree of image resolution for micrometric-scale measurements. The X40 HPF lens was chosen to avoid errors in the measurement process that could have resulted from using a lower powered lens. The measurements were calibrated using a Mshot micrometer slide with a calibrated scale of 0.1 mm.

A mean of 5-10 microscopic fields were analyzed on each slide, in the selected cases, with a mean of 50 well-visualized nuclei measured on each microscopic field. Nuclei in areas with fragmented or overlapping nuclei, as well as stromal cell nuclei, were excluded. Ten nuclear morphometric parameters were measured using the drawing tools of the Digimizer v.5 software (MedCalc Software Ltd.), followed by the processing of the obtained data (Fig. 1).

Statistical analysis. The collected data were processed using Minitab v.19 (Minitab LLC). The measured parameters included the area, perimeter, average intensity, red average intensity, green average intensity, blue average intensity, length, width, hue and roundness. $P<0.05$ was considered to indicate a statistically significant difference. A two-way ANOVA and Bonferroni's post hoc test were utilized to assess the mean differences among the normal groups and the groups with follicular adenomas, follicular carcinomas, follicular variant papillary carcinomas and undifferentiated carcinomas Comparisons between the nuclear area and perimeter of normal cells and between benign and malignant cells were conducted using the paired t-test.

Results

Case group composition. The analyzed group comprised 36 selected cases, including 10 cases of follicular adenomas, 10 cases of follicular carcinomas, 14 cases of papillary carcinomas (10, the follicular variant of papillary thyroid carcinomas and 4, the diffuse sclerosing variant of papillary thyroid carcinomas), as well as two cases of undifferentiated carcinomas. It was found that two of the cases of papillary carcinomas were associated with autoimmune thyroiditis.

Statistical analysis results. Aggressive papillary carcinomas showed significantly higher nuclear parameter values than the normal thyrocyte group and thyroid adenomas.

The nuclear areas of the analyzed follicular carcinomas were larger than in the control group and larger than the follicular adenomas. Table I presents a synoptic presentation of the morphological and morphometric characteristics of the cases analyzed.

The two-way ANOVA test was applied to compare means between the normal groups and follicular adenomas group, normal groups and follicular carcinomas group, normal groups and follicular variant papillary carcinomas group and normal groups and undifferentiated carcinomas group, as shown in Table I.

The nuclear morphometry parameters used in the statistical calculations were area, perimeter, roundness, average intensity red, average intensity green, average intensity blue and average intensity and hue.

Table I provides the results obtained from the post hoc analysis, specifically Bonferroni, concerning the nuclear morphometry parameters. Notably, means distinguished by different letters signify statistically significant differences between the groups at a significance level of $P<0.05$. The utilization of distinct letters following the means indicates that they are statistically different from one another.

In the case of undifferentiated carcinomas, the nuclear size morphological parameters were slightly increased

Table I. Comparison of nuclear morphometric parameters between normal cell groups and neoplastic cell groups. Different superscript letters indicate statistical significance.

	Normal cell group/follicular adenoma cell group		Normal cell group/follicular carcinoma cell group		Normal cell group/Follicular variant papillary carcinoma cell group		Normal cell group/undifferentiated carcinoma cell group	
Variable, mean ± SD	Normal (n=2,754)	Follicular adenoma (n=2,754)	Normal (n=2,488)	Follicular carcinoma n=2488	Normal (n=2,384)	Follicular variant papillary carcinoma (n=2,384)	Normal (n=472)	Undifferentiated carcinoma (n=472)
Area	12.908^{e}±3.279	24.841^{c}±2.061	13.035^{e}±3.688	33.524^{b}±8.143	13.250^{e}±3.342	52.081^{a}±11.147	12.963^{e}±3.153	20.018^{d}±9.474
Perimeter	13.440^{e}±1.693	18.097^{c}± 3.981	13.560^{e}±1.873	21.68^{b}±2.616	13.562^{e}±1.695	26.665^{a}±2.92	13.425^{e}±1.635	17.177^{d}±4.338
Roundness	0.887^{b}±0.062	0.911^{a}±0.054	0.877^{b}±0.073	0.885^{b}±0.066	0.894^{b} ±0.060	0.914^{a}±0.085	0.893^{b}±0.059	0.815^{c}±0.131
Mean intensity	0.420^{d}±0.048	0.560^{c}±0.081	0.421^{d}±0.050	0.633^{b}±0.058	0.423^{d}±0.048	0.691^{a}±0.072	0.421^{d}±0.048	0.387^{e}±0.062
Meanintensity, red	0.375^{d}±0.050	0.650^{c}±0.096	0.375^{d}±0.053	0.746^{b}±0.054	0.378^{d}±0.051	0.816^{a}±0.072	0.376^{d}±0.050	0.373^{d}±0.069
Mean intensity, green	0.366^{b}±0.058	0.336^{c}±0.081	0.366^{b}±0.060	0.394^{a}±0.071	0.369^{b}±0.058	0.872^{a}±0.059	0.367^{b}±0.059	0.349^{c}±0.064
Mean intensity, blue	0.520^{d}±0.044	0.694^{c}±0.090	0.521^{d}±0.044	0.757^{b}±0.058	0.522^{d}±0.043	0.872^{a}±0.059	0.521^{d}±0.043	0.438^{e}±0.062
Hue	242.17^{d}±7.98	293.150^{b}±5.070	242.54^{d}±7.810	298.50^{a}±2.74	242.71^{d}±8.09	293.83^{b}±2.45	242.39^{d}±8.39	258.09^{c}±20.12

In the table, each unique subscript letter assigned to different parameters indicates a statistically significant difference. The subscript letters 'a' represent the highest values of the morphometric parameters, while 'e' represents the lowest values. The intermediate values are represented by 'b,' 'c,' and 'd,' in descending order.

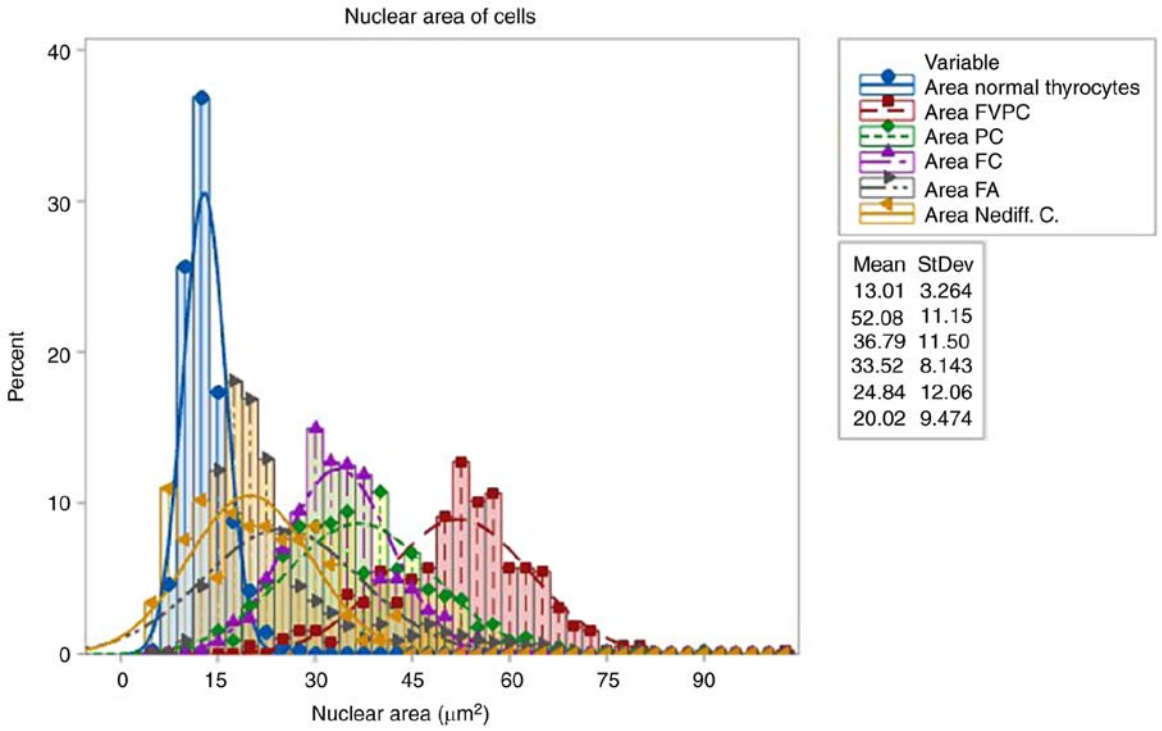

Figure 2. Comparative histogram of the nuclear area parameter for normal, benign and malignant cell groups.

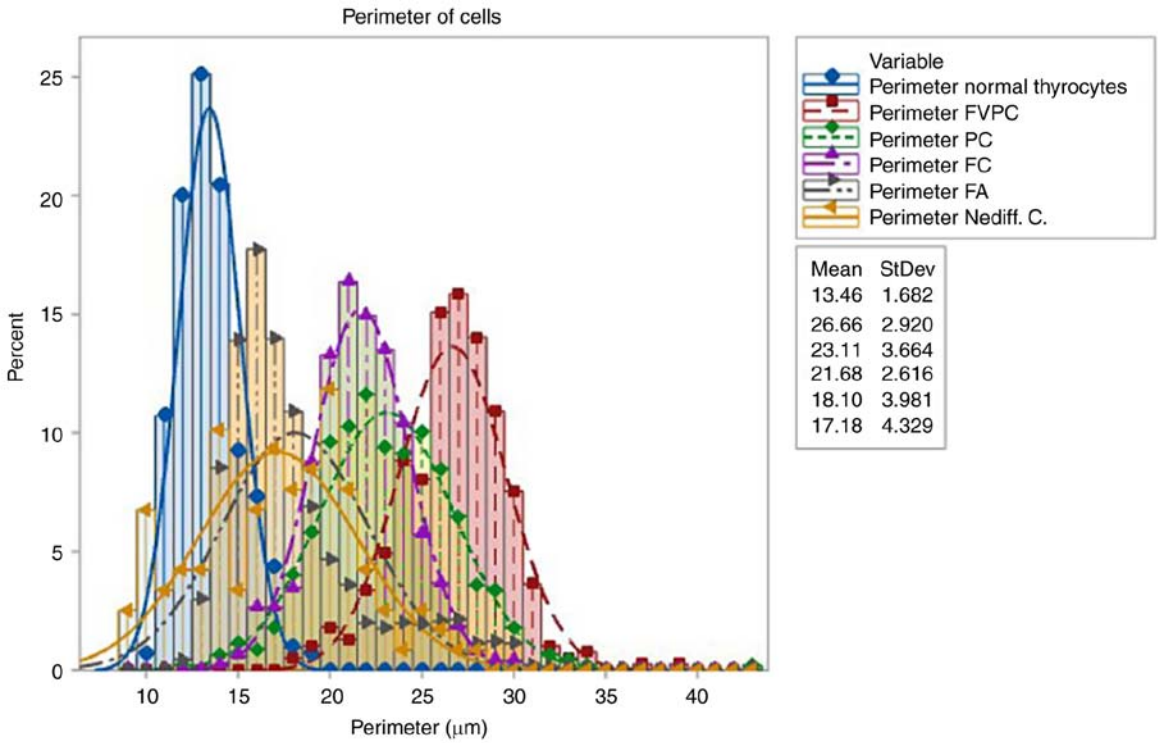

Figure 3. Comparative histogram of the nuclear perimeter parameter for normal, benign and malignant cell groups.

as compared with normal. For these cases, the roundness parameter was also worth taking into account, with a significantly increased standard deviation value of 0.131 as compared with the values of the other cases. This parameter shows the increased degree of irregularity of the nuclear membrane observed in undifferentiated carcinomas. Microscope images illustrating morphometric analyses can be found in Figs. 2, 3 and 4.

In the Digimizer software, the roundness parameter is defined as a value between 0 and 1, where values closer to 1 indicate a more circular shape. For instance, a perfect circle has a roundness value of 1.

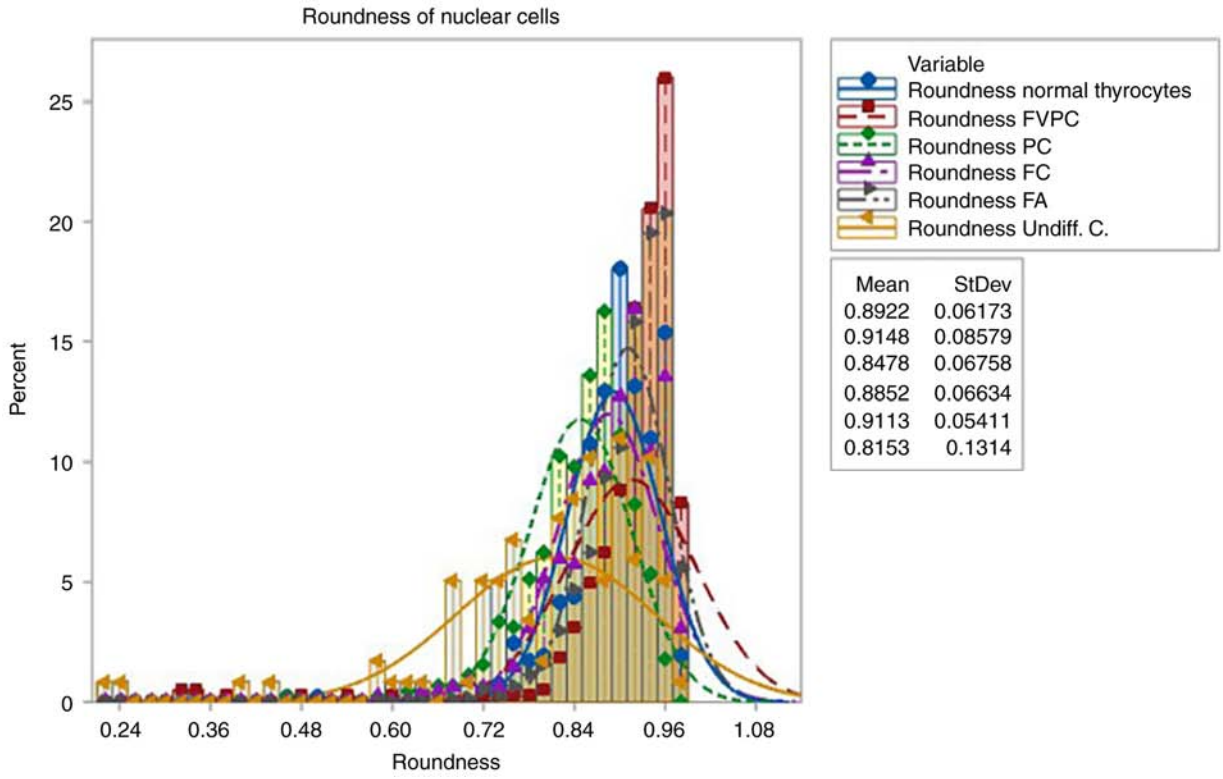

Figure 4. Comparative histogram of the roundness of nuclear cell parameter for normal, benign and malignant cell groups.

Furthermore, the Digimizer software can calculate the average intensity of an area, with an all-white area having an average intensity of 1 and an all-black area having an average intensity of 0.

In terms of color, the hue attribute in the Digimizer software refers to the property that allows a color to be categorized as a distinct color in the visible spectrum, such as red, green, yellow, or blue. This attribute is determined by the frequency of the corresponding wavelength and is expressed as a number between 0 and 360, as depicted in Fig. 5.

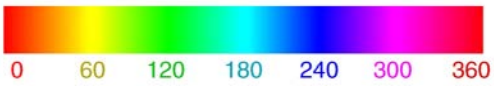

Figure 5. Correspondence between colors and hue value in the visual spectrum.

The present study performed a statistical analysis of the areas and perimeters within the normal group compared with the benign group and subsequently compared the normal group with the malignant group using paired t-tests.

Table II presents the results of paired t-tests for the nuclear morphometry parameters comparing the normal group with the benign group and also for the comparison within the normal group.

Statistically significant differences between groups were identified based on the means ($P<0.05$).

The control group consisted of nuclei from normal thyrocytes found in the tissue adjacent to the lesion. These nuclei exhibited a nuclear area with a mean of 13,010 μm^2.

The group with neoplastic lesions, specifically that with follicular adenomas, showed a nuclear area with a mean of 24.841 μm^2. These values were significantly higher compared with those in the control group.

The group with follicular carcinomas had nuclear areas with a mean of 33.626 μm^2.

The control group consisted of nuclei from normal thyrocytes found in the tissue adjacent to the lesion. These nuclei exhibited a nuclear perimeter with a mean of 13,457 μm.

The group with neoplastic lesions, specifically that with follicular adenomas, showed a nuclear area with a mean of 18.254 μm. These values were significantly higher compared with those in the control group.

The group with follicular carcinomas had nuclear areas with a mean of 39.112 μm.

Patient characteristics. The characteristics of the patients, such as age, sex, tumor location (unilateral or bilateral) and tumor size, are presented in Table III.

During the sampling process, no account was taken of any medications used for different types of thyroid diseases. The mean age of benign cases was of 65.4 years (range, 52-87 years), whereas for malignant cases it was of 55.08 years (range, 28-93 years). The majority of benign cases were encountered in women, with a male-to-female ratio of 3:7, most of the malignant cases also being encountered in women, with a male-to-female ratio of 9:17.

The tumor size for benign cases ranged between 1.5 and 2.5 cm with a mean ± SD of 1.76±0.33, while for malignant cases, it ranged between 2.8 and 8.2 cm with a mean ± SD of 3.4±1.45.

The present study found that the most effective sensitivity and specificity parameters were area, perimeter and roundness.

Table II. Paired t-tests for morphometric parameters (area and perimeter) between normal cell group and benign cell group, and normal cell group and malignant cell groups.

	Normal cell group/benign cell group			Normal cell group/malignant cell group		
Variable, Mean ± SD	Normal (n=2,754)	Benign (n=2,754)	P-value t-test	Normal (n=5,344)	Malignant (n=5,344)	P-value (t-test)
Area	12.908±3.279	24.841±12.061	<0.01	13.082±3.394	39.112±14.683	<0.01
Perimeter	13.440±1.693	18.097±3.981	<0.01	13.515±1.734	23.077±4.360	<0.01

However, the parameters average intensity, red average intensity, green average intensity, blue average intensity, length, width and hue could not be used because their P-value was >0.05.

Discussion

Complementary immunohistochemistry techniques do not provide any tested markers with a significant role in discriminating malignant lesions from benign ones. The CK19 or HBME-1 immunohistochemical markers are considered good predictors of malignancy, but cannot be taken for granted as markers of absolute certainty (17). Additionally, Galectin3, being negative in the case of these types of lesions, cannot provide any benefits in elucidating the diagnosis (4,18,19).

The most common histological subtype of thyroid papillary carcinoma is the follicular variant of papillary thyroid carcinoma, which is found in >24% of cases (20). Diagnosis is easily established based on the specific features of the nucleus, which have the appearance of optically bare or frosted glass nuclei, the degree of invasiveness and the presence of lymph node metastases. In one third of cases, they do not show extrathyroidal infiltration, being completely encapsulated. In some situations, these cases may present specific nuclear changes only focally, which is a common feature in benign lesions, or processing artifacts can be visualized by using a hyperconcentrated formaldehyde solution (4,21).

The histopathological diagnosis of non-encapsulated carcinomas remains one of the most controversial surgical thyroid pathologies. A correct diagnosis in these cases is crucial because these tumors may have the most controversial lymphatic and distant thyroid metastatic potential. Also, the interpretation of a follicular adenoma as a follicular carcinoma may expose the patient to unnecessary aggressive surgery (22).

In practice, many confusing situations arise in which follicular lesions are difficult to assess. Subjective interpretation of atypical cytological features is not a reliable criterion for the malignancy of thyroid lesions because these changes may be present in benign lesions, such as adenomatous hyperplasia and follicular adenoma. Often, overdiagnosis of these lesions can lead to overtreatment, which may be too aggressive (surgical or radioactive iodine) in the case of non-recurrent lesions that are considered malignant. Therefore, an objective morphological analysis would be of great value in differentiating benign from malignant lesions (4,18,23).

Computer-assisted image analysis can vary from simple software programs, which require human intervention, to complex applications involving artificial intelligence. The current state of software development allows for the integration and processing of histopathological images with automatic measurements. This acts as an expert system that allows for the automatic querying of a complex database, facilitating the activity of morphopathology (2,3,7,11,13,14,17,24).

However, specialized literature rarely provides concrete data on how authors created software applications for automated morphometric measurements.

The aim of the computerized morphometric study of thyroid histological smears was to identify and validate efficient morphometric parameters that distinguish the differences between benign and malignant epithelial cells. The most reliable parameters among them were area, perimeter, length, width, the degree of roundness and irregularity of the nuclear membrane. The present study predominantly focused on follicular architecture cases as follicular tumors cause the greatest diagnostic problems in thyroid pathology.

The present study found that cases in the group with malignant thyroid lesions (follicular carcinomas, papillary carcinomas, follicular versions, papillary carcinomas and the diffuse sclerosing variant, as well as undifferentiated carcinomas) had significantly higher values of nuclear morphometric parameters when compared with benign groups. The highest values were found in the follicular variant of papillary thyroid carcinomas. Undifferentiated carcinomas also had a higher value of the roundness parameter, which measures the nuclear membrane irregularity degree. It was concluded that the morphometric parameters that characterize benign and malignant lesions in the smears that were evaluated in the present study can be considered within the normal data presented in specialized literature (11).

Computerized morphometry is a useful and cost-effective tool that can be implemented in the anatomopathological management of thyroid gland investigations due to its simplicity of execution and safety (5). Nuclear morphological changes define the characteristics of a malignant tumor. The most important characteristics are enlarged nuclei, hyperchromasia, irregular, pulverized chromatin and prominent nucleoli.

In some cases, these characteristics may be interpreted subjectively. Computerized morphometry can be useful in these cases. Additionally, computerized morphometry appears to be an inexpensive and reproducible tool for evaluating the histological characteristics of various lesions (25,26).

Table III. Characteristics of patients.

Case	Histopathological diagnosis	Age, years	Sex	Tumor location	Tumor diameter, cm
1	Follicular adenoma	54	F	Right thyroid lobe	1.5
2	Follicular adenoma	78	F	Right thyroid lobe	1.8
3	Follicular adenoma	85	M	Left thyroid lobe	2.0
4	Follicular adenoma	57	F	Right thyroid lobe	1.5
5	Follicular adenoma	62	M	Right thyroid lobe	2.5
6.	Follicular adenoma	52	F	Left thyroid lobe	1.5
7	Follicular adenoma	57	F	Right thyroid lobe	1.5
8	Follicular adenoma	87	F	Left thyroid lobe	2.0
9	Follicular adenoma	54	F	Right thyroid lobe	1.8
10	Follicular adenoma	68	M	Left thyroid lobe	1.5
11	Follicular carcinoma	57	F	Right thyroid lobe	3.0
12	Follicular carcinoma	54	F	Left thyroid lobe	2.8
13	Follicular carcinoma	60	M	Left thyroid lobe	2.5
14	Follicular carcinoma	52	F	Left thyroid lobe	3.0
15	Follicular carcinoma	56	M	Left thyroid lobe	2.5
16	Follicular carcinoma	76	F	Left thyroid lobe	2.5
17	Follicular carcinoma	79	F	Right thyroid lobe	2.8
18	Follicular carcinoma	55	M	Right thyroid lobe	3.0
19	Follicular carcinoma	57	F	Right thyroid lobe	2.8
20	Follicular carcinoma	54	F	Right thyroid lobe	3.0
21	FVPC	42	F	Right thyroid lobe	3.5
22	FVPC	57	F	Left thyroid lobe	2.8
23.	FVPC	38	F	Left thyroid lobe	2.5
24	FVPC	35	M	Left thyroid lobe	1.5
25	FVPC	54	F	Left thyroid lobe	2.5
26	FVPC	60	M	Right thyroid lobe	2.8
27	FVPC	39	F	Right thyroid lobe	2.5
28.	FVPC	65	F	Right thyroid lobe	3.5
29	FVPC	67	F	Left thyroid lobe	2.5
30	FVPC	78	M	Left thyroid lobe	3.5
31	DSV	43	M	Both thyroid lobes	5.0
32	DSV	32	M	Both thyroid lobes	4.0
33	DSV	28	F	Both thyroid lobes	5.8
34	DSV	35	M	Both thyroid lobes	3.5
35	Undiff. C.	76	F	Both thyroid lobes	6.5
36	Undiff. C.	83	F	Both thyroid lobes	8.2

FVPC, follicular variant of the papillary thyroid carcinoma; DSV, diffuse sclerosing variant of the papillary thyroid carcinoma; Undiff. C., undifferentiated carcinoma; F, female; M, male.

Morphometric nuclear parameters such as the nuclear perimeter and the nuclear area can be useful for differentiating between different thyroid lesions (27,28).

Although the use of morphometric analyses in thyroid cytology is not a common practice, it appears to only be used in studies conducted in the case of scientific research, not for clinical use (4).

Tseleni-Balafouta *et al* (16) showed in their study that the nuclear area is larger in follicular carcinoma compared with the nuclear area found in adenomatous lesions and in thyroid hyperplastic nodules. They found significantly higher morphometric parameters in thyroid papillary carcinomas as compared with thyroid follicular carcinomas, similar to the measurements found in the present study.

Wright *et al* (29) discovered altered morphological parameters in Giemsa-stained preparations when measuring papillary and follicular carcinomas as compared with benign thyroid lesions.

Khatri *et al* (3) found significant differences in nuclear morphological parameters between benign and malignant thyroid lesions, concluding that cytomorphological features of thyroid lesions can be quantitatively estimated by performing nuclear measurements.

Atypical lesions of undetermined significance, including heterogeneous entities, are classified into subgroups based on cytologic and architectural atypia.

Those with cytological atypia are associated with an increased risk of malignancies (30).

Mathur *et al* (30) suggest that in cases with lesions of undetermined significance, the encountered atypical nuclear characteristics should be thoroughly evaluated to distinguish between benign and malignant lesions, as the follow-up and treatment procedures are different.

Eliminating the category of undetermined significance lesions leads to a significant decrease in sensitivity regarding the detection of thyroid lesions.

Sensitivity to detecting papillary carcinoma decreases from 100 to 7% when undetermined significance cases are not investigated (31).

Failing to investigate undetermined significance cases can result in an increase in false-negative and false-positive diagnoses, with ≤53% of neoplastic thyroid lesions and 37% of thyroid papillary carcinomas being misdiagnosed as benign. On the other hand, 38% of benign lesions may be misdiagnosed as follicular neoplasms or suspected follicular neoplasia. A thorough examination of the unknown significance lesion category is necessary in order to avoid misdiagnosis. Nuclear morphometry can be used to quantify a wide range of parameters that characterize nuclear size and shape and can facilitate a more precise diagnosis of thyroid pathology. Studies have suggested that nuclear morphometric parameters, such as nuclear area and perimeter, improve diagnosis, treatment and outcomes in a variety of neoplasms, including mammary gland carcinoma, bladder carcinoma, skin lymphomas and soft tissue sarcomas (32-35). Morphometric analysis is reproducible and inexpensive and objective information obtained through quantification of the characteristics of nuclear morphological parameters can be useful in classifying different lesions (2,16,36,37).

Shih *et al* (36) and Aiad *et al* (4) performed retrospective studies on thyroid cytological preparations, performing multivariate analyses of computerized morphometry correlated with clinical data. Parameters related to the size and shape of nuclei had significantly increased values in the follicular variant of the papillary thyroid carcinoma as compared with the follicular neoplasm. Wright *et al* (29) found significant differences in the values obtained in the measurements of nuclear areas and perimeters between cases with multinodular goiter as compared with follicular adenomas, as well as follicular and papillary carcinomas. Nuclear morphometry is useful for distinguishing malignant from benign lesions. The limitations of the present study are the relatively small sample of patients and the fact that it was conducted only in one hospital.

The present study employed a semi-automatic system of nuclear morphometry, which can be easily replicated in any laboratory setting. This technique offers substantial advantages in terms of cost-effectiveness and time efficiency for each case. In comparison to laborious immunohistochemical methods that require extended work duration and significantly higher costs, our approach stands out due to its minimal expenses and reduced diagnostic time. The present study cannot be considered an absolute diagnostic criterion as it relies solely on statistical differences. The utilization of morphometric analysis in thyroid pathology is still limited in clinical research. Computerized morphometry could positively effect diagnostic accuracy by allowing better clinical and imaging correlation.

In conclusion, the use of morphometry in cytological smears for suspected malignant follicular lesions leads to increased accuracy in clearly establishing suspicious malignant diagnoses for follicular lesions.

Parameters such as area, perimeter and intensity have good sensitivity and specificity for detecting malignancy.

Nuclear morphometry provides an unbiased point of view that increases diagnosis accuracy and differentiation between lesions bordering on malignancy and benignity. Computerized morphometry can positively influence diagnostic accuracy, allowing a better correlation with clinical and imaging data.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

IM and MC were responsible for conceiving the current study. IM, ZC and MC performed validation of data and formal analysis. IM and MC confirm the authenticity of all the raw data. BS, AC and DS made substantial contributions to conception and design. IM, BS, AC, DS, ZC and MC reviewed and edited the manuscript and supervised the current study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board/Ethics Committee of Braila Emergency County Hospital (approval no. 37948/08.10.2020).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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