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Full Length Article

Diagnostic criteria of well differentiated thyroid tumor of uncertain malignant potential; a histomorphological and immunohistochemical appraisal

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KEYWORDS

Well-differentiated thyroid tumors uncertain malignant potential;
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Abstract *Background:* Well differentiated thyroid tumor of uncertain malignant potential (WDT-UMP) represents a true "gray zone" of "follicular patterned" thyroid lesions, that needs to be characterized in order to outright the diagnosis of carcinoma and avoid unnecessary aggressive treatment.
Aim: To emphasize on the histomorphological criteria for more accurate diagnosis of WDT-UMP. Also to compare the immunohistochemical expression of CK19 of WDT-UMP versus adenoma and papillary thyroid carcinoma (PTC).
Materials and methods: The study included 60 thyroid specimens; 18 WDT-UMPs, 24 PTC (18 classic variant and 6 follicular variants) and 18 benign thyroid lesions (8 adenoma, 6 Hashimoto's thyroiditis and 4 hyperplastic nodules). H&E stained sections were assessed according to the published major and minor criteria of malignancy in the thyroid. CK 19 immunostaining was examined and evaluated according to the proportion and intensity scores.
Results: We could detect the absence of nuclear inclusions, presence of characteristic nuclear groove, nuclear clearing, ovoid nuclei, nuclear crowdness, nuclear enlargement and pleomorphism as important reliable features for diagnosis of WDT-UMP with p value (<0.0001 for each). WDT-UMP showed moderate to strong CK 19 immunostaining with proportion scores 3 and 4; an intermediate expression profile; higher than adenoma and less than papillary carcinoma ($p < 0.0001$).

Abbreviations: WDT-UMP, well differentiated thyroid tumor of uncertain malignant potential; PTC, papillary thyroid carcinoma; PTC-Ns, papillary thyroid carcinoma-type nuclear changes; EFVPTC, encapsulated follicular variant papillary thyroid carcinoma

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Conclusion: The constellations of both major and minor criteria of malignancy are important clues for WDT-UMP diagnosis which could be ascertained by CK 19 immunostaining.

Introduction

Williams, 2000 proposed a diagnostic terminology in thyroid pathology; a well differentiated tumor of uncertain malignant potential (WDT-UMP) for capsulated follicular pattern tumors with diffuse equivocal or focal unequivocal papillary thyroid carcinoma type nuclear changes (PTC-Ns) and without definite invasion. This group of tumors has been suggested to be borderline in nature [1].

PTC-type nuclear changes (PTC-Ns) are cited in the literature and textbooks as the diagnostic criteria for malignancy in thyroid tumors, regardless of whether or not the tumor has a capsule, is invasive, or has a papillary growth pattern [2], they included cytoplasmic pseudo inclusions, nuclear grooves, nuclear clearing, elongated overlapping nuclei and nuclear irregularity [3].

When PTC-Ns were observed equivocally, such as only nuclear clearing and nuclear grooves without nuclear pseudo inclusions, thyroid lesions will be classified as WDT-UMP. When unequivocal PTC-Ns were seen in the entire part of the tumor it will be as encapsulated follicular variant papillary thyroid carcinoma. When unequivocal PTC-Ns were found in only part of the tumor, it will be classified as WDT-UMP, as explained in (Fig. 1) [3].

The immunohistochemical studies have been reported as a helpful tool in confirming diagnosis of malignancy in thyroid lesions with special reference to CK19; is one of the most commonly used keratin to investigate thyroid lesions with high sensitivity (92%) and specificity (97%) when used as a single marker [4–6].

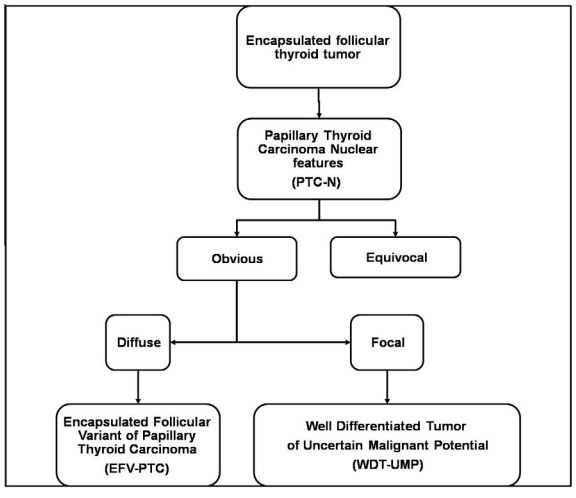

Figure 1 Nomenclature for encapsulated thyroid follicular tumors; if PTC-N are equivocal, it should be WDT-UMP, if PTC-N are unequivocal but focal, it should be WDT-UMP and if PTC-N are unequivocal but diffuse it should be EFV-PTC (modified from Williams' proposal) [3].

WDT-UMP is still a controversial entity among expert pathologists [3], so the purpose of this study is to emphasize on the histomorphological criteria for more accurate diagnosis of WDT-UMP. Also to compare the immunohistochemical expression of CK19 of WDT-UMP versus adenoma and papillary thyroid carcinoma (PTC).

Material and methods

Patients and tissue samples

This was a retrospective study carried out in the Pathology Department, Sohag University Hospital, during the period from Jan 2011 till June 2014.

The hematoxylin and eosin (H&E) stained sections of 200 specimens of the thyroid nodules were reviewed, 60 out of 200 cases were selected and categorized into three groups according to their final diagnosis which was based on the terminology of the Chernobyl Pathologist Group and modified Williams' proposal [1].

The first group (n = 18)

All cases were encapsulated, follicular patterned tumors with diffuse equivocal or focal unequivocal PTC-N features, without capsular or vascular invasion and well demarcated from the surrounding thyroid parenchyma. *They were diagnosed as WDT-UMPs.*

The second group (n = 24)

They are formed of follicular patterned lesions with diffuse unequivocal PTC-N features; diagnosed as encapsulated follicular variant of PTC (EFV-PTC, $n = 6$), and 18 cases were classic PTC. This group was considered a control group of *malignant thyroid nodules.*

The third group (n = 18)

All cases were follicular patterned lesions lacking PTC-N features (8 cases were diagnosed follicular adenoma, 6 cases were Hashimoto thyroiditis and 4 cases were hyperplastic nodules). This group was considered a control group of *benign thyroid lesions.*

Immunohistochemistry

Representative formalin-fixed, paraffin embedded routinely processed, tissue sections from each specimen were stained with 1:100 a mouse monoclonal CK19 antibody (clone A53-B/A2.26, Lab vision, USA). Briefly, tissue sections were deparaffinized and rehydrated, antigens were retrieved by incubating sections in sodium citrate buffer (pH 6.0) in an

800w microwave for 10 min. After blocking nonspecific reactions by endogenous hydrogen peroxidase, sections were incubated at 40 °C with CK19 for an hour. Visualization of staining was conducted using streptavidin–biotin; ABC staining kit (Catalog # TA-015-HP, Lab-Vision Corporation Fremont, USA), according to manufacturer's instructions. Immunohistochemical reactions were developed with 3,3-diaminobenzidine; chromogen peroxidase substrate (DAB). Counterstaining of tissue sections was done using Myer's Hematoxylin and mounted using DPX and cover slipped. Both positive and negative controls were consistently immunoreactive and lacking reactivity respectively. This confirms the validity of the staining results.

Immunohistochemical evaluation

Evaluation included the proportion of reactive cells within the lesion (the percentage) as well as the staining intensity and its distribution pattern. *The proportion score* described the estimated fraction of positively stained cells (0; no cytoplasmic staining in any tumor cell, 1+; cytoplasmic staining in less than 5% tumor cells, 2+; cytoplasmic staining in 5–50% tumor cells, 3+; cytoplasmic staining in 50–95% tumor cells, 4+; cytoplasmic staining in more than 95% tumor cells) [7].

The intensity score described the staining intensity (0; no staining, 1+; weak, 2+; moderate, 3+; strong). The distribution of immunoreactive cells was classified as diffuse (when most of cells were stained by CK19) or focal (when some portion of the tissue presented clusters of stained cells while other proportions did not) [8].

Statistical analysis

Statistical package for social sciences (SPSS), version 22 IBM-Chicago, USA (2013) was used for statistical data analysis. Pair-wise comparisons were done between groups with downward adjustment of alpha, *p*-value was considered significant if <0.05 and highly significant when <0.001.

Results

The current study compared the histopathological features and the pattern of immuno-histochemical expression of CK19 among three groups of thyroid lesions; the WDT-UMP, the malignant and the benign.

All WDT-UMPs (18/18) were follicular patterned nodules with no vascular or capsular invasion. Nine cases of WDT-UMPs showed diffuse equivocal (not well developed) PTC-N type changes (Fig. 2A and B). The other 9 cases showed focal unequivocal (obvious nuclear features were found in only part of tumor) (Fig. 2C and D). PTC-N type changes were diffusely found in all cases of the 2nd group (6 FVPC and 18 classic PTC) (Fig. 3).

According to Chan and Lloyd et al., literatures, the histomorphological features of malignancy in thyroid- including the PTC-N changes – were categorized into major and minor criteria. We compared these features between WDT-UMP and both malignant and benign groups (Table 1). The features were ordered in a descending way according to their importance in diagnosis [9,10].

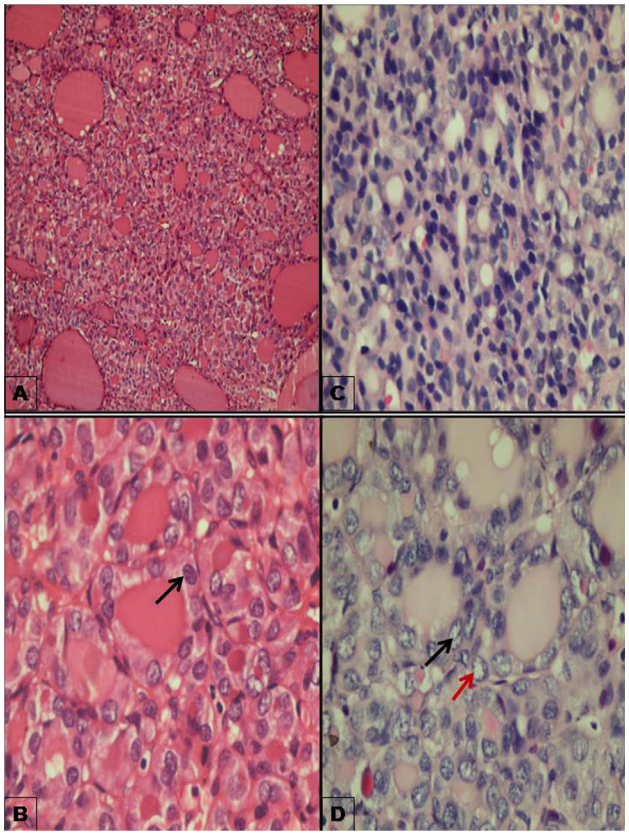

Figure 2 Diagnostic nuclear features of WDT-UMP either diffuse equivocal PTC-Ns (A and B) or focal unequivocal PTC-Ns (C and D). Thin incomplete nuclear grooves (black arrows), nuclear clearing (Red arrow), enlarged ovoid nuclei, mild to moderate nuclear crowdness and nuclear pleomorphism (HE; ×200, ×400, ×400, ×400, original).

Major criteria

(1) *The nuclear pseudo inclusions* (the nuclear invagination, defined as a definite inclusion with sharp borders) could not be found in any cases of WDT-UMP (0/18) (Fig. 2), detected in 83% (20/24) of PTC cases (2nd group) (Fig. 3C and D) and were absent in the 3rd group (benign lesions).
(2) 2-*The nuclear groove:* sixteen out of eighteen (89%) cases of WDT-UMPs showed faintly visible, thin and incomplete nuclear groove (Fig. 2B and D). This was opposite to the PTC group (2nd group), 24/24 cases showed frequent thick complete nuclear groove, which was running across the long axis of the nucleus (complete). The third group (18/18) was lacking this nuclear feature.
(3) *Nuclear clearing* was detectable in 100% (18/18) of WDT-UMP cases (Fig. 2D) and in all cases (24/24) of the 2nd group (Fig. 3) and occasionally seen in the 3rd group (5/18).
(4) *Nuclear shape:* ovoid nuclei were detected in all WDT-UMPs (18/18) (Fig. 2C) and PTC cases (2nd group, 24/24) and it was occasionally seen in the 3rd group (2/18) (Fig. 4).

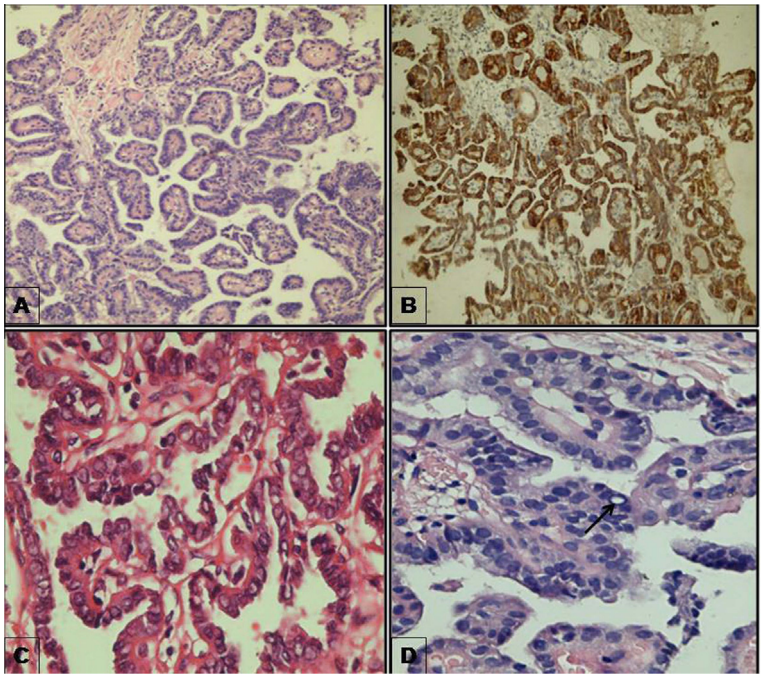

Figure 3 Classic PTC; true papillae (A), strong diffuse expression of CK19 (B), obvious PTC-Ns; nuclear clearing, pseudoinclusion (black arrow), enlarged ovoid nuclei, marked pleomorphism and overlapping (C and D) (HE; ×200, ×400, ×400-CK19; ×200, original).

Table 1 The major and minor diagnostic criteria in the different groups:

Studied groups	WDT-UMP $N = 18(\%)$	Malignant group $N = 24(\%)$	Benign group $N = 18(\%)$	Chi-Square P value
		Major criteria[*]		
1. Nuclear pseudoinclusion	0(0)	20(83)	0(0)	< 0.0001
2. Nuclear groove	16(89)	24(100)	0(0)	< 0.0001
3. Nuclear clearing	18(100)	24(100)	5(28)	< 0.0001
4. Ovoid nuclei	18(100)	24(100)	2(11)	< 0.0001
5. Nuclear crowdedness				
Moderate	12(66.7)	1(4.2)	2(11)	< 0.0001
Severe	6(33.3)	23(95.8)	0(0)	
6. Nuclear enlargement	17(94.4)	23(95.8)	2(11)	< 0.0001
7. Nuclear pleomorphism				
Mild	9(50)	0(0)	2(11)	
Moderate	9(50)	0(0)	0(0)	< 0.0001
Severe	0(0)	24(100)	0(0)	
		Minor criteria		
1. Distorted follicles	14(78)	24(100)	14(78)	0.09
2. Papillae	2(11)	20(83)	5(28)	< 0.0001
3. Necrosis	10(55.5)	20(83)	4(22)	< 0.0001
4. Dense colloid	18(100)	24(100)	16(89)	0.166
5. Fibrosis/sclerosis	12(66.6)	23(96)	9(50)	0.006

[*] Ranked in descending order according to the importance. [9,10].

(5) *Nuclear crowdness and overlapping* were moderate to severe in WDT-UMPs (18/18) (Fig. 2D), while all cases of PTC (24/24) showed a severe degree of nuclear crowdness and overlapping except one case which showed a moderate degree (Fig. 3D). Two case of the 3rd group showed mild to moderate degrees of crowdness (Fig. 4).

(6) *Nuclear enlargement:* there is a nuclear enlargement in 17/18 of WDT-UMPs in comparison to adjacent nuclei of follicular cells and small nuclei of the adenoma. Nuclei of PTC showed a marked nuclear enlargement (23/24) (Fig. 4).

(7) *Nuclear pleomorphism:* There are mild to moderate degrees of nuclear pleomorphism in all WDT UMPs (18/18) and a marked degree in PTC (23/24) cases. Very mild degree of nuclear pleomorphism could be seen in the benign group (2/18) (Fig. 4).

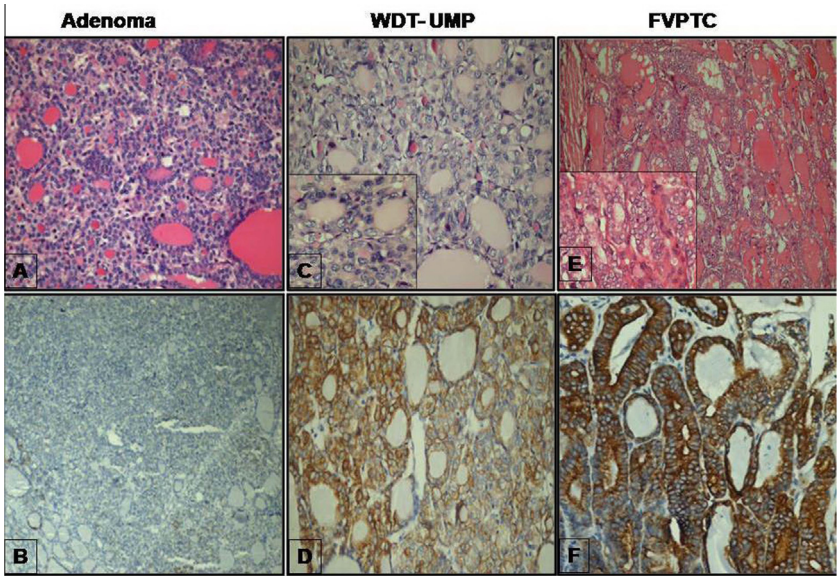

Figure 4 Diagnostic features of WDT-UMP versus adenoma and follicular variant of PTC (A, C and E), moderate multifocal expression of CK 19 in WDT-UMP (D) in comparison to negative expression in adenoma (B) and strong diffuse staining in follicular variant of PTC (F) (HE; ×400, ×400, ×400-CK19; ×200, ×400, ×200, original).

There was a statistically significant association between the previous 7 nuclear features and the diagnosis of WDT-UMP with p values (< 0.0001 for each) (Table 1). The absence of nuclear pseudoinclusion, presence of characteristic nuclear groove, nuclear clearing, ovoid nuclei, moderate to severe degrees of nuclear crowdness, nuclear enlargement and mild to moderate degrees of nuclear pleomorphism are important features for the diagnosis of WDT-UMP (Fig. 5).

Minor criteria

(1) *Distorted follicles*: fourteen out of eighteen (78%) cases of WDT-UMP showed disturbed follicular pattern compared with 100% of the PTC group and 77.8% in the benign group (Table 1) (Fig. 4).

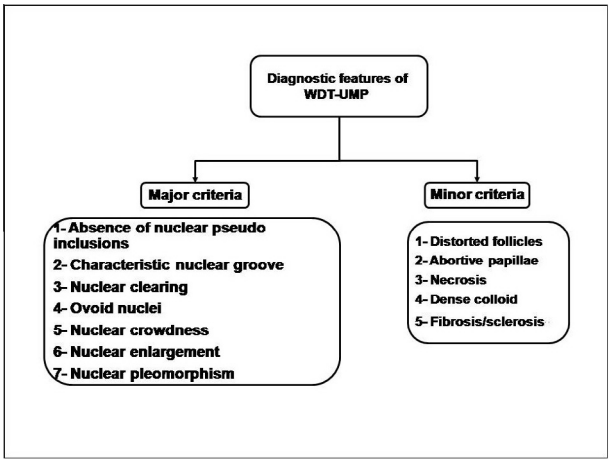

Figure 5 The major and minor diagnostic criteria of WDT-UMP.

(2) *Abortive papillae:* were detected in 11% of WDT-UMPs compared with the true well formed papillae in the classic variant of papillary carcinoma of the 2nd group (20/24) (Fig. 3). Three cases of hyperplastic nodules and 2 cases of Hashimoto's thyroiditis showed abortive papillae (Table 1).
(3) *Necrosis*: the necrotic areas could be seen in 55.5% (10/18) of WDT-UMP cases, in 83% (20/24) of the malignant group and in 22% (4/18) of the benign group (Table 1).
(4) *Dense colloids:* were seen in 100% cases of WDT-UMP compared with similar percent in the malignant group and 88.9% in the benign group (Table 1).
(5) *Fibrosis/sclerosis:* could be seen in 66.6% (12/18) of WDT-UMPs with a higher percent (96%) (23/24) being detected in the malignant group and a lower percent (50%) (9/18) in the benign group (Table 1).

The presence of papillae (either true or abortive) ($p < 0.0001$), necrosis ($p < 0.0001$) and fibrosis/sclerosis ($p = 0.006$) could be found in WDT-UMPs with the steady increase toward the PTC group (Table 1 and Fig. 5).

Immunohistochemical features of CK19

The staining pattern was predominantly pancytoplasmic with membranous accentuation, the immunoreactivity of CK19 was explained according to the applied proportion and intensity scores (Table 2).

The first group (WDT-UMP, n = 18)

Nine cases out of 18 (50%) of WDT-UMPs showed strong CK19 staining with a proportion score 2+ and the other 50% (9/18 cases) expressed a moderate staining with a

Table 2 Comparison between the studied groups as regards CK 19 expression.

Studied groups	Benign group $N = 18(\%)$	WDT-UMPs $N = 18(\%)$	Malignant $N = 24(\%)$	Kruskal–Wallis test P value
Proportion score of CK 19				
0	12(66.7)	0(0)	0(0)	< 0.0001
1+	6(33.3)	0(0)	0(0)	
2+	0(0)	9(50)	0(0)	
3+	0(0)	9(50)	3(12.5)	
4+	0(0)	0(0)	21(87.5)	
Median*	0	70	98	
Intensity score of CK 19 expression				
0	12(66.7)	0(0)	0(0)	< 0.0001
1+	4(22.2)	0(0)	0(0)	
2+	2(11)	9(50)	3(12.5)	
3+	0(0)	9(50)	21(87.5)	
Median**	0	2.5	3	

* *Median of proportion score*: Benign = 0 (nil expression), WDT-UMP = 70% (score 3), malignant group = 98% (score 4).
** *Median of intensity score*: Benign = 0 (nil expression), WDT-UMP = 2.5 (moderate to strong), malignant group = 3 (strong).

proportion score 3+, as shown in (Fig 4D), the adjacent normal thyroid follicles showed negative staining of CK19.

The second group (papillary thyroid carcinoma, n = 24)

The classic PTC (18/18) cases showed a strong expression of CK19 with a proportion score 4+, (Fig. 3B), the follicular variant (6/6) cases showed moderate to strong staining with proportion scores ranging between 3+ and 4+, (Fig. 4F).

The third group (benign thyroid lesions, n = 18)

The expression of CK19 in adenoma and hyperplastic nodules were negative except in 2 cases of adenoma which showed a weak focal positivity (Fig. 4B), Hashimoto's thyroiditis (4/6) showed weak to moderate immunostaining with a proportion score 1+.

CK 19 immunostaining of WDT-UMP showed an intermediate expression profile; higher than adenoma and less than PTC with P value (< 0.0001) (Table 2).

An interesting phenomenon was noticed within WDT-UMPs; areas with obvious PTC nuclear features showed more intense expression of CK19 than the surrounding areas of less atypical nuclear features. This gave an impression of a nodule (with more atypia) within the nodule (WDT-UMP) (Fig. 6). It is similar to what happens in dysplastic changes of the liver.

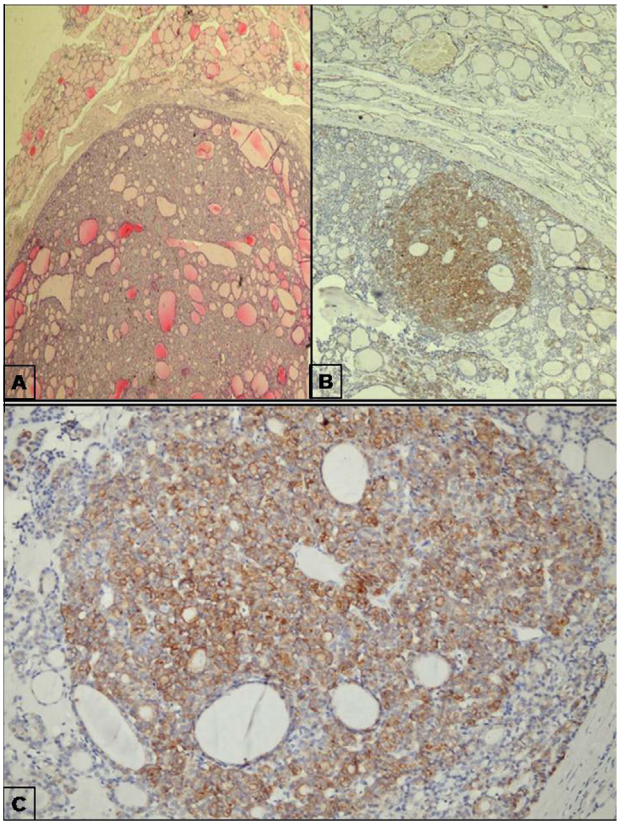

Figure 6 WDT-UMP (A) showed intense expression of CK19 in areas with atypical nuclear features more than the surroundings (B and C) (HE; ×40-CK19; ×100, ×200, original).

Discussion

The current study compared the histopathological criteria of WDT-UMP lesions versus benign and malignant lesions. More light was also shed on the pattern of immunohistochemical expression of CK19; a proven helpful distinguishing marker between the three lesion types.

The term "well differentiated tumor of uncertain malignant potential or WDT-UMP" was proposed for follicular patterned lesions of the thyroid that cannot be readily diagnosed as benign or malignant because of questionable nuclear changes and absent capsular or vascular invasion [1]. The main reason behind this proposal was to avoid extensive treatment (total thyroidectomy followed by radioactive iodine) of thyroid tumors which clinically behave in a benign fashion and carry excellent prognosis [11].

Although no single morphological feature is pathognomonic of malignancy in thyroid lesions; a constellation of

features has to be considered, basically, the nuclear features of PTC. Chan in his literature, categorized the histomorphological features of malignancy into; major and minor (subsidiary) criteria [9].

The current study emphasized on both major and minor criteria of WDT-UMPs. On the top of the list of major criteria is the nuclear pseudoinclusions which is considered as a major exclusive feature, it was found only in PTC cases (83%) and absent in both the WDT-UMPs (0/18) and benign group (0/18). Its presence is a diagnostic clue for malignancy. Our results were close to what was mentioned by Liu et al., as they reported the absence of nuclear pseudoinclusion in all WDT-UMPs (30 cases) opposite to 3–8% were observed in follicular variant of PTC [3]. Furthermore, Lloyd et al., in a survey study for diagnostic criteria of malignancy, documented the cytoplasmic pseudoinclusion as one of the three most important diagnostic features of malignancy (the others; abundant nuclear grooves and ground glass nuclei) [10].

The second most important major criteria is the nuclear groove as reported by Nishigami and his colleagues in a cyto-morphological study, they clarified the incidence of nuclear grooves in broad spectral thyroid lesions; they did not find the nuclear grooves in adenomatous goiter or follicular adenoma while they found the incidence of nuclear groove increased gradually from WDT-UMPs (4.5%) to PTC (6.5%). They added that the difference between the nuclear grooves of WDT-UMP which were rare, thin and faintly visible while that of PTC were dense, running across the long axis of the nucleus [12]. These results were confirmed by the current study, as we did not found any grooves in the benign group (adenoma and goiter) while we found frequent complete grooves in all cases of the malignant group (PTC; 100%). Furthermore, in WDT- UMPs; 16 out of 18 cases (89%) showed characteristic incomplete thin groove with irregular distribution.

The nuclear clearing or ground glass nuclei- as a third feature- was observed in 100% of WDT-UMPs and 100% of malignant cases in our study, the difference was clear in comparison to the hyper chromatic nuclei of adenoma. These figures came in accordance with Liu and his colleagues, as they reported nuclear clearing in 30/30 cases of WDT-UMPs [3]. Other supportive studies ranked the nuclear clearing as one of the most important diagnostic features of thyroid malignancy among experts [9,13].

However, we could not consider the previous feature as an isolated reliable criterion for the diagnosis of WDT-UMP, because we recognized it in 28% of the benign group (Hashimoto thyroiditis and hyperplastic nodule). So it should be incorporated with other major features. Rosai and his colleagues reported the vesicular nuclei in wide range of benign and malignant thyroid disorders and they considered it of no diagnostic significance by itself [14].

Nishigami and his colleagues, in the morphometric study of spectral thyroid lesions clarified the degree of nuclear circularity of WDT-UMP; it was found to be statistically smaller than that of PTC. This finding was detected roughly in our study, whereas the ovoid nuclei were apparent in 100% of all cases of WDT- UMPs and in 100% of the malignant group while the nuclei of adenoma were more circular [12].

Both Chan and Elsheikh and their colleagues reported the ovoid nuclei on the top of the most important list of PTC-N features [9,13].

In the current study; the epithelium of thyroid follicles in WDT-UMPs still had polarity but the cells were crowded (moderate to severe degrees). This was opposite to the PTC group in which the polarity was lost and the nuclei were overlapped (more severe crowdness), and the benign group missed this feature. Liu and his colleagues, found the same feature in thirty cases of WDT-UMPs; preservation of the polarity with nuclear crowdness but without overlapping which was seen in frank malignancy [3]. Another supportive study by Chan, reported the nuclear crowdness as the second most important criteria of nuclear atypia [9].

Liu and his colleagues reported the nuclear size of WDT-UMP was 2–4 times that of normal thyroid follicular cells. This came in agreement with our results where we found variable degrees of nuclear enlargement in most cases of WDT-UMPs (94.4%) and PTC (95.8%) in comparison to 11% of the adenoma group [3].

The last major criterion is the nuclear pleomorphism; all cases of WDT-UMPs showed mild to moderate degrees while 95.8% of PTC revealed a marked degree of nuclear pleomorphism. Very mild degree of nuclear pleomorphism could be seen in 4.2% of the benign group. Our results were supported by Elsheikh et al., where they detected this feature clearly in WDT-UMPs [13].

The current study considered the presence of characteristic nuclear groove, nuclear clearing, ovoid nuclei, moderate to severe degrees of nuclear crowdness, nuclear enlargement and mild to moderate degrees of nuclear pleomorphism are important reliable features of WDT-UMP' diagnosis.

Chan' study considered the irregularly shaped follicles, presence of sclerosis, dense colloid, necrosis and abortive papillae as minor (subsidiary) criteria, especially if one or more features of the major ones were lacking [9]. We could detect the minor criteria in variable percent in study groups, however, we could not consider them as separate dependable features for diagnosis of WDT-UMP.

In the current study, the necrotic areas were focally seen in the surrounding stroma or within the thyroid nodules. They attributed to several causes; processing artifact at the sites of previous fine needle aspiration cytology or biopsy, secondary to cystic degeneration [9,10], or due to infarction.

We could see focal areas of fibrosis/sclerosis in 66.6% of WDT-UMPs. They were recognized in proximity to lymphocytic thyroiditis or hyperplastic nodules. So we considered them an associated condition rather than pattern of growth for WDT-UMP. Hoffmann and his colleagues confirmed our findings [15].

Additional evidence came from Elsheikh and his colleagues' study; they listed the distorted follicular architectures, fibrosis, dense colloid and abortive follicles as secondary features for the diagnosis of malignancy in follicular lesions of the thyroid as cited by experts [13].

On the basis of the criteria proposed by the Chernobyl Pathologist Group [1], we developed an intimate description of nuclear changes of WDT-UMP including; the absence of nuclear pseudoinclusion, presence of characteristic nuclear groove (89%), nuclear clearing (100%), ovoid nuclei (100%), nuclear enlargement (94.4%), nuclear crowdness (moderate–severe) and nuclear pleomorphism (mild to moderate), either with focal and well developed or diffuse and equivocal distribution. So the nucleus of WDT-UMP has more atypia than follicular adenoma

but less than PTC nucleus. Moreover, minor criteria were considered as alarming subsidiary features (Fig. 5).

We used CK19 as a proven useful marker to confirm the diagnosis especially when the cyto-morphological features were equivocal and did not offer conclusive diagnosis. The distribution and the intensity of CK 19 staining were very helpful for accurate interpretation and differential diagnosis [16,17].

Cytokeratin 19 displayed several patterns of immunoreactivity in the current study;

The classic PTC (18/18) showed strong immunoreactivity of CK19 with distribution more than 95% of tumor cells. While 50% of the cases of follicular variant showed strong staining with distribution more than 50% and the other 50% of cases showed moderate intensity with distribution more than 95% of the tumor. Several authors agreed with us and demonstrated strong, diffuse CK19 immunoreactivity in 80–100% of PTC cases [16,17], while Bukhari and his colleagues' study reported a larger number of cases (60 cases of follicular variant of papillary carcinoma) expressed 4 + (> 95% cells were CK19 positive) [18]. The negative staining of CK19 was considered strong evidence against diagnosis PTC [19].

Our study demonstrated CK19 immunoreactivity in 100% of WDT-UMPs; 50% of cases showed moderate staining of CK19 with distribution more than 50% up to 95% of the tumor. The other 50% of WDT-UMPs showed strong immunoreactivity in 50% of the follicular lesion. Thus, we considered the pattern of immunoreactivity of CK19 in WDT-UMPs varied from moderate diffuse to strong multifocal immunostaining.

Although a few immunohistochemical studies on WDT-UMPs were found in the literature, their results were close to ours. Bukhari & his colleagues and Barut et al. [18,20] demonstrated a significant positivity (strong diffuse) of CK19 in WDT-UMPs; they found wide proportions of the stained cells among 35 cases (ranged from 5% to 95%). However, a lower expression of CK19 was reported by Scognamiglio [21] and by Hoffmann et al. [15]; they recorded that 63–64% of WDT-UMPs expressed CK19.

Many additional studies supported the previous immunohistochemical profile of WDT-UMP and concluded that these lesions might be biologically borderline lesions which represent an early phase of carcinogenesis and precursor lesions of PTC [22–24].

Our study showed a weak focal positivity of CK19 in 2 cases of adenoma and 4 cases of Hashimoto thyroiditis. These findings came in agreement with others [18,25–27] who reported nil or focal positivity of CK19 in follicular adenomas. The follicular cell expression of CK19 within lymphocytic thyroiditis could be explained by the presence of reactive follicular epithelium around areas of degeneration especially at the sites of previous needle biopsy [25].

In our study, the immunostaining profile of CK19 in WDT-UMPs ranged from moderate to strong staining with a proportion score range of 50–95% which is higher than adenoma and less than carcinomas. This came in agreement with Scognamiglio and his colleagues [13] who performed immunohistochemical studies using a panel of four antibodies (HBME-1, GAL3, CK19 and CITED1). They reported 5/11 cases of WDT-UMPs with an intermediate expression profiles between PTC and FA.

In the current study, areas with obvious PTC nuclear features that showed more intense expression of CK19 than the surrounding of less atypical nuclear features in WDT-UMPs, giving an impression of a nodule (with more atypia) inside the nodule (WDT-UMP). The association between PTC nuclear features distribution and the immunoreactivity pattern of CK19 could be explained from the molecular point of view. This is because RET/PTC rearrangements have been restricted to the focal areas of unequivocal nuclear features of PTC, representing an early development of PTC in a pre-existing benign lesion [28].

Although the molecular characterization of WDT-UMP is still under great observer variability, it had distinct molecular features compared to PTC and benign thyroid lesions [3]. Some authors considered the WDT-UMP as an early phase of carcinogenesis (pre-cancerous), borderline and a very low grade malignancy [22].

We concluded that WDT-UMP is an intermediate lesion; differs from adenoma and benign lesions of the thyroid and shares the PTC to certain extent. However, a constellation of both histomorphological features and immunohistochemical CK19 findings can provide reliable features for the diagnosis of WDT-UMP.

More histopathological and molecular studies on bigger number of specimens with long term follow up data are recommended for better understanding of the neoplastic nature of the WDT-UMP thyroid lesions.

Conflict of interest

Author declares no conflict of interest.

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