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Immunohistochemical Expression of MDM2 and p16 in Adipocytic Neoplasms Measuring Ten Centimeters or More in Diameter Among Filipino Patients In a Public Tertiary Hospital From 2017 to 2019*

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ABSTRACT

Introduction. A size of more than 10 cm suggests that a soft tissue tumor might be malignant. Pertinent ancillary diagnostic testing, such as immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH), may be done to confirm the diagnosis. Several studies have shown that size may be a useful criterion in determining which tumors are candidates for further molecular testing. MDM2 and p16 are IHC markers for atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLPS).

Objectives. The primary objective of this study is to determine the proportion of tumors signed out as "lipomas" from 2017 to 2019, and measuring at least 10 cm, that express MDM2 and p16 on IHC and warrant revision as ALT/WDLPS.

Methodology. This is a descriptive, retrospective cohort study in which all lipomas from 2017 to 2019 that measured at least 10 cm were included. The size, age of the patient, and location of each tumor were documented. The slides of all eligible cases were reviewed and immunohistochemically stained for MDM2 and p16. For each case, the intensity and immunoreactivity of each stain were assessed using a modified, four-tier scoring system. Fisher's exact test was used to determine if a significant number of tumors expressed MDM2 or p16.

Results. Thirty (30) cases satisfied the inclusion and exclusion criteria. The average size of these tumors is 15.10 cm. There is no sex predilection. The most common location of these tumors is the extremities. None of the tumors expressed MDM2, and only one case was p16-positive. The case positive for p16 also showed cytologic atypia and variability in cell size, resulting in the revision of its diagnosis from lipoma to atypical lipomatous tumor. The rate of diagnosis revision after slide review and IHC studies is 3.33%.

Conclusion. None of the adipocytic tumors that measured at least 10 cm in diameter and were signed out as lipomas was MDM2 positive, and only one case was p16-positive. Thus, morphology remains the cornerstone in the diagnosis of adipocytic tumors. Careful microscopic evaluation is necessary to establish the diagnosis of malignancy in these tumors. Ancillary tests should only be considered in cases where the pathologic features are equivocal.

Key words: Neoplasms, Adipose Tissue; Lipoma; Liposarcoma; Extremities

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INTRODUCTION

Soft tissue tumors are neoplasms of fat, muscle, peripheral nerves, blood vessels, fibrous tissues, and also tumors with uncertain histogenesis. It is estimated that the annual incidence of benign soft tissue neoplasms is as high as 3000 cases per one million population, in contrast to soft tissue sarcomas, which are reported to have an incidence of about 50 cases per one million population. In a majority of cases, the etiology of most benign and malignant soft tissue tumors is unknown. Although specific genetic abnormalities are found in certain entities, suggesting a familial basis, most tumors appear to arise *de novo*.[1]

Lipomas are among the usual specimens encountered by pathologists in their daily practice. This tumor is the most common soft tissue neoplasm in adults. It usually arises in the subcutaneous tissue of the trunk and extremities. Several morphologic variants of lipomas exist. Some of

these variants have characteristic clinical features. For instance, most lipomas are painless, but angiolipoma is painful, with pain correlating with the degree of vascularity.[2] Spindle cell lipoma and pleomorphic lipoma are commonly found in the subcutis of the upper back, posterior neck, and shoulder region of middle-aged males.[3] Other variants may mimic certain sarcomas, such as chondroid lipoma, which may mimic chondrosarcoma.[2] Conventional lipomas are soft, mostly painless, and easily cured by excision. The prognosis is excellent.[4]

Atypical lipomatous tumors/well-differentiated liposarcomas (ALT/WDLPS) are among the most common malignant soft tissue tumors in adults. Three main subtypes of ALT are recognized: adipocytic (lipoma-like), sclerosing, and inflammatory. These patterns may be seen simultaneously in one lesion. Lipoma-like ALT is the most common subtype. The presence of substantial variation in cell size and nuclear atypia in fat cells or stromal cells should separate lipoma-like ALT from lipoma. In some cases, the atypia may be so focal that thorough sampling of the tumor should be considered and ancillary testing may be warranted.[5]

ALT/WDLPS are locally aggressive tumors that are more common in the 5th to 7th decade of life. They are usually encountered in the proximal lower extremities and in the retroperitoneum. They may recur locally if inadequately excised. The metastatic potential of ALT/WDLPS is nearly zero; however, this increases if the tumor undergoes dedifferentiation.[2,4]

Lipomas do not undergo malignant transformation; however, lipoma-like ALT/WDLPS resembles the former grossly and histologically.[2,6] Oftentimes, histologic criteria are sufficient to diagnose an atypical lipomatous tumor. Problematic cases include tumors measuring more than 10 cm in greatest dimension, lesions with equivocal atypia, recurrent lipomas, "lipomas" located in the retroperitoneum and deep abdominal or pelvic viscera, and cases with worrisome clinical or radiologic features.[7]

The World Health Organization (WHO) states that "all superficial soft tissue lesions measuring >5 cm, and all deep-seated lesions, are statistically likely to be sarcoma."[1] Johnson et al., recommends that all soft tissue tumors be considered malignant until proven otherwise if they have any of the following clinical features: increase in size, size more than 5 cm deep-seated, or painful. A size of less than 5 cm is said to be the best indicator of a benign lump.[8] These recommendations are reiterated by several guidelines on the diagnosis and management of soft tissue tumors.[9-11] Of these, size is the only feature that can be independently determined by the pathologist during gross examination.

Subcutaneous lipomas may occasionally grow beyond 5 cm.[8,12,13] Lipomas growing larger than 10 cm are rare.[14] The number of ALT/WDLPS is significantly higher in cases where the tumor size is >10 cm; therefore, adequate sampling is necessary to rule out ALT/WDLPS.[7,14-18] The College of American Pathologists (CAP) recommends submitting one section per centimeter of maximum dimension for histologic evaluation, but this guideline is not always strictly followed.[19]

Ancillary testing is sometimes necessary to confirm the diagnosis of ALT/WDLPS. Detection of *MDM2* amplification via fluorescent *in situ* hybridization (FISH) is the gold standard in differentiating lipomas from ALT/WDLPS.[15,16,18] Immunohistochemistry (IHC) is used as an alternative test if FISH is not available. MDM2, p16, and CDK4 are three IHC markers that may be used to support the diagnosis of ALT/WDLPS. p16 has the highest sensitivity (96.8%) of the three markers.[20] One hundred percent of ALTs express at least two of these markers.[21]

No definite consensus guidelines exist on when to do FISH or IHCs. Several authors have advocated testing large tumors, i.e.at least 10 cm, deep-seated lesions, and those with equivocal atypia, especially if the sample is limited, e.g., core biopsies.[15-18,22] In some Western centers, a size of at least 10 cm triggers testing for *MDM2* amplification. In one study, for adipocytic tumors that underwent ancillary molecular testing because of a size more than 10 cm, 68 out of 187 tumors (36%) proved to be ALT/WDLPS.[7] In the Philippine General Hospital (PGH), the diagnosis of lipoma relies mainly on gross and microscopic examination. The possibility of an ALT/WDLPS being signed out as a lipoma should therefore be considered in tumors with worrisome clinical features.

This study aims to evaluate the immunohistochemical expression of MDM2 and p16—the two stains for ALT/WDLPS that are currently offered by the Department of Laboratories—among tumors with a size of at least 10 cm that were signed out as "lipomas" in PGH from 2017 to 2019. Specific objectives of this study are: to determine the basic demographic information of eligible cases; to compare the characteristics (size, male:female ratio, age, and location) of eligible cases with those with a final diagnosis of malignant adipocytic tumors from the same time period; to determine the number of lipomas measuring at least 10 cm that express MDM2 or p16 via IHC; to determine if there is a significant difference between the immunohistochemical expression of MDM2 and p16 in adipocytic tumors; and to determine the degree of concordance between the original diagnoses and the diagnoses after slide review and IHC studies.

METHODOLOGY

This study is a descriptive, retrospective cohort study that involves surgical pathology cases signed out as "lipoma" from 2017 to 2019. Prior to the implementation of the study, ethical clearance was secured from the University of the Philippines – Manila Research Ethics Board.

Inclusion and Exclusion Criteria

This study included all adipocytic tumors that were submitted to the Department of Laboratories from 2017 to 2019 with a final diagnosis of "lipoma" or its variants. Furthermore, these tumors fulfilled all of the following conditions: the surgical procedure done to the specimen was at least an excision or resection; the tumor size was at least 10 cm, based on the gross description of the pathology resident and consultant in charge of the case; and no prior ancillary studies, either IHC or FISH, were done on the specimen.

Any of the following criteria excluded a specimen from this study: lipomas from patients with recurrent tumors, at least one of which was eventually signed out as liposarcoma, whether ancillary studies were done or not; lipomas diagnosed on core needle, incision, or wedge biopsies; and cases for which the microscopic slides and/or paraffin blocks could not be retrieved (e.g., slide reviews, missing blocks), or were not fit for further evaluation or testing (e.g., damaged paraffin blocks, minimal residual tissue within paraffin block that was not sufficient for IHC).

Data Collection Procedures

The surgical pathology reports of all soft tissue tumors with a definite histopathologic diagnosis of "lipoma" or one of its variants from 2017 to 2019 were reviewed. All cases that satisfied the inclusion and exclusion criteria were assigned unique code numbers. Data collected from the surgical pathology reports included the age and sex of the patient, location of the tumor as specified by the attending physician, size of the tumor based on the gross description, and the number of sections taken. The hematoxylin and eosin slides of these cases, as well as their corresponding paraffin blocks, were retrieved with the assistance of the staff of the Surgical Pathology Division.

Slide Review

To maintain anonymity, the microscopic slides were given new study-specific code numbers. All slides were evaluated by the principal investigator for the quality of their staining. All slides with poor staining quality were restained with the assistance of the Surgical Pathology Division staff. The investigators evaluated the microscopic slides of all cases for the two features of ALT/WDLPS: 1) presence of focal atypia in either the adipocytes or stromal cells, and 2) heterogeneity of cell size. Suitable paraffin blocks, based on the initial histopathologic evaluation, were submitted for further IHC testing.

Immunohistochemistry Studies

Antibodies against MDM2 (Bio-SB mouse monoclonal antibody BSB-64) and p16 (DB Biotech mouse monoclonal antibody clone R15-A) were used to stain the chosen paraffin blocks. Immunohistochemical staining of the slides were performed as per the manufacturer's protocols, as follows:

MDM2

Formalin-fixed paraffin-embedded tissues were cut and fixed on positively charged slides, followed by air-drying for 2 hours at 58°C. The tissues were deparaffinized, dehydrated, and rehydrated. Tissues were subjected to heat-induced epitope retrieval (HIER) using a suitable retrieval solution. Tissues were heated using water bath method. After heat treatment, slides were transferred in ImmunoDNA Retriever EDTA to room temperature. Automated staining methods were performed according to the instrument manufacturer's instructions. Between each step of IHC staining, slides were washed with ImmunoDNA washer solution. Slides were mounted for observation.

p16

Formalin-fixed paraffin-embedded tissues were deparaffinized, dehydrated, and rehydrated. Endogenous peroxidase was blocked by incubating the tissue in 3% hydrogen peroxide for 10 minutes. The slides were immersed in Tris-EDTA buffer at pH 9.0 and incubated at 95-97°C in a water bath for 25 minutes. The slides were allowed to cool for 15 minutes. The slides were stained with p16 using automated staining methods. The slides were then mounted for observation.

Interpretation of Immunohistochemical Expression of MDM2 and p16

The IHC slides were reviewed independently by the principal and co-investigators. MDM2 and p16 are nuclear stains; therefore, inconsistent staining patterns, e.g., cytoplasmic or membranous only, were considered as negative results. Nonspecific staining patterns were also documented but were still considered negative.

Each tumor was assessed using a modified version of the method by Thway et al. This method consists of a four-tier scoring system based on the intensity of reaction and immunoreactivity.[20] After quantification, a positive result was given if 1) there was at least moderate intensity for tumors with at least 11% of cells stained (patchy to diffuse), or 2) there was strong staining if only 1% to 10% of cells are stained (focal). Tumors with weakly staining nuclei and a focal pattern of staining were considered negative.[23] Table 1 presents a summary of this method.

In cases where the immunostains gave a positive result, the case was independently reviewed by the investigators and referred to a bone and soft tissue pathologist for concurrence.

Data Analysis

Microsoft Excel was used to tabulate the data on patient demographics (age, sex, and location of the tumor) and tumor characteristics (presence of atypia, cell size heterogeneity, and expression of MDM2 and p16). An independent statistician was consulted for data analysis. Fisher's exact test was used to determine if there was a significant number of tumors that expressed MDM2 or p16.

RESULTS

Demographics of Patients with Adipocytic Tumors in a Tertiary Hospital from 2017 to 2019

A total of 938 resected adipocytic tumors were submitted to the PGH Department of Laboratories for pathologic evaluation, including IHC studies, from 2017 to 2019. Of the 904 adipocytic tumors that were diagnosed as benign (96.38%), 36 cases satisfied the inclusion and exclusion criteria. The other 868 tumors were either benign, measured less than 10 cm, already diagnosed as malignant, or sampled using incision or core biopsy procedures. Of the 36 cases that satisfied the inclusion and exclusion criteria, the paraffin blocks of six cases, all from 2017, were not retrievable even after a diligent search by the Surgical Pathology staff; therefore, only 30 cases were included in the study. These were processed for IHC studies with MDM2 and p16.

The categories for tumor location follow the recommended anatomic primary site distribution of the AJCC Cancer Staging Manual, 8th edition.[24] The most common location

Table 1. Four-tier system for assessment of staining patterns*

Intensity	Reactivity	%	Interpretation (Intensity + Reactivity)	
Absent	Absent	0	No staining	Negative
Weak	Focal	1-10	Weak Intensity, Focal Immunoreactivity	
Moderate	Patchy	11-50	Moderate Intensity, Patchy to Diffuse Immunoreactivity	Positive
Strong	Diffuse	>50	Strong Intensity, **Any** Reactivity (Focal, Patchy, Diffuse)	

*The system is based on the 1) intensity and 2) reactivity of the stains. This system applies to both MDM2 and p16. Interpretation is done on atypical stromal or adipocytic cells with nuclear staining. If cells other than stromal or adipocytic cells show some degree of staining, the interpretation is *nonspecific*, which is equivalent to *negative*.

Table 2. A comparison between the eligible cases and the liposarcomas that were evaluated by the Department of Laboratories from 2017 to 2019

Characteristics	Lipomas Measuring at least 10 cm*	Malignant Adipocytic Tumors**
Number (N)	30	34
Average Size	15.10 cm (Median: 14 cm; Range: 10 to 40 cm)	18.70 cm (Median: 19 cm; Range: 4 to 31 cm)
Number of Sections taken for Microscopic Evaluation***	9 sections per 10 cm	8 to 9 sections per 10 cm
Male to Female Ratio	1:1	1:1.3
Mean Age (in years)	40	54
Most common location	Extremities	Extremities
Other locations	Trunk wall, head and neck	Retroperitoneum, abdominal and thoracic visceral organs, trunk wall, head and neck
Most common diagnosis	Lipoma****	ALT/WDLPS (14), Myxoid Liposarcoma (9), DDL (7)

* This column includes only the cases that had available paraffin blocks for further testing.
** All malignant adipocytic tumors from 2017 to 2019 were included for comparison.
*** This refers to the average number of tissue sections taken by the pathology resident during specimen grossing, as indicated in the surgical pathology report.
**** Part of the inclusion criteria is to have a diagnosis of lipoma.

is the extremities (13; 43.33%), followed by the trunk wall (12; 40%) and the head and neck region (5; 16.67%). None of the tumors have a visceral or retroperitoneal location (i.e., abdominal, pelvic, and retroperitoneal organs).

On the other hand, out of the 938 cases, 34 cases were signed out as malignant adipocytic tumors, comprising 3.62% of all tumors. Sixteen of these cases were established as liposarcomas through further IHC studies. The most common type of liposarcoma is ALT/WDLPS (14 cases; 41.18%). The most common location for malignant adipocytic neoplasms is the extremities (15 cases; 44.12%), followed by the retroperitoneum (8 cases; 23.53%), abdomen and thoracic visceral organs (5; 14.71%) trunk wall (4; 11.77%), and the head and neck region (2; 5.88%).

Table 2 shows the comparison between the eligible cases and the malignant adipocytic tumors from 2017 to 2019.

Pathologic Findings of Adipocytic Tumors Measuring at Least 10 Centimeters

The thirty lipomas that qualified for this study had an average size of 15.10 cm (95% CI: 13 to 17.2), ranging from 10 cm to 40 cm, and a median size of 14 cm. All adipocytic tumors were signed out as lipomas or one of its variants; one was described as having fat necrosis, while another tumor was signed out as osteolipoma. The osteolipoma case was also the largest of the eligible tumors, measuring 40 cm in gross tumor dimension based on the gross description.

On microscopic examination, eight lipomas presented with cellular atypia, including the lipoma with fat necrosis and osteolipoma. Five of these tumors were located in the extremities, including the osteolipoma, which was located in the right thigh. Except for the osteolipoma, which had a moderate degree of stromal cell atypia, the atypia seen in these tumors were at most mild, i.e., minimal variation in nuclear size and absence of hyperchromasia. Most of the tumors did not present with variability in cell size, except for the lipoma with fat necrosis, which showed mild variability, and the osteolipoma, which had moderate variation in cell size.

The collected data for all 30 cases included in this study are summarized in Table 3.

Expression of MDM2 and p16 in Adipocytic Tumors

None of the lipomas were positive for MDM2 via IHC using the modified method by Thway et al. Four lipomas presented with weak, focal staining with MDM2, but these were not sufficient to be interpreted as positive. Of these four lipomas, three also presented with mild to moderate cellular atypia, as described, including the osteolipoma. Similarly, all but one of the specimens did not present with positive p16 immunostaining. Because most of the IHC results for MDM2 and p16 were negative, Fisher's exact test could not be performed to determine if there is any significant difference between the expression of the two stains.

However, a significant number of cases (n=19; p-value=0.035; α=0.05) presented with nonspecific staining for p16, in which nuclear and cytoplasmic staining was observed in cells or tissues other than the atypical stromal cells. These include the cytoplasm and membranes of benign adipocytes, inflammatory cells, endothelial cells, and areas of fat necrosis. Figure 1 shows representative photomicrographs of the various staining patterns observed with p16 immunostain.

The one case that presented with strong, diffuse nuclear positivity with p16 in the atypical stromal cells is the osteolipoma case. The results for this case were interpreted as p16-positive. After consultation with other pathologists, including a bone and soft tissue subspecialist, the diagnosis was revised to ALT/WDLPS. Therefore, the rate of diagnosis revision after p16 IHC and case review is 3.33% (1/30). Figure 2 shows representative photomicrographs of the aforementioned case.

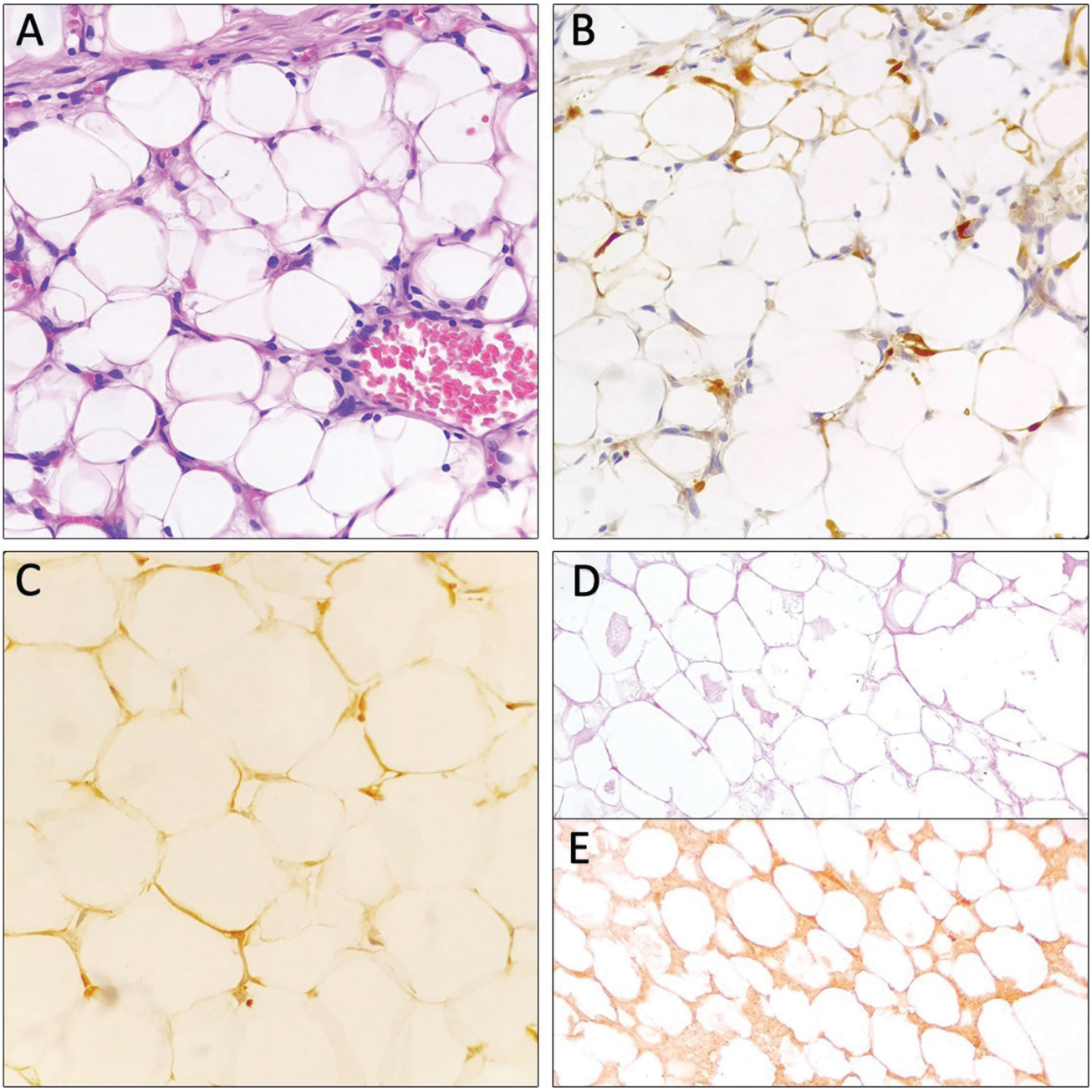

Figure 1. Representative photomicrographs of the different lipoma cases reviewed. **(A)** Lipomas are composed of mature adipocytes with or without the presence of other mesenchymal derivatives, such as fibrous tissues and blood vessels. The nuclei of the adipocytes are small and pushed to the periphery. In this photomicrograph, the adipocytes are roughly the same size, while the prominent nuclei belong to endothelial cells (H&E, 400x). **(B)** to **(E)** p16 may present with non-specific staining patterns. **(B)** The nuclei of endothelial cells are nonspecifically stained with p16 (HRP, 400x); **(C)** Occasionally, the peripheral cytoplasm and cellular membranes of adipocytes show moderate staining with p16 (HRP, 400x); **(D)** Fat necrosis is characterized by the lack of nuclear staining, adipocyte dropout, and cytoplasmic vacuolization (H&E, 100x); **(E)** p16 shows diffuse, moderate cytoplasmic staining in areas of fat necrosis (HRP, 100x).

Table 3. Tumor Characteristics, Pathologic Features, and Immunohistochemistry Profiles*

Study number	Age	Sex	Size	Sections	Adequacy of Sections (%)	Original Diagnosis	Specific Location	General Location	Atypia	Variability of Cell Size
MCM-01	31	F	14.5	5	34.48	Lipoma	Back	Trunk	N	N
MCM-02	44	F	12.3	16	130.08	Lipoma	Shoulder, right	Extremity	N	N
MCM-03	27	F	10	8	80.00	Lipoma	Upper back	Trunk	N	N
MCM-04	68	M	10.5	8	76.19	Lipoma	Shoulder, left	Extremity	N	N
MCM-05	60	M	13	10	76.92	Lipoma	Back	Trunk	N	N
MCM-06	41	M	11.5	20	173.91	Lipoma	Neck, right	Head and neck	Y (mild)	N
MCM-07	43	M	13	13	100.00	Lipoma	Shoulder, right	Extremity	N	N
MCM-08	25	M	14	8	57.14	Lipoma	Upper back	Trunk	N	N
MCM-09	44	M	12	8	66.67	Lipoma	Axilla, left	Extremity	N	N
MCM-10	40	F	20	20	100.00	Lipoma with fat necrosis	Lower back	Trunk	Y (mild)	Y (mild)
MCM-11	54	F	12	10	83.33	Lipoma	Back	Trunk	N	N
MCM-12	40	F	13	9	69.23	Lipoma	Back	Trunk	N	N
MCM-13	43	F	14.5	15	103.45	Lipoma	Gluteal area	Extremity	N	N
MCM-14	53	F	16.2	19	117.28	Lipoma	Flank, right	Trunk	N	N
MCM-15	72	M	14.5	17	117.24	Lipoma	Trunk	Trunk	N	N
MCM-16	44	F	27.5	28	101.82	Lipoma	Thigh, left	Extremity	Y (mild)	N
MCM-17	19	F	14	13	92.86	Lipoma	Lower abdomen	Trunk	N	N
MCM-18	79	F	40	6	15.00	Osteolipoma	Thigh, right	Extremity	Y (moderate)	Y (moderate)
MCM-19	10	M	20	25	125.00	Lipoma	Shoulder, right	Extremity	N	N
MCM-20	61	F	12	10	83.33	Lipoma	Arm, right	Extremity	Y (mild)	N
MCM-21	58	F	11	11	100.00	Lipoma	Back	Trunk	N	N
MCM-22	46	M	15	16	106.67	Lipoma	Thigh, left	Extremity	Y (mild)	N
MCM-23	34	M	13	16	123.08	Lipoma	Inguinal area, left	Extremity	N	N
MCM-24	3	M	17.5	16	91.43	Lipoma	Neck, anterior	Head and neck	Y (mild)	N
MCM-25	4	F	15	16	106.67	Lipoma	Chest wall	Trunk	N	N
MCM-26	66	F	11	12	109.09	Lipoma	Supraclavicular area	Head and neck	N	N
MCM-27	47	M	16	11	68.75	Lipoma	Occipital area	Head and neck	N	N
MCM-28	12	M	15	15	100.00	Lipoma	Arm, left	Extremity	Y (mild)	N
MCM-29	2	M	15	12	80.00	Lipoma	Thigh, left	Extremity	N	N
MCM-30	38	M	10	1	10.00	Lipoma	Occipital area	Head and neck	N	N

*This summary table presents the tumor characteristics, pathologic features, and staining patterns with MDM2 and p16 of the cases that were included in the study.

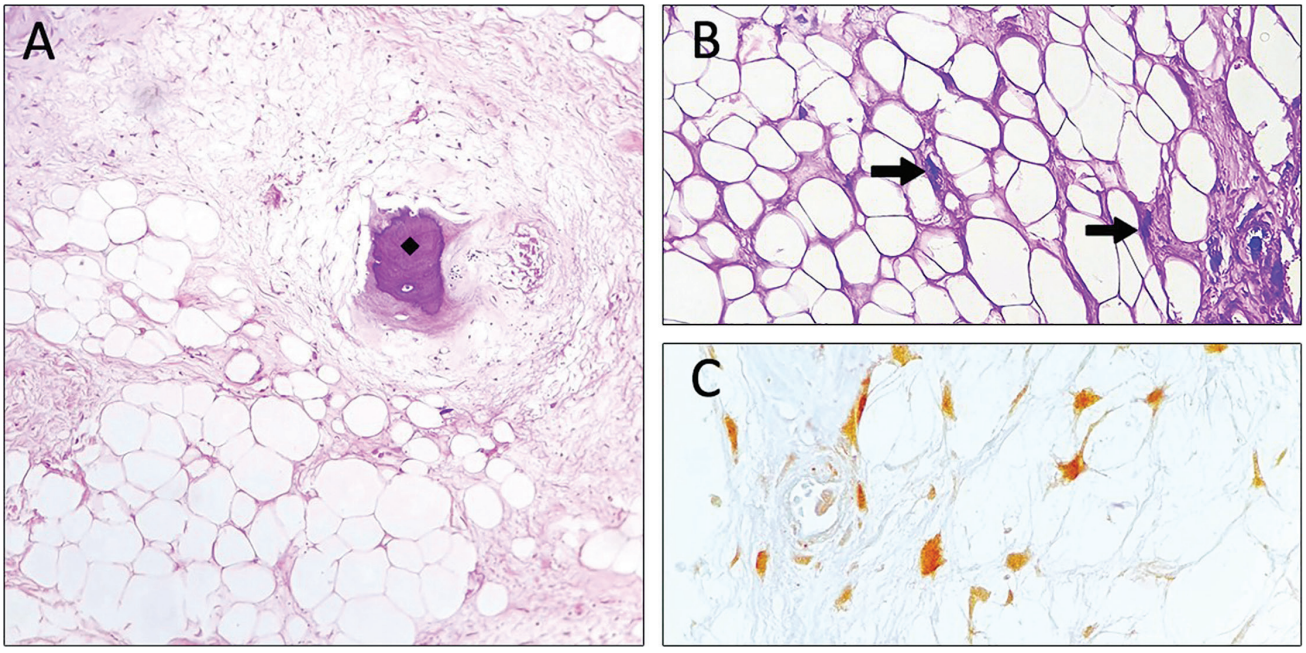

Figure 2. Atypical lipomatous tumor with osseous metaplasia. **(A)** This tumor from the thigh of a 70-year-old female was initially signed out as an osteolipoma due to the presence of mature lamellar bone (◆) admixed with lipomatous areas (H&E, 40x); **(B)** On slide review, variability in cell size was noted, as well as atypical stromal and adipocytic cells (→; H&E, 100x); **(C)** Immunostaining with p16 showed strong, diffuse, nuclear staining in the atypical stromal cells (HRP, 400x).

MDM2			p16			Revised Diagnosis
Intensity	Immunoreactivity	Interpretation	Intensity	Immunoreactivity	Interpretation	
Absent	Absent	Negative	Moderate	Diffuse	Non-specific	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Moderate-Strong	Patchy-Diffuse	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Diffuse	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Weak	Focal	Non-specific	No Revision
Absent	Absent	Negative	Moderate	Focal	Non-specific	No Revision
Absent	Absent	Negative	Moderate	Focal	Non-specific	No Revision
Weak	Focal	Negative	Strong	Diffuse	Positive	Atypical Lipomatous Tumor
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Weak	Focal	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Moderate-Strong	Focal	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Weak	Focal	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Weak	Focal	Negative	Weak-Moderate	Focal	Non-specific	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision

DISCUSSION

Size as Basis for the Immunohistochemical Evaluation of Adipocytic Tumors

Current practices in the pathologic evaluation of soft tissue tumors make use of size primarily for staging purposes.[24] ALTs are suspected in the setting of a large, slow-growing tumor, especially when located in the proximal extremities or the trunk. ALT/WDLPS in the retroperitoneum or thoracoabdominal cavity may go unnoticed until it reaches a size of 20 cm.[5] All lipomatous tumors with a retroperitoneal or visceral location evaluated in our institution were given a diagnosis of ALT/WDLPS.

The size cut-off for suspecting an ALT/WDLPS varies, ranging from 5 cm to 15 cm.[5,7,15,17,22,25] Ten centimeters was chosen as the cut-off for this study because most of the previous studies reviewed utilized this size. This is also the cut-off used by Clay et al., and Thway et al., to prompt recommendation for further tests for *MDM2* amplification, by FISH or surrogate IHC markers.[15,22] The location of the tumor also affects the cut-off for size. For masses located in the deep soft tissues and the retro-peritoneum, most authors agree that 10 cm is a reasonable cut-off for suspecting ALT/WDLPS.[26,27]

In this study, there is a difference in the average size between the eligible lipomas and the diagnosed cases of liposarcomas (15.10 cm vs. 18.70 cm); however, this study demonstrates that size should not be used as the sole basis for reflex testing with MDM2 and p16 IHC. Most of the eligible cases, even though they measure more than 10 cm, demonstrated no or minimal atypia and cell size heterogeneity. The lack of atypia correlates well with the absence of staining with MDM2 in all cases and the nonspecific or absent staining with p16 in 29 out of 30 cases. Therefore, in the clinical setting of a large mass located in the proximal extremities, correlation with cellular atypia and cell size heterogeneity on microscopic evaluation is of utmost importance and remains the foundation in diagnosing lipoma-like ALT/WDLPS. In one study involving 405 extremity-based tumors, a cut-off of 15 cm was recommended for doing ancillary tests for tumors without diagnostic cytologic atypia.[28] In relation to the results of this study, this cut-off might be more suitable as a basis for further molecular testing of tumors with neither cytologic atypia nor heterogeneity in cell size.

Nonetheless, a large size remains a vital clue to the diagnosis of ALT/WDLPS. The sole case that had its diagnosis revised from osteolipoma to atypical lipomatous tumor measured 40 cm, which was an outlier via the interquartile method (IQR=3; upper bound=19.50 cm). The tumor also showed strong, diffuse, nuclear positivity for p16 and weak, focal, nuclear staining for MDM2. A review of the pathology report of the case revealed that only six sections were taken. Cases such as this will benefit from the CAP recommendation of taking one section per centimeter, allowing a more thorough microscopic evaluation for cellular atypia and heterogeneity of cell size, which are both present in the case.

One other caveat for this case is that an atypical spindle cell/pleomorphic lipomatous tumor (ASPLT) should

be included in its differential diagnoses. ASPLT and ALT have overlapping features, including a persistently enlarging mass, predilection for the thigh, presence of atypical spindle cells and adipocytes, heterologous differentiation, and positivity for p16. However, ASPLT does not demonstrate *MDM2* amplification, either by FISH or IHC. The case demonstrated focal, weak staining with MDM2. Nevertheless, along with the revised diagnosis, a recommendation was made to perform *MDM2* FISH to fully rule out an ASPLT. Differentiating ASPLT from ALT is important because the risk of recurrence for ASPLT is low (10-15%) even if the lesion incompletely excised, and there is no documented risk for metastasis or dedifferentiation.[29,30]

The Limited Utility of Immunohistochemistry Studies Using MDM2

The characteristic cytogenetic aberration seen in ALT/WDLPS is a supernumerary ring and/or giant marker chromosome. Although lipomas and ALT/WDLPS affect the same chromosomal region, lipomas are characterized by translocations of 12q13-15. In ALT/WDLPS, there is amplification of the genes located in 12q13-15.[31] Among the genes amplified in the 12q13-15 region are the oncogenes *MDM2* and *CDK4*. The gene product of *MDM2* is an inhibitor of p53, which is important in cell cycle arrest, senescence, and apoptosis. On the other hand, the gene product of *CDK4* mediates the progression of the cell cycle through the G1 phase, eventually leading to cellular proliferation. In the presence of increased CDK4 expression, p16 is upregulated to perform its inhibitory function.[32]

Initial studies on the sensitivity and specificity of MDM2 showed promising results for the diagnosis of ALT/WDLPS and DDL, with a sensitivity and specificity of 97% and 92%, respectively.[31] Succeeding studies comparing the performance of MDM2 IHC with *MDM2* FISH showed that the latter is superior in detecting ALT/WDLPS. As such, FISH for *MDM2* amplification remains the *prima facie* evidence for well-differentiated and dedifferentiated liposarcomas, with a sensitivity and specificity of 92-94% and 96-100%, respectively.[33] The cost of FISH may deter patients from availing it; therefore, IHC with known ALT markers may still be considered as an alternative test to establish the diagnosis of ALT/WDLPS.

The results of the study showed that all tumors had a negative result for MDM2 IHC; however, the absence of staining does not entirely preclude the diagnosis of ALT. Of note, four of these tumors showed weak, focal staining with MDM2. Three of these tumors showed mild to moderate cytologic atypia. One tumor also showed a positive result for p16, with its diagnosis ultimately being revised from osteolipoma to ALT. The latter finding suggests that weak, focal staining with MDM2 might be demonstrated in some ALT cases; however, in line with the results of previous studies, it might be more prudent to use at least two IHCs markers like CDK4 and p16 in order to clinch the diagnosis.[31,34] Although CDK4 is not available locally, this IHC has a reported sensitivity and specificity of 86% and 89%, which are less than those of p16 but higher than those of MDM2.[20]

The results of the study also suggest that in general, large lipomas with minimal or no atypia and without cell size variability might benefit less from further testing with IHCs. FISH remains an option should there be a strong suspicion for a malignant lesion on clinical grounds, especially in cases with limited material for ancillary tests, e.g., core needle biopsies.[16]

Nonspecific Staining with p16: A Potential Pitfall

Several studies have indicated that p16 can be used as another marker to differentiate ALT/WDLPS from deep-seated lipomas and lipomas with equivocal atypia.[18,20,35,36] One study even showed that p16 is more sensitive than MDM2 and CDK4. The combination of p16 and CDK4 is more sensitive than the combination of either with MDM2.[20] Only one case showed a definite positive result for p16 in this study, as previously discussed. This case, which had its diagnosis revised from osteolipoma to ALT/WDLPS, showed strong and diffuse staining for p16. This staining pattern is consistent with the recommended criteria by several authors for a positive p16 interpretation.[20,35]

A significant number of lipomas (19/30; $p=0.035$) exhibited nonspecific staining with p16, which is characterized as staining of any intensity and any localization, i.e., nuclear, membranous, or cytoplasmic in non-atypical stromal cells. Nonspecific staining was observed in endothelial cells, cell membrane and cytoplasm of mature adipocytes, inflammatory cells, and areas of fat necrosis. This is in line with the findings of previous studies, in which they noted that lipomas with secondary changes have a propensity to stain nonspecifically with p16.[35,37,38] Therefore, when using p16 for the diagnosis of ALT/WDLPS, careful interpretation is warranted in order to avoid misinterpretation of nonspecific staining patterns.

Because of nonspecific staining, it is imperative to do p16 along with another ALT marker. Using a panel of markers is also useful in the setting of DDLs, because other malignancies may be positive for p16. These tumors include leiomyosarcoma, undifferentiated pleomorphic sarcoma, desmoid tumors, endometrial stromal sarcomas, sarcomatoid carcinomas, and gastrointestinal stromal tumors.[36]

Evaluation of Adipocytic Tumors: Surgical Pathology Practice Recommendations for a Tertiary Government Institution

Confirming the diagnosis of an ALT/WDLPS is beneficial for patients. In the setting of a lipoma-like ALT, the patient may benefit from closer surveillance and appropriate surgical intervention, e.g., marginal excision. The latter is particularly important because marginal resection is indicated for ALTs to avoid recurrence and dedifferentiation, and to reduce the morbidity associated with wide resection.[39] Even in DDLs with no well-differentiated component, the results of IHC or FISH can help identify the possible lineage of a tumor. This is important in determining if other treatment modalities, such as chemotherapy and radiotherapy, should be employed. Based on the preceding discussion, the following guidelines are recommended to maximize the pathologic evaluation of adipocytic tumors:

1. Size should not be used solely as a trigger for reflex IHC testing, especially in the absence of cytologic atypia and cell size heterogeneity.
2. One section per centimeter should be taken during the gross examination of lipomatous tumors, especially for extremity-based masses with a size of at least 15 cm, or those located in suspicious anatomic primary sites such as the retroperitoneum.
3. For lipoma-like ALT/WDLPS, careful evaluation for stromal atypia and heterogeneity of cell size should be done before considering ancillary tests. If the atypia is equivocal, FISH for *MDM2* amplification is preferred over IHCs.
4. When evaluating the immunohistochemical expression of MDM2, a negative IHC result does not entirely rule out a malignancy.
5. For immunostaining with p16, careful evaluation must be done to exclude the possibility of nonspecific staining.
6. If, for socioeconomic reasons, *MDM2* FISH is not an option, and ALT/WDLPS is a strong consideration, a panel of at least two liposarcoma markers should be requested to overcome their respective limitations.

CONCLUSION

None of the adipocytic tumors that measured at least 10 cm in diameter and were signed out as lipomas was MDM2 positive, and only one case was p16-positive. In contrast to the malignant adipocytic tumors, these lipomas are smaller, have no sex predilection, and are not seen in the retroperitoneum and thoracoabdominal viscera. Expression of p16 and MDM2 on IHC was mostly negative, precluding the determination of any significant difference between the immunohistochemical expression of MDM2 and p16. The diagnosis rendered based on morphological evaluation alone remained unchanged in the vast majority of cases even after immunohistochemical studies. The rate of diagnosis revision after slide review and IHC studies is 3.33%, indicating a high degree of concordance between the original and reviewed diagnoses.

As this study suggests, size alone should not automatically trigger further testing with either IHC or FISH. Morphology remains the cornerstone in the pathologic diagnosis of adipocytic tumors. A thorough gross examination should be done for larger tumors, ensuring a sufficient number of sections for careful microscopic examination. Correlation with clinical features, such as tumor location, is also helpful to establish the diagnosis. Only in cases where the histopathologic features are equivocal should ancillary tests be considered. *MDM2* FISH is preferred over IHCs; however, a panel composed of MDM2 and p16 may be considered if FISH is not available or accessible.

RECOMMENDATIONS

To fully evaluate IHCs as an acceptable alternative to FISH, the following recommendations are made:

1. Further studies with a larger sample size, which includes both benign and malignant adipocytic tumors, may be done to better assess the correlation between MDM2 and p16 expression;
2. A prospective study on how the immunohistochemical expression of liposarcoma markers correlate with other clinical features (e.g., pain, rapid growth) may be pursued;
3. Studies using p16 on established cases of liposarcomas, whether ALT or DDL, may be done to determine its utility as an alternative marker to FISH; and
4. The sensitivity and specificity of IHC studies with MDM2 and p16 vis-à-vis FISH for established cases of liposarcomas in the local setting may be explored.

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