ORIGINAL ARTICLE

DIAGNOSTIC UTILITY OF FISH FOR MDM2 IN ADIPOCYTIC NEOPLASMS

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Background: The 2013 World Health Organization (WHO) classification of soft tissue and bone tumours recognizes benign entities such as lipoma and four major liposarcoma subtypes: atypical lipomatous tumour/well differentiated liposarcomas (ALT/WDL), dedifferentiated liposarcomas (DDL), myxoid liposarcoma and pleomorphic liposarcoma. This classification of atypical and malignant adipocytic tumours has evolved significantly over the past few decades owing to contributions from cytogenetics, molecular genetics and Immunohistochemical correlates. Most ALT/WDLs can be diagnosed on histology; however, some of the biopsies may be underdiagnosed due to focal atypia or limited nature of tissue for the biopsy. Fluorescence in situ hybridization (FISH) for MDM2 (located at 12q14-15) gene amplification has emerged as gold standard for diagnosis in cases with limiting histological factors. **Methods:** We studied MDM2 amplification by FISH in 55 such problematic adipocytic tumours with overlapping morphological features and a retrospective analysis was made against their corresponding histological features. **Results:** MDM2 amplification correctly identified 11 of 17 ALT/WDLs (64.71% concordance) and 8 of 10 Lipomas (80% concordance). We were able to differentiate liposarcomas from other high grade sarcomatous lesions and sub-classified these lesions into pleomorphic and dedifferentiated types. **Conclusion:** FISH for MDM2 amplification should be used as a gold standard in adjunction with morphology and immunohistochemistry for problematic adipocytic neoplasms

Keywords: FISH; MDM2 amplification; Lipoma; Liposarcoma.

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INTRODUCTION

Adipocytic neoplasms are commonly encountered soft tissue tumours in the routine histopathology practice. These tumours are seen occurring in almost every age and anatomical site.[1]

The 2013 World Health Organization (WHO) classification of soft tissue and bone tumours recognizes benign entities such as lipoma and four major liposarcoma subtypes: atypical lipomatous tumour/well differentiated liposarcoma (ALT/WDL), dedifferentiated liposarcoma (DDL), myxoid liposarcoma and pleomorphic liposarcoma. This classification of atypical and malignant adipocytic tumours has evolved significantly over the past few decades owing to contributions from cytogenetics, molecular genetics and immunohistochemical correlates.[2] Most adipocytic neoplasms can be diagnosed on histology, however, some of the biopsies may be problematic due to presence of focal atypia on morphology, deeper or retroperitoneal location, large size, worrisome radiological features or scant amount of tissue available for evaluation.[3] Fluorescence in situ hybridization (FISH) for murine double minute 2 (MDM2) gene located at 12q13-15 amplification has emerged as a useful ancillary tool and a gold standard for diagnosis in such cases with limiting histological and clinical features.[4–6] It is also used to distinguish (DDL) from other soft tissue sarcomas.[7–8]

Both, ALT/WDLs and DDLs harbour a characteristic supernumerary ring and giant marker chromosome composed of amplified MDM2 and CDK4 gene sequences.[9] As much as it helps evaluation of challenging adipocytic neoplasms, MDM2 amplification is not unique to these tumours. It has also been associated with some other soft tissue sarcomas such as osteosarcomas, myxofibrosarcomas and malignant peripheral nerve sheath tumours (MPNST).[10–21] We investigated the diagnostic utility of MDM2 gene amplification by FISH in various problematic adipocytic neoplasms with indecisive histological and clinical features submitted in our institution from year 2017–2020. Objective of the study was to assess diagnostic utility of FISH for MDM2 in differentiating ALT/WDs and DDLs from other problematic benign and malignant adipocytic neoplasms. It was a retrospective observational study.

MATERIAL AND METHODS

Full texts of Previously published literature addressing diagnostic utility of FISH for MDM2 were obtained and systematically reviewed via Ovid, Higher Education Commission National Digital Library, BioMed central, PubMed and Google scholar. An approval from Shaukat Khanum Memorial Cancer Hospital and Research Centre's internal review board was obtained and 55 Resection and incisional biopsies on which FISH for

MDM2 was performed were retrospectively identified using the institution's database from 2017 to 2020.

The inclusion criteria incorporated all the incisional and excisional biopsies of adipocytic tumours along with cases referred from outside institutes for review submitted in Shaukat Khanum Memorial Cancer Hospital and Research Centre possessing any one of the following features:

a) Equivocal atypia on histology
b) Lesion size ≥ 10 cm
c) Deep and retroperitoneal location
d) High grade Spindle cell morphology with immunohistochemical evidence of adipocytic differentiation
e) Worrisome radiological features

The exclusion criteria included specimens with poor fixation and processing artifacts. These cases were identified by microscopic evaluation and showed autolysis precluding optimal cytomorphological evaluation of the tissue and were excluded from the study. Haematoxylin and eosin-stained slides of 4–5-micron thick sections were prepared using Leica Peloris for processing, Thermo Histostar for Embedding, Leica RM 2245 for microtomy, Leica ST 5020 for staining and Leica CV 5030 for coverslips. An Olympus 75 microscope was used for assessment of morphology of tumour and each case was independently evaluated by two experienced pathologists of our institute prior to evaluation of FISH results. MDM2 FISH was performed on formalin fixed paraffin embedded tissue with commercially available Vysis MDM2/CEP12 FISH Probe Kit.[6] A Dual colour probe designed was used to detect copy number of LSI MDM2 probe target located on chromosome 12q15. Data obtained from all 55 Cases was Statistically evaluated using IBM SPSS Statistics 20.

RESULTS

Data collected from 55 cases was analysed. The age range was 21–74 years with a mean of 45.81 years. 28 subjects were males and 27 were females. Age and size range of ALT/WDL and lipomas is given in table-1. The cases were divided into three major categories. In the first category, concordance of FISH MDM2 was analysed against their histological opinions to differentiate between lipomas (including subtypes) and ALT/WDLs. In second category, other high-grade sarcomas, spindle cell sarcomas and undifferentiated malignant neoplasms were analysed for FISH MDM2 where liposarcoma was considered as a differential diagnosis due to location, mixed morphology or equivocal immunohistochemical features. Third category included cases in which FISH MDM2 was performed to differentiate between pleomorphic and dedifferentiated liposarcoma.

Twenty-seven cases were included in the first category, 17 of which were histologically ALT/WDL and 10 were Lipomas. Eleven of 17 cases (64.71%) with morphology suggestive of ALT/WDL showed MDM2 amplification by FISH whereas, only 2 of 10 cases (20%) showing morphology consistent with lipoma showed MDM2 amplification rendering a final diagnosis of liposarcoma. In both these cases FISH for MDM2 was recommended because of retroperitoneal location and greater than 10 cm tumour size. (Table-2)

Twenty-four cases were included in the second category which included histologically high grade sarcomas, undifferentiated malignant neoplasms and spindle cell sarcomas. MDM2 was amplified in 13 cases leading to a final diagnosis of dedifferentiated liposarcomas. In 11 cases, MDM2 gene amplification was not detected leading to a final integrated diagnosis of Undifferentiated spindle cell sarcoma in 6 cases, leiomyosarcoma in 4 cases and synovial sarcoma in 1 case {supported by FISH for t(X:18)}. (Table-3)

There were 4 cases in the third category, 2 (50%) of which were histologically suggestive of pleomorphic liposarcoma and 2 (50%) were suggestive of dedifferentiated liposarcoma. MDM2 was not amplified in any of these cases rendering a diagnosis of pleomorphic liposarcoma in all these cases (50% concordance ratio).

Table-1: Comparison of tumour size and site with MDM2 amplification-assisted diagnoses

Lipoma		**ALT/WDL**	
Tumour Size (cm)	Number of cases	Tumour Size (cm)	Number of cases
0–10	3	0 to 10	4
10–20	3	10 to 20	3
20+	1	20+	5
Mean	11.16	Mean	16.5
Range	3 to 28	Range	3 to 26

Table-2: Category 1: Lipomatous lesions (lipoma and ALT/WDL) and their correlation with FISH MDM2

Histological Opinion	**n**	**FISH MDM2 status**		**Concordance ratio**
		Negative	Positive	
Atypical lipomatous tumour/well differentiated liposarcoma	17	6	11	64.71%
Lipoma	10	8	2	80%

Table-3: Statistical breakdown of cases in category 2

MDM2 status	Number of cases (24)	FISH integrated diagnosis
Amplified	13	Dedifferentiated liposarcoma
Not amplified	11	Undifferentiated spindle cell sarcoma (6)
		Leiomyosarcoma (4)
		Synovial sarcoma (1)

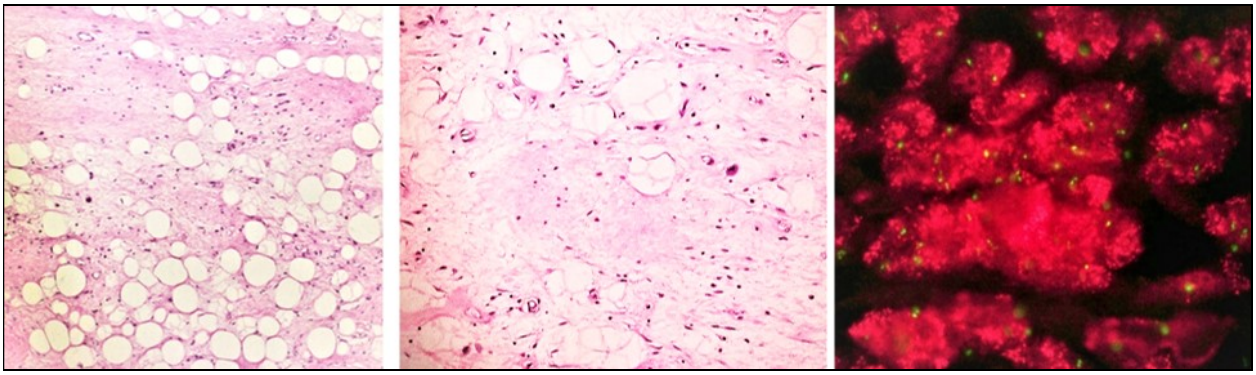

Figure-1: H&E section of a lipomatous tumour showing mature adipocytes and few lipoblasts and unequivocal cytological atypia (left). FISH analysis showing amplified target MDM2 signals in red against centromere region in green. Target/Centromere ratio >2 in this case is diagnostic of MDM2 amplification.

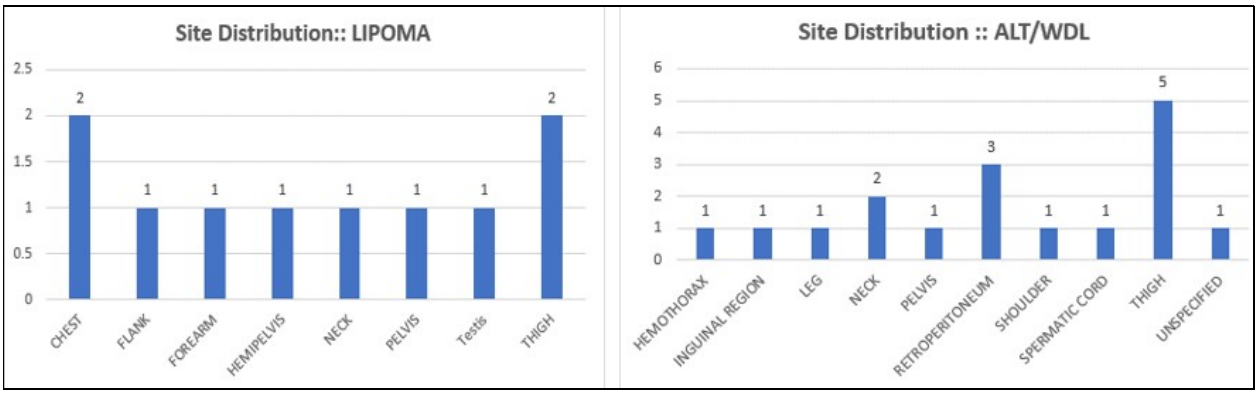

Figure-2: Site distribution

DISCUSSION

Most lipomas can be differentiated from liposarcoma comfortably on the basis of H&E morphology. However, problematic lipomatous tumours are often encountered in soft tissue pathology posing difficulties and such tumours are challenging to diagnose.[21–27] Lipoblasts and cellular atypia may not be present in ALT/WDLs, and alternatively, various subtypes of lipomas may show worrisome histological features. Identification of high-grade sarcomas with scarce/absent lipomatous component and subtyping of liposarcomas into dedifferentiated and pleomorphic types pose similar difficulties due to overlapping morphological features and equivocal immunohistochemistry results.

ALT/WDLs, dedifferentiated liposarcomas and pleomorphic liposarcomas included in our study showed a strong predilection for deep/retroperitoneal sites with mean tumour size of 16.6 cm. Whereas, benign lesions showed propensity for superficial sites such as chest wall, neck and thigh. Of 55 cases, MDM2 gene amplification assisted in correctly identifying 30 liposarcomas.

In a study of 301 cases by Michael R. Clay *et al*. in 2015, Analysis of FISH testing criteria was sorted out. They concluded that FISH testing in adipocytic tumours should be recommended on recurring "lipomas", deep extremity tumours, tumours larger than 10 cm, retroperitoneal and intra-abdominal locations and tumours with equivocal cytological atypia.[28] In comparison, our study includes all the problematic adipocytic lesions requiring FISH analysis on a broader spectrum of biopsy-related, clinical and radiological scenarios and findings are comparable with the aforementioned study. Mean tumour size in our FISH-concordant

ALT/WDLs is 16.5 cm whereas in spindle cell lipomatous lesions, 16.9 cm (greater than 10 cm). 10 out of 17 (58.82%) ALT/WDLs are located in deep extremities, retroperitoneal and pelvic locations indicating predilection of atypical lipomatous tumours for these sites.

In another study of 347 patients, Khin Thway *et al*. in 2015 noted that of 122 cases with benign morphological diagnosis, 113 showed no MDM2 amplification giving out a concordance ratio of 92.6%. Similarly, of 73 cases with morphological features of ALT/WDL, 71 showed MDM2 amplification (97.3% concordance ratio).[29] Lower, yet comparable concordance ratios (80% and 64.71% respectively) in our study can be attributed to availability of lesser number of cases available for molecular evaluation on a wider range of histological diagnoses; a regional and financial limitation.

Another study by Joshua Weaver *et al*. in 2008 addressed diagnostic utility of FISH MDM2 in a broader spectrum of lipomatous tumours. They concluded that 13 out of 13 morphologically ALT WDL and 14 out of 14 dedifferentiated liposarcomas showed MDM2 amplification by FISH. Whereas, all 10 Lipomas did not harbor MDM2 amplification. They also evaluated MDM2 amplification in non-lipomatous tumours.[30] Author states that their study included only well-characterized tumour samples and predicted that the MDM2 FISH assay will become a valuable tool in the evaluation of difficult lipomatous lesions. In comparison, our study includes problematic lipomatous tumours with comparable concordance ratio for Lipomas (80%) and ALT/WDL (64.71%) and dedifferentiated liposarcomas (50%).

In summary, our experience of MDM2 amplification by FISH for suspected ALT/WDLs and spindle cell lesions with lipomatous differentiation shows a high concordance with histological opinion. However, FISH testing is not widely available tool in clinical laboratories. Most centres, especially in developing world, rely on IHC alone as an ancillary diagnostic tool. FISH testing, in general, is uneconomical and has high turnaround time. Hence, it cannot be performed for every lipomatous tumour encountered in routine practice. Whilst our study does call attention to some shared characteristics of these tumours, wider research on a larger case number is required to devise a criterion for FISH testing on such lesions.

We suggest that this test should be used as a gold standard in adjunction with morphology and immunohistochemistry for problematic adipocytic neoplasms in soft tissue pathology.

AUTHORS' CONTRIBUTION

SS: Data retrieval and analysis, manuscript writing SM: Conceptualization, methodology, formal analysis and review. UH: Data and draft review. H M: Draft review. RQ: Data analysis and draft review.

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