CLINICOPATHOLOGICAL CHARACTERISTICS OF LIPOSARCOMA-TEN YEARS EXPERIENCE IN A SINGLE INSTITUTION

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LIST OF ABBREVIATIONS

AJCC: American Joint Committee on Cancer

ALT/WDLS: Atypical lipomatous tumour/Well-differentiated liposarcoma

DDLS: Dedifferentiated liposarcoma

PLS: Pleomorphic liposarcoma

MLS : Myxoid liposarcoma

MPLS: Myxoid pleomorphic liposarcoma

OS: Overall survival

MDM2: Mouse double minute 2 homolog

CDK4: Cyclin-dependent kinase 4

FISH: Fluorescence in situ hybridization

PCR: Polymerase chain reaction

SPSS: Statistical Package for the Social Sciences

RB: Retinoblastoma

NGS: Next Generation Sequencing

SNP: Single nucleotide polymorphisms

STS: Soft tissue sarcoma

JEPeM: Jawatankuasa Etika Penyelidikan Manusia

H&E: hematoxylin and eosin

FNCLCC: Fédération Nationale des Centres de Lutte Contre le Cancer

ABSTRAK

Liposarkoma adalah sejenis kanser sarkoma yang jarang berlaku dan ia berasal daripada tisu lemak. Berdasarkan klasifikasi WHO 2020, ia dibahagikan kepada lima kategori. Liposarkoma mempunyai pelbagai bentuk histomorfologi sehinggakan ia menjadi salah satu cabaran kepada pakar patologi dalam mendiagnosa sekaligus berisiko untuk terjadi kesalahan dalam perawatan pesakit. Oleh itu, kajian ini bertujuan menyiasat liposarkoma dari aspek klinikopatologi serta hasil klinikal. Sebanyak 79 kes ketumbuhan lipoma telah dikumpulkan secara retrospektif (saiz berukuran lebih dari 10 cm), dari Jabatan Patologi, Hospital Universiti Sains Malaysia, yang didiagnosa dalam tempoh Januari 2010 sehingga Disember 2020. Slaid kes telah dikaji dan diklasifikasi serta dibahagikan semula berdasarkan jenis histologinya. Data klinikopatologi telah diambil dari unit rekod perubatan serta laporan histopatologi. Hasilnya, terdapat 52 kes telah diklasifikasi sebagai lipoma sementara 27 kes lagi merupakan kanser liposarkoma. Umur median pesakit yang mempunyai lipoma dan liposarkoma adalah sama (52 tahun). Lipoma yang tidak tipikal/liposarkoma dengan ciri yang baik (ALT/WDLS) dan liposarkoma miksoid merupakan dua jenis tumor yang paling lazim, menyumbang kepada 40.7% kes. Kadar terjadi semula sakit liposarkoma adalah 37%. Tahap histologi merupakan faktor prediktor yang utama untuk terjadi semula penyakit liposarkoma ini. Hasil kajian menunjukkan bahawa terdapat hubungan yang signifikasi antara kes berulang penyakit ini dengan jenis kanser liposarkoma yang berciri tidak baik serta miksoid liposarkoma. Walaubagaimanapun, kajian kami menunjukkan tiada kaitan yang signifikasi antara penyebaran tumor dan jenis histologi tumor ($p = 0.115$). Begitu juga dengan hasil klinikal, tiada perbezaan ditunjukkan antara kes lipoma besar dan ALT/WDLS selepas pembedahan dilakukan. Kerana keterbatasan waktu, maka kami tidak menjalankan kajian berhubung perkembangan dari WDLS ke DLS. Kesimpulannya, tumor lipoma yang mencurigakan agak mencabar untuk didiagnosa; oleh itu, ujian molekular genetik MDM2 dan CDK4 menjadi semakin penting agar membantu mendapatkan diagnosa yang lebih tepat.

Kata kunci: liposarkoma, lipoma, klinikopatologi, sakit liposarkoma berulang, penyebaran liposarkoma

ABSTRACT

Liposarcomas are a rare adipocytic soft tissue sarcoma. It is primarily classified into five subtypes according to the recent 2020 WHO classification. However, its heterogeneous morphology consistently presents a diagnostic problem to most pathologists, leading to inappropriate patient management. The study focuses on our institution's clinicopathologic aspects of liposarcoma and its clinical outcome. We retrospectively collected 79 cases of archived lipomatous tumours (larger than 10 cm in size), diagnosed from January 2010 to December 2020, from the Pathology Department at HUSM. The histopathology slides were reviewed. The clinicopathological data were retrieved from the medical records and histopathology reports. Results: Fifty-two of the 79 cases were histologically evaluated as lipomas. The remaining 27 cases were liposarcomas. The median age of patients with lipoma and liposarcoma was similar (52 years). Atypical lipomatous tumour/well-differentiated liposarcoma (ALT/WDLS) and myxoid liposarcoma were the two most prevalent subtypes, accounting for 40.7% of cases. The overall recurrence rate for liposarcoma was 37%, with histological grade being the most significant predictor for recurrence [$\beta = 5.93$, $p < 0.001$]. However, distant metastasis did not show a significant association with histological subtypes ($p = 0.115$). There is no significant difference in the clinical outcomes of large lipomas and ALT/WDLS after surgical excision. Due to their morphologic heterogeneity, lipomatous tumours can be challenging to diagnose. Molecular testing of the MDM2 and CDK4 genes is increasingly important to reduce morbidity and mortality. Future research should examine whether amplified MDM2 and CDK4 have synergistic or opposite effects on prognosis and targeted treatment.

Keywords: Liposarcoma, lipoma, clinicopathology, recurrence, metastasis

CHAPTER 1

1.0 INTRODUCTION

Liposarcomas are malignant mesenchymal tumours with adipocytic differentiation. They are the most common soft tissue tumours. They are usually predominantly middle-aged adults, with a higher prevalence rate than females. They affect different body parts, the most common sites being the extremities and retroperitoneal region. (1) According to the latest WHO classification, there are five subtypes of liposarcoma: atypical lipomatous tumour/well-differentiated liposarcoma, myxoid liposarcoma, dedifferentiated liposarcoma, pleomorphic liposarcoma, and myxoid pleomorphic liposarcoma. (2) There are no known etiological factors for liposarcomas, but there are risk factors associated with liposarcoma, including radiation exposure, toxic chemicals, trauma, and familial cancer syndromes. (3)

There are differences in pathogenesis and molecular patterns of the different subtypes of liposarcomas. ALT/WDLS and Dedifferentiated liposarcomas amplify segments of chromosome 12, including MDM2 and CDK4 genes. Most myxoid liposarcomas have translocations involving the FUS gene. Pleomorphic liposarcoma has complex genetic rearrangement with no pathogenomic structural rearrangements. Myxoid pleomorphic liposarcoma has been associated with numerical chromosomal aberrations and inactivation of the RB1 tumour suppressor gene. (4–7) The clinical presentations of liposarcomas are primarily dependent on tumours locations. Retroperitoneal tumours usually have late presentation until they become huge. The symptoms of tumours in other body locations are mainly due to the mass effect on surrounding tissue. Presentations due to distant metastasis are not common. (3,4)

Even though histopathology is the cornerstone for differentiation between the subtypes of liposarcomas, the diagnosis of liposarcoma remains a challenge for most pathologists

due to its overlapping features between the subtypes, benign and malignant adipocytic tumours and with other soft tissue sarcomas. Therefore, it poses more difficulty, especially in the small biopsy tissue, whereby the representative tissue might show other heterologous mesenchymal differentiation. The diagnosis is relatively simple on imaging studies, but the type of tumour is much harder to define. In addition, no specific immunohistochemistry stains could help the pathologists solve the dilemma, which leads to a dilemma for the treatment team for further surgical intervention and follow-up management.

Immunostaining for MDM2 and/or CDK4 has been adopted as a surrogate marker because of its high concordance rate with FISH, which is the gold standard for MDM2 and CDK4. However, the IHC for both markers is less reliable, often gives non-specific or false positive staining, and is considered insensitive for diagnosing ATL/WDLS. (8) Surgical excision is the mainstay of treatment. Wide and deep surgical excision, adjuvant radiation, and/or chemotherapy may be necessary for high-grade lesions, with prognosis depending on several factors, including histologic subtypes, the grade of the tumour, the tumour location, and the status of surgical margins.(9–11) The survival rate of different subtypes of liposarcomas varies, with an atypical lipomatous tumour having the best prognosis and pleomorphic liposarcoma with the worst prognosis with a 5-year survival rate of around 57%. (1,2,5)

1.1 LITERATURE REVIEW

1.1.1 Overview on Liposarcoma

Although no comprehensive studies comprise all of Asia, studies from China and Singapore show statistics similar to those in other parts of the world regarding incidence rate, gender, and age distribution for liposarcoma. (12) In Malaysia, the National Cancer Registry 2012-2016 put soft tissue sarcoma incidence (including liposarcomas) at 1.4% of all new cancers. (13) Dr. Rudolph Virchow first described liposarcoma in 1857. The first WHO histological classification of soft tissue sarcomas, including liposarcoma, was published by Dr. Franz M Enzinger in 1969, with subsequent updates in 1994, 2002, 2013, and 2020. The clinical behaviour of the tumours was central to the classification. The new classifications put huge emphasis on the genetics and molecular properties of the tumours. There was an introduction of a new entity in the malignant lipomatous tumour in the latest classification with the introduction of myxoid pleomorphic liposarcoma. (14) Liposarcomas are classified into different subtypes; the classification is based on the histologic, cytogenetic, biologic, and molecular features of the tumours. The latest 2020 classification from the World Health Organization proposes classifying malignant adipocytic tumours into five entities: well-differentiated liposarcoma/atypical lipomatous tumour, dedifferentiated liposarcoma, myxoid liposarcoma, pleomorphic liposarcoma, and myxoid pleomorphic liposarcoma. (1,2)

Well-differentiated liposarcoma (WDLS) / Atypical lipomatous tumour (ATL) represents 40–50% of all liposarcomas, it is a locally aggressive tumour with tumours in the extremities and trunk named ATL and those located in the retroperitoneum named well-differentiated liposarcoma. The difference in terminology is related with expected behaviour of the lesion. The ALT is located in an excisable site where complete surgical resection is possible, while WDLPS are located either in mediastinum or retroperitoneal

regions where complete resection may not be possible, with the subsequent recurrence and progression of the tumour. (15) The macroscopic appearance of ATL usually consists of a large, well-circumscribed lobulated mass with variable consistencies, firm grey to gelatinous areas, and often foci containing fat necrosis and punctate haemorrhages. Histologically, there are three main subtypes: adipocytic, sclerosing, and inflammatory. The most important features are the variations in sizes and shapes of adipocytes with atypical stromal cells. A varying number of lipoblasts may be found, but sometimes their presence is barely demonstrated. However, its presence is not necessary for the diagnosis. The inflammatory and sclerosis subtypes have increased chronic inflammatory cell infiltration and sclerosis, respectively. (2,16–18)

Dedifferentiated liposarcoma is an ATL/WDLS showing progression, with the risk of progression being higher in deep-seated lesions. It represents about 10% of liposarcoma cases, with most cases in the retroperitoneal region, 90% of which are primary and 10% of which are recurrent. (2) Macroscopically, they are large multinodular yellow masses containing discrete, solid, often tan-grey areas in dedifferentiated areas. The histological hallmark is an abrupt transition from ATL/WDLS to non-lipogenic sarcoma. The non-lipogenic sarcoma can be high-grade (most of the time) or low-grade. Dedifferentiated areas exhibit variable histological pictures but most frequently resemble an undifferentiated pleomorphic sarcoma. Finding the well-differentiated lipomatous component can be challenging. (2,19)

Myxoid liposarcoma (MLS) accounts for 20–30% of liposarcomas, mainly among adults in the fourth to fifth decades. It is also the most common liposarcoma subtype in children. They are typically large (>10 cm), circumscribed, multinodular intramuscular neoplasms. The cut surface is smooth, gelatinous, and glistening. Histologically, MLS are moderately

cellular lobulated tumours with increased periphery cellularity and pattern-less arrays of uniform, small ovoid cells. The stroma is highly eosinophilic myxoid with a plexiform, delicate arborizing capillary network. They usually lack atypia and mitosis. Lipoblasts may be rare or even absent. High-grade MLS exhibit diminished myxoid matrix, cellular overlap, and increased mitotic activity in more than 5% of the tumour. (2,20)

Pleomorphic liposarcomas are high-grade sarcomas with variable numbers of pleomorphic lipoblasts and no areas of atypical lipomatous tumour or well-differentiated liposarcoma. They account for less than 5% of liposarcomas, with a peak age of incidence in the 7th decade of life. They are usually large, with a median size of 8–10 cm; they are well-demarcated but not encapsulated. They are usually in the extremities in two-thirds of the cases. Histologically, they are infiltrating lesion that contains a varying proportion of pleomorphic lipoblasts against a background of high-grade pleomorphic sarcoma. (21,22)

Myxoid pleomorphic liposarcoma is exceptionally rare and a new entity in the 2020 WHO classification. It is an aggressive adipocytic neoplasm typically occurring in children and adolescents and shows a preference for the mediastinum. They are non-encapsulated tumours with ill-defined margins. Histologically, the tumour exhibits variable areas of a myxoid matrix with scattered lipoblasts, relatively bland primitive round to oval cells, and a delicate plexiform capillary network. Pleomorphic spindle or ovoid cells with hyperchromatic nuclei may be scattered through the tumour. (7,23)

1.1.2 Pathogenesis of liposarcoma

No apparent etiological factor can be attributed as a cause for the development of most soft tissue sarcomas. Still, several factors have been associated with sarcoma development.

These factors include hereditary syndromes like Li-Fraumeni syndrome, occupational and environmental chemical exposure, and radiation. (24,25)

Atypical Lipomatous Tumour/Well Dedifferentiated liposarcoma is characterised by a supernumerary ring and giant marker chromosome that contain amplified sequences originating from the long arm of chromosome 12. Dedifferentiated liposarcoma is cytogenetically related, sharing the same basic genetic abnormality as ATL and WDLPS with identical features, although the amplicons may be incorporated into the chromosomes. The amplified region of chromosome 12 is in the 12q13–15 region; this region includes several genes, including CDK4 and MDM2, HMGA2, CPM, SAS/TSPAN31, and YEATS4. (26,27)

MDM2 (Murine Double Minute 2) is a proto-oncogene discovered as a highly amplified genome region in a spontaneous tumorigenic mouse cell line. Its human homolog is in the q15 region of chromosome 12. MDM2 is consistently amplified and overexpressed and is considered to represent one of the earliest events in the formation of WDLS/DDLS. The protein encoded by MDM2 is a nuclear phosphor protein with an inhibitory effect on the TP53 pathway, which plays a central role in regulating DNA repair, cell cycle arrest, and apoptosis to maintain genomic integrity. MDM2 binds to TP53 to block its transcriptional activity, and it can also induce TP53 protein degradation through its function as the E3 ubiquitin ligase. (4,28,29)

Cyclin-dependent kinase 4(CDK4) gene is another oncogene located in the amplified q13-15 region of chromosome 12 and amplified in up to 90% of ALT/ WDLS and DDLS. CDK4 is a member of the cyclin-dependent kinase family. It is involved in the retinoblastoma (RB) pathway, which is associated with cell cycle regulation and tumorigenesis. RB gene is a tumour suppressor gene that regulates the cell cycle by

preventing S phase entry through binding and inactivating the transcription factor E2F1. CDK4 interacts with several types of Cyclines during the G1 phase of the cell cycle, and these CDK4- Cyclines complexes subsequently hyper-phosphorylate RB, resulting in the release of E2F1, which then promotes cell entry into the S phase and subsequent cell proliferation. (4,30)

In addition to MDM2 and CDK4, several other amplified genes of 12q13-15 are consistently detected in WDLS/DDLS and include HMGA2, TSPAN31, CPM, and YEATS4. HMGA2 (High mobility group AT-hook 2) has an amplification ratio of 72% in DDLS cases. HMGA2 encodes a protein that belongs to the non-histone chromosomal high mobility group protein family and functions as an architectural factor and a critical component of the enhanceosome, which enhances gene transcription. HMGA2 may have both oncogenic and anti-oncogenic abilities. The deletion, amplification, or rearrangement of HMGA2 is associated with various benign tumours, especially mesenchymal ones. HMGA2 is thought to play a role in adipogenesis and mesenchymal differentiation. (31,32) Unfortunately, the other three commonly amplified genes of interest, TSPAN31, CPM, and YEATS4, are less well-studied. (4)

The amplification profiles of genes in the 12q13–15 region vary significantly between WDLS and DDLS, with more frequent high-level amplifications and substantially higher mean amplification ratios in DDLPS compared with WDLPS. Differences in amplification profiles between WDL and DDL are likely related to tumour progression and dedifferentiation. (33)

Myxoid liposarcoma is characterised by a recurrent translocation and gene fusion, the t (12;16) (q13; p11), seen in over 90% of cases, which fuses the 5 ' half of the FUS gene on chromosome 16 with the entire reading frame of the DDIT3 gene on chromosome 12. A

much smaller fraction of myxoid liposarcoma cases harbours a similar variant translocation and gene fusion, the t(12;22) (q13;q12), which fuses the EWSR1 gene to the DDIT3 gene. FUS and EWSR1 are similar genes, ubiquitously expressed, with a transcriptional activation domain in their 5' end that is fused to the entire coding region of DDIT3, which encodes an apparent DNA-binding and dimerization domain. This novel chimeric transcription factor is oncogenic for myxoid liposarcoma and inhibits adipocytic differentiation. Translocations include portions of the RNA-binding domain of FUS or EWSR1, and the oncogenic gene fusion may alter RNA splicing. (6,34)

Pleomorphic liposarcoma has a complex and non-specific molecular profile; it lacks the specific alterations of other liposarcomas and resembles UPS or myxofibrosarcoma. By conventional cytogenetics, Pleomorphic liposarcoma invariably demonstrates a complex karyotype with numerous structural rearrangements and imbalances. Copy number alterations are seen across the genome and are more frequently gains than losses, which can be detected on array comparative genomic hybridization and SNP arrays as well as next-generation sequencing (NGS) approaches. (5,35)

Myxoid pleomorphic liposarcomas show complex chromosomal alterations, including recurrent significant chromosomal gains involving chromosomes 1, 6, 7, 8, 18, 19, 20, and 21, and losses involving chromosomes 13, 16, and 17. Losses in chromosome 13, in particular a loss in 13q14 (including the RB1, RCTB2, DLEU1, and ITM2B genes), were observed in half of the cases analysed by wide genome sequencing. In addition, monoallelic RB1 deletion was confirmed by FISH in 2/3 of tumors. Moreover, nuclear Rb expression was deficient in most tumours, further emphasising that inactivation of the RB1 tumour suppressor gene is a consistent finding and pathogenetically paramount in this tumour type. (7,36)

1.1.3 Ancillary Tests

As mentioned, diagnosing liposarcoma is always challenging for most pathologists due to its overlapping morphology. In addition, the understanding of the subtype of liposarcoma is still lacking. Therefore, the molecular approach may provide important insight into tailored specific surgical management and early multidisciplinary targeted approach. Thus, the molecular approach by identifying these MDM2 and CDK4 genes has been recognised to supplement the pathological diagnosis of liposarcoma and help the treatment team personalise the targeted approach based on their amplification using the molecular technique.

Fluorescent In Situ Hybridization (FISH) demonstrated amplification of MDM2 and CDK4 has more than 90% sensitivity and specificity for ALT/WDLS and DDLS using Formalin-fixed paraffin-embedded tissue blocks. Immunohistochemistry using MDM2 and CDK4 antibodies is less costly than FISH, with a sensitivity of 45-50% and specificity reaching 90%. Quantitative PCR has sensitivity and specificity figures similar to FISH but is more expensive. (8,37)

For myxoid liposarcoma, the demonstration of recurrent translocations enables diagnostic confirmation using reverse transcription-polymerase chain reaction (RT-PCR) or fluorescence in situ hybridization (FISH), typically with break-apart probes designed to detect rearrangement of DDIT3, FUS, or EWSR1. In new studies, nuclear anti-DDIT3 immunoreactivity is found to be a sensitive marker for myxoid liposarcoma, which, when diffusely present among tumor cells, is also highly specific. (38)

Immunohistochemistry plays a limited role in pleomorphic liposarcoma due to its non-specific immunoprofile and variable expression of SMA (often focal), Desmin, and CD34 positivity. S-100 protein is positive in adipocytes and maybe occasionally useful for highlighting lipoblasts in a tumour that resembles undifferentiated pleomorphic liposarcoma in all other respects. As previously stated, pleomorphic liposarcoma has a complex and non-specific molecular profile, and FISH cannot detect any specific chromosomal alterations. (5)

Immunohistochemistry for myxoid pleomorphic liposarcoma showed diffuse CD34 and p16 expression, loss of nuclear RB expression, and was negative for MDM2. FISH can be used to demonstrate monoallelic deletion of RB1 and to rule out WDLS and DDLS. (7)

1.1.4 Clinical Management

As mentioned above, the difficulty in differentiating the benign and malignant subtypes of liposarcoma and other soft tissue sarcoma leads to a considerable risk for local recurrence with inappropriate surgical resection. Low recurrence rates can be expected with appropriate excision as low-grade liposarcomas or atypical lipomatous tumours show low to no metastatic potential. The prognosis is excellent, given the favourable outcomes with surgery alone, radiation therapy and systemic therapy are not routinely recommended. (9,11)

WDLS rarely metastasizes with a tendency for local recurrence, especially retroperitoneal WDLS, which effects morbidity and impacts overall survival; thus, efforts to improve local control are essential. Trials assessing the impact of perioperative radiation therapy

compared to surgery alone showed that preoperative radiotherapy followed by surgery or surgery alone demonstrated a similar 3-year recurrence-free survival. (39)

Stage-directed perioperative radiation therapy and/or chemotherapy may be considered for extremity DDLS. DDLS has a poor chemosensitivity, but new evidence suggests that adjuvant chemotherapy may improve outcomes in high-risk patients with extremity/trunk Soft Tissue Sarcoma and a predicted 10-year overall survival (OS) of 51% or less. (40) Preoperative chemotherapy for high-grade retroperitoneal soft tissue sarcoma is recommended for patients with good performance status and borderline resectable or recurrent tumours where tumour shrinkage may improve surgical outcomes. (9)

For unresectable or metastatic DDLS, the recommended first-line therapy remains an anthracycline-based chemotherapy regimen. Doxorubicin monotherapy or doxorubicin in combination with ifosfamide is recommended. More patients responded and had a more prolonged progression-free survival with the combination of both drugs. (40)

There is a new trend of using novel therapies based on the molecular understanding of the pathogenesis of liposarcomas. CDK4/6 inhibitors induce growth arrest, upregulate the chromatin remodelling enzyme ATRX, and decrease expression of the negative P53 regulator, MDM2, resulting in cell senescence. Initial clinical studies showed modest activity using the CDK4/6 inhibitor palbociclib but with haematological toxicity leading to a dose reduction. There are other CDK4/6 inhibitors abemaciclib and Ribociclib, which show promise with increasing disease-free survival in clinical trials with use individually or use with other agents. (41–43)

MDM2 inhibitors in tumours with MDM2 overexpression or amplification showed manageable toxicity and some activity (mainly stable disease and a few partial responses) in WDLS/DDLS. (44) There are multiple new therapies in trials with variable results. These

therapies include Selective inhibitors of nuclear export, Multikinase inhibitors, and Immunotherapy.(45–47)

The treatment of extremity and trunk MLS closely aligns with that of WDLS/DDLS. MLS tend to be especially radiosensitive relative to other liposarcomas, with 91% of patients achieving a pathologic response, defined as greater than 50% treatment effect. The use of perioperative anthracycline-based chemotherapy in select patients with large, high-grade, localized MLS of the extremity and trunk wall is recommended, with trials showing improvement in both disease-free and overall survival rates. (40,48)

FUS-DDIT3 fusion leads to the upregulation of several oncogenic pathways with targeted therapy for some of these pathways. FUS-DDIT3 activates various kinases, suggesting a potential role for multikinase inhibitors in treating MLS; however, studies have failed to demonstrate the activity of TKIs in MLS. DDIT3 fusion drives high expression of PPAR-γ, likely through suppression of signalling downstream of PPAR-γ, which correlates with poor outcomes in MLPS. The PPAR-γ agonist efatutazone was studied in trials enrolling patients with advanced malignancies and demonstrated a markedly long-lasting effect in a patient with MLS. (49,50)

For pleomorphic liposarcoma, the cornerstone of treatment for patients with high-risk localized PLS is complete surgical resection when feasible and radiation therapy for STS. In the metastatic setting, the agents described for WDLS/DDLS anthracycline-based chemotherapy regimen are used. Doxorubicin monotherapy or doxorubicin in combination with Ifosfamide is recommended, demonstrating moderate activity in PLS. There are no studies on targeted therapies and novel approaches for PLS. (9,51)

Since Myxoid pleomorphic liposarcoma is a newly classified entity, there are no consensus recommendations for the standard of care concerning local and systemic therapies. (9)

1.1.5 Prognosis

WDLS and DDLS have an early recurrence rate of 18-39% and 33-58%, respectively, and a late recurrence rate of 60% in both diseases. The early metastatic rate of WDLS is 0%, and for DDLPS, 9-44%. WDL has a late metastasis rate of 8%, compared 28% for DDLS. In pleomorphic liposarcoma, studies show Local recurrence and metastatic rates of around 30–50% and 50%, respectively, and 5 Five-year survival of approximately 57%. (5,52) Prognostic factors associated with the overall survival (OS) are age, gender, tumour size, invasion of adjacent structures, the radicality of the surgical resection, involvement of margins, histological subtype, grade, primary location inside or outside the retroperitoneum, and the presence of metastatic disease. (10,52)

Poor overall survival (OS) factors included primary tumour size greater than 10 cm, higher tumour grade, older patient age, and higher stage and metastatic disease. MDM2/CDK4 amplification is associated with worse disease-specific and disease-free survival in WDL and DDLS. (53,54) Myxoid liposarcoma has 13–33% local recurrence rates, distant metastasis rates of 11–38%, and 10-year OS rates of 55–86%. In addition to the previously mentioned prognostic factors in WDLPS/DDLPS, myxoid liposarcoma shows responsiveness to radiotherapy by decreasing the size of many tumours significantly. However, it did not influence local recurrence. (55)Although the data for myxoid pleomorphic liposarcoma is limited, they appear to be particularly aggressive, with metastases and death from a disease within 40 months in all patients with available clinical follow-up. (7)

1.1.6 Staging and Grading

The staging of soft tissue sarcomas is based on histological and clinical information. The major staging systems used were developed by the Union for International Cancer Control (UICC) and AJCC and are clinically useful and of prognostic value.

The American Joint Committee on Cancer (AJCC) has used available evidence-based literature to construct staging systems for many cancers. In addition to the three variables that comprise the foundation of most cancer staging systems - tumour size (T), nodal status (N), and distant metastases (M) – sarcoma staging has included grade (G) and tumour depth (superficial/deep). (56)

There have been some changes in the AJCC 8th Edition with greater emphasis on the primary anatomic site of soft tissue sarcomas. There are now separate staging systems for tumours on (1) extremity and trunk, (2) retroperitoneum, (3) head and neck, and (4) visceral sites. With less impact on the outcome, the superficial/deep category formerly used for tumours in non-retroperitoneal locations is no longer used. For the tumour size, the T1 category is for preserved tumours less than or equal to 5 cm, tumours that are greater than 5 cm but less than or equal to 10 cm are T2, tumours that are greater than 10 cm but less than or equal to 15 cm are T3, and tumours that are greater than 15 cm are T4. Size should be regarded as a continuous variable, with 5 cm, 10 cm, and 15 cm used merely as arbitrary divisions that make it possible to group the patients. (57)

Lymph node metastases (N1) are uncommon in individuals with soft tissue sarcomas, although the true prevalence across histologic subtypes and disease sites remains unknown at the time of diagnosis. In the assignment of stage group, patients whose nodal status is

not proven to be tumour positive either clinically or pathologically should be classified as N0 and not as NX. (57,58) CT or MRI imaging is the modality used to assess tumour metastasis. The lung is the most common site for soft tissue tumours to metastasise. (57)

Histologic grading is the most important prognostic factor and the best indicator of metastatic risk in adult soft tissue sarcomas. The most commonly used systems are the French grading and the National Cancer Institute grading. The NCI system, published in 1984, was based on assessing six histological parameters (histological type, mitoses, necrosis, pleomorphism, cellularity, and intercellular matrix). The final form of this system uses a group of predefined grade 1 and grade 3 sarcomas and, in tumours not automatically categorised, the amount of necrosis is used to distinguish grade 2 from grade 3 tumours, with a cut-off point of 15% for the extent of necrosis. (59–61)

However, the FNCLCC system appears to be more precisely defined and potentially more reproducible, and it is therefore the most widely used. The French Sarcoma Group proposed a system based on assessing three independent prognostic factors: tumour differentiation, mitotic index, and extent of necrosis (Table 1.0). These parameters are scored 1–3 for differentiation and mitotic index and 0–2 for necrosis. A three-grade system is obtained by summing the scores for these three parameters. Grade 1 is defined as a total of 2 or 3; grade 2 as a total of 4 or 5; and grade 3 as a total of 6–8. Differentiation is the most controversial parameter and is, in fact, a mixture of histological type and subtype and/or true differentiation. A score of 1 is currently assigned to sarcomas closely resembling normal adult tissue to such a degree as to be confused with benign tumours, such as a well-differentiated leiomyosarcoma. A score of 3 is given to embryonal and poorly differentiated sarcomas, sarcomas of doubtful histological type, synovial sarcoma, primitive neuroectodermal tumour, osteosarcoma, pleomorphic rhabdomyosarcoma, and

pleomorphic liposarcoma. Other histological types, such as myxoid liposarcoma are scored 2. (Table 1.1). (60–62)

1.1.7 Study limitation

This study was part of a big research project on the molecular properties of liposarcoma and their associations with clinicopathological data. The project, which in under RUTop Down grant , was approved by JEPeM, entitled "The characterisation of MDM2 and CDK4 gene amplifications and their role in survival among liposarcoma patients". The cornerstone of the project was to study the clinicopathological characteristics of liposarcoma and the association with MDM2 and CDK4 gene amplification using FISH. Unfortunately, due to the variability between tissue types and DNA probes, an optimised FISH protocol is mandatory to avoid unsatisfactory results. Optimisation of pre-analytical and analytical parameters such as deparaffinisation, digestion, denaturation, and hybridisation conditions, as well as post-hybridisation washes are crucial steps in order to get a good signal without background staining due to non-specific bounds.

We had many technical setbacks in several pre-analytical and analytical steps, including the inability to optimise digestion , denaturation, and hybridisation, resulting in unsatisfactory results. In addition, the technical challenges in establishing a molecular laboratory for solid tumours in the pathology department at HUSM also contributed to the delay in the research project. It took almost a year, limiting the possibility of incorporating the molecular portion of the analysis in this study. Although the molecular analysis is not included, the clinicopathological characteristics of liposarcoma are the first phase of the overall research project. The project's next step will undoubtedly have more complete results with the incorporation of molecular testing in liposarcoma and management in the

next stage. We hope the results will guide clinicians in the appropriate steps for management.

Table 0.0: The French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading system.

Parameter	**Score**	**Description**
Tumour differentiation	1	Sarcoma histologically very similar to normal adult mesenchymal tissue
	2	Sarcoma of defined histological subtype (e.g., myxofibrosarcoma)
	3	Sarcoma of uncertain type, embryonal and undifferentiated sarcomas
Mitosis count	1	0-9 / 10 HPF
	2	10-19 / 10 HPF
	3	>20 / 10 HPF

Microscopic tumour necrosis	0	No necrosis
	1	<50% tumour necrosis
	2	>50% tumour necrosis
Final histological grade	1	Total score 2 or 3
	2	Total score 4 or 5
	3	Total score 6, 7, or 8

Note: Adopted from WHO Classification of Tumours of Soft Tissue and Bone. 2013

Table 0.1: Individual tumour differentiation scores according to the FNCLCC system.

Well-differentiated liposarcoma	1
Well-differentiated leiomyosarcoma	1
Malignant neurofibroma	1
Well-differentiated fibrosarcoma	1
Myxoid liposarcoma	2
Conventional fibrosarcoma	2
Conventional MPNST*	2
Myxofibrosarcoma	2
Myxoid chondrosarcoma	2
Conventional leiomyosarcoma	2

Conventional angiosarcoma**	2
High-grade myxoid (round cell)	3
Pleomorphic liposarcoma	3
Dedifferentiated liposarcoma	3
Poorly differentiated/epithelioid	3
Poorly differentiated MPNST*	3
Malignant Triton Tumour	3
Poorly differentiated/pleomorphic	3
Synovial sarcoma	3
Rhabdomyosarcoma	3
Mesenchymal chondrosarcoma	3
Poorly differentiated/epithelioid	3
Extra skeletal osteosarcoma	3
Extra skeletal Ewing sarcoma	3
Alveolar soft part sarcoma	3
Malignant rhabdoid tumour	3
Clear cell sarcoma	3
Undifferentiated (spindle cell and	3

Note: Adopted from WHO Classification of Tumours of Soft Tissue and Bone. 2013

CHAPTER 2: OBJECTIVES

GENERAL OBJECTIVES:

- To describe the clinicopathological characteristics of liposarcoma

SPECIFIC OBJECTIVES:

- To determine the proportion of liposarcoma cases according to histological subtype
- To study the association between the liposarcoma subtypes and clinicopathological data

CHAPTER 3: MANUSCRIPT.

TITLE PAGE

CLINICOPATHOLOGICAL CHARACTERISTICS OF LIPOSARCOMA- TEN YEARS EXPERIENCE IN A SINGLE INSTITUTION

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ABSTRACT

Introduction: Liposarcomas are a rare adipocytic soft tissue sarcoma. It is primarily classified into five subtypes according to the recent 2020 WHO classification. However, its heterogeneous morphology consistently presents a diagnostic problem to most pathologists, leading to inappropriate patient management. The study focuses on our institution's clinicopathologic aspects of liposarcoma and its clinical outcome. **Materials and methods:** We retrospectively collected 79 cases of archived lipomatous tumours (larger than 10 cm in size), diagnosed from January 2010 to December 2020, from the Pathology Department at HUSM. The histopathology slides were reviewed. The clinicopathological data were retrieved from the medical records and histopathology reports. Results: Fifty-two of the 79 cases were histologically evaluated as lipomas. The remaining 27 cases were liposarcomas. The median age of patients with lipoma and liposarcoma was similar (52 years). Atypical lipomatous tumour/well-differentiated liposarcoma (ALT/WDLS) and myxoid liposarcoma were the two most prevalent subtypes, accounting for 40.7% of cases. The overall recurrence rate for liposarcoma was 37%, with histological grade being the most significant predictor for recurrence [$\beta = 5.93$, $p < 0.001$]. However, distant metastasis did not show a significant association with histological subtypes ($p = 0.115$). There is no significant difference in the clinical outcomes of large lipomas and ALT/WDLS after surgical excision. **Conclusion:** Due to their morphologic heterogeneity, lipomatous tumours can be challenging to diagnose. Molecular testing of the MDM2 and CDK4 genes is increasingly important to reduce morbidity and mortality. Future research should examine whether amplified MDM2 and CDK4 have synergistic or opposite effects on prognosis and targeted treatment.

Keywords: Liposarcoma, lipoma, clinicopathology, recurrence, metastasis

3.1 INTRODUCTION

Lipomatous tumours are the most prevalent soft tissue tumours, most of which are lipomas. Liposarcoma accounts for 20% of all soft tissue sarcomas. According to the Malaysian National Cancer Registry for 2012-2016, soft tissue sarcoma incidence (including liposarcoma) accounted for 1.4% of all new malignancies in the country. (1,2)

Since the initial soft tissue sarcoma classification was published in 1969, there have been modifications to the categorisation of the lipomatous tumour. The refinement of classification schemes plays a crucial role in improving the quality of pathologic diagnosis and, therefore, therapeutic options. The recent 2020 World Health Organisation classifying malignant adipocytic tumours into five subtypes: Atypical Lipomatous Tumours /Well-Differentiated Liposarcoma (ALT/WDLS), Dedifferentiated Liposarcoma (DDLS), Myxoid Liposarcoma (MLS), Pleomorphic Liposarcoma (PLS), and Myxoid Pleomorphic Liposarcoma (MPLS). (3,4)

ALT/WDLS account for 40 to 50 percent of liposarcomas. It resembled lipoma grossly, and the interpretation is always tricky on biopsy samples and can be misinterpreted. It is a locally aggressive cancer with local recurrence, and its metastatic potential is debatable. (5) The terminology "ALT" and "WDLS" is based on a tumour's location. They are morphologically and genetically identical but have different behaviours, clinical outcomes, and treatments. Thus, the variation in terminology is intended to avoid both undertreatment and overtreatment. (6)

ATL refers to the tumours in the extremities and trunk, which are usually resectable with no risk for dedifferentiation. On the contrary, WDLS refers to tumours in deep-seated organs, e.g., retroperitoneum, mediastinum, and spermatic cord (6). Achieving negative

margins is significantly diminished, and the risk of local recurrence, dedifferentiation is increased with poorer clinical outcomes. ALT/WDLS can be diagnosed by histologic criteria alone in the presence of large, atypical, hyperchromatic cells and adipocytic sizes variation. However, the atypical stromal may be focal, the diagnosis may be missed. (3,7,8)

Differentiating ALT/WDLS from huge lipomas, spindle cell lipomas, pleomorphic lipomas, and atypical spindle cell/pleomorphic lipomatous tumours, especially in large and deeply seated lesions, can be difficult. The recent WHO classification reclassified the lesion previously known as spindle cell liposarcoma as an atypical spindle/pleomorphic lipomatous tumour (APLT). It is a benign tumour whose morphology closely resembles ALT/WDLS. The diagnosis would be challenging without evidence of amplified MDM2 and CDK4 genes. The same goes for other benign lipomas with degenerative changes like fat necrosis, which will also render diagnostic problems because it mimics ALT/WDLS. Therefore, amplification of the MDM2/CDK4 genes (located at 12 q12–15) evaluated by Fluorescence In Situ Hybridization (FISH) has emerged as an essential ancillary diagnostic test. (9) Given the favourable prognosis, low recurrence rates, and no evidence of dedifferentiation associated with ALT in the extremities, radiation therapy, and systemic therapy are not typically recommended. However, the WDLS seldom metastasise; nonetheless, local recurrence of retroperitoneal WDLS causes morbidity and affects overall survival; hence, efforts to enhance local control are essential. (10)

Myxoid liposarcoma (MLS) is the second most frequent type of liposarcoma They account for 20-30% of all liposarcomas. They primarily affect adults in their fourth and fifth decades. It is also the most prevalent subtype of liposarcoma in young people. Typically, they are big (> 10 cm), confined, multinodular intramuscular tumours. (3,11) A recurrent translocation and gene fusion characterise them at t (12; 16) (q13; p11), which is seen in over 90% of cases. A much smaller fraction of MLS cases harbour a similar variant