MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF ANATOMICAL PATHOLOGY

TITLE: COMPLETENESS OF HISTOPATHOLOGICAL REPORTING OF MALIGNANT MELANOMA AT MUHIMBILI NATIONAL HOSPITAL.

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LIST OF ABBREVIATIONS

ALM	Acral lentiginous melanoma
AJCC	American Joint Committee on Cancer
CM	Cutaneous melanoma
CPL	Central Pathology Laboratory
FFPE	Formalin Fixed Paraffin Embedded
H&E	&Eosin
HoD	Head of Department
LM	Lentigomaligna melanoma
MM	Malignant melanoma
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of health and Allied Sciences
SR	Synoptic report
NR	Narrative report
SSM	Superficial spreading melanoma
SOP	Standard Operation Procedure
SRM	Sakura Model Microtomy
UVR	Ultra violet radiation
USA	United States of America
WHO	World Health Organization

ABSTRACT

Background: Malignant melanoma is an aggressive tumor arising from melanocytes. Its incidence and mortality rate has been increasing worldwide though it is generally high in white population in comparison to Asian and African populations. Histological reporting remains to be the gold standard for the confirmatory diagnosis of malignant melanoma and is one of important prognostic tools. Therefore, pathology report should document all histological features that are of diagnostic and prognostic importance.

Majority of pathology centers in low and middle resource setting including those in Tanzania employ narrative reporting practice over the currently advocated standardized synoptic reporting system. Nevertheless, narrative histopathological reporting approach may cause misinterpretation due to lack of important information and structure. On the other hand, synoptic reports are pages long, requiring extensive text editing and multiple intermediate steps. Therefore, there is a need to substantiate whether the traditional narrative reporting approach has justifiable shortcomings to warrant the change.

The aim of this study is to assess whether the current histomorphological reporting system at Muhimbili National Hospital (MNH) in Tanzania, incorporates all important diagnostic and prognostic characteristics of malignant melanoma in order to form an unbiased decision to opt to synoptic reporting system.

Broad objective: To assess the completeness of histopathological narrative reporting of malignant melanoma at MNH in order to objectively obtains an informed decision on the use of synoptic reporting approach.

Methodology: This will be a cross sectional hospital based descriptive study conducted at MNH for 5 years' duration from January 2015 to December 2019. Convenience sampling method will be used to obtain 99 cases of malignant melanoma retrieved from the archive. All histopathological reports present during the five years of the study period will be retrieved and there after slides for each case will be reviewed by the investigator and a senior pathologist. In each case the microscopical features on tissue section(s) will be filled in a prepared synoptic

template and then be compared with features reported in a narrative report. Obtained data will be analyzed using Statistical Package for Social Sciences (SPSS) version 20. **Correlation between microscopic features gathered in narrative reporting system versus those in synoptic template will be done using Pearson's correlation coefficient(r) test.**

Budget

The estimated budget for this study will be 2,305,000 Tanzanian shillings this will be funded by Ministry of Health, Community, Development, Gender, Elderly and Children (MoHCDEC) of Tanzania.

CHAPTER ONE

1.0 INTRODUCTION

Background

Worldwide, the incidence of malignant melanoma has steadily increased over the last several decades (1). Annual incidence has risen as rapidly as 4–6% in many fair-skinned populations (3,4). Though there is paucity of data on true prevalence of malignant melanoma in Africa, several studies have reported that malignant melanoma is not infrequently encountered (276, 28,29). In Tanzania, a study done in Northern part of the country reported a 67.5% of all dermatological malignancy to be malignant melanoma, which was predominated by nodular subtype. Similarly, 50% of cases **reported by Vuhahula *et al*** in Tanzania had characteristics of aggressiveness as evidenced by high proliferation index and frequent loss of p16 antigen (14).

Currently the complexity of cancer pathology reporting including malignant melanoma reporting has significantly increased (15). The adequate surgical pathology report not only documents the presence and accurate typing of the malignancy but also contains information related to tumor grade, size, local extent, vessel involvement, marginal status along with other morphologic and sometimes ancillary results including tumor markers (6,7). For the malignant melanoma essential histopathological features to be included in the pathology report are as follows, surgical margin or tissue edge status, Breslow thickness, ulceration, mitotic count, satellites, lympho-vascular invasion, desmoplastic melanoma component and perineural invasion. Recommended or optional histopathological features to be included in the pathology report of malignant melanoma are extent of ulceration, Clark level, tumor-infiltrating lymphocytes, tumor regression, tumor regression margins, associated melanocytic lesion, intraepidermal melanoma growth pattern and melanoma subtype (6,7).

These features can easily be captured in synoptic format because it is much easier to decipher the information than ones that are presented in a narrative or paragraphed style, therefore minimizing the risk of misinterpretation and clinical error (16,17). Synoptic reporting can save time since all important diagnostic and prognostic factors are laid out in a list or table with

headers and responses rather than being buried in paragraphed text fields (17). While cancer pathology reports including that of malignant melanoma are principally prepared for clinicians, other groups use information in these reports for a variety of important purposes. Tumor registries, government agencies and health planners, epidemiologists and others involved in quality improvement activities and population-based research all utilize cancer pathology information (35, 36, 37). These various groups prefer to have cancer pathology reports presented in a standardized synoptic format that will ensure completeness (16,17).

The aim of this study is to assess whether the current histomorphology reporting system of malignant melanoma at MNH incorporate all important diagnostic and prognostic characteristics in respect to clinical management guidance of malignant melanoma.

1.1 Problem statement

Histopathologic parameters for malignant melanoma is of paramount importance for management and prognosis guidance.While many pathologist in developed countries have adopted a synoptic formulaic or tabular format for malignant melanoma reporting ,most anatomical laboratories in developing countries including MNH in Tanzania still use a traditional descriptive transcription of the histopathological findings on the final report. Important information which is essential in guiding management plan and prognosis may be hidden in paragraphic form or even not reported at all therefore the prognosis and management will not be guided in required manner. If a pathologist is not guided by a tumor-specific standardized template or checklist, it is easy to omit information that may be required for clinical decision making therefore standardized and synoptic cancer pathology reporting has recently been advocated**. Hence the narrative report format at MNH will be evaluated to assessed to what extent essential parameters required in histopathology report for malignant melanoma are documented in concordance with synoptic reporting format. The obtained result will provide an evidence based decision on about adoption to new reporting format at MNH.**

1.2 Rationale of the study

This study will attempt to establish and shows the usability of synoptic reporting format on malignant melanoma by assessing the documentation and capturing of important parameters like margins status, Breslow thickness, ulceration, perineural invasion and vascular invasion, which are essential for guiding management and prognosis of the patients at **MNH. In addition, the results of this study will show the evidence on the usability of synoptic reports since they are easier to interpret through their consistency because they increase report completeness, increase report readability, reduce medical record errors and improve turnaround times.**

On the other hand, synoptic reports are pages long, requiring extensive text editing and multiple intermediate steps. Therefore, there is a need to substantiate whether the traditional narrative reporting approach has justifiable shortcomings to warrant the change. Also synoptic report format needs time and attention of pathologist to fill all required elements in the final report.

Hypothesis

Narrative reporting system of malignant melanoma at MNH doesn't include all essential features for guiding management and prognosis, therefore a need of synoptic report format.

1.3 Research questions

1. **What are the essential diagnostic grossing features missed in narrative report?**
2. **What are the essential histormophological diagnostic and prognostic features missed in the narrative report?**
3. What are the challenges encountered by the pathologist in attempt to implement synoptic reporting system?

1.4 Broad objective

To assess the completeness of histopathological narrative reporting of malignant melanoma at Muhimbili National Hospital in order to objectively obtains an informed decision on the use of synoptic reporting approach.

1.4.1 Specific objectives

1. To identify the essential features in the narrative gross sheets of malignant melanoma cases at MNH.

2. To ascertain the essential diagnostic and prognostic histormophological features of malignant melanoma that are not included in narrative report system at MNH.

3. To compare the completeness of diagnostic and prognostic histomorphological features of malignant melanoma cases between synoptic versus narrative reporting format at MNH.

4. To establish any challenges a pathologist at MNH may face in adopting synoptic reporting from the traditional narrative reporting system for malignant melanoma.

CHAPTER TWO

2.0 LITERATURE REVIEW

Accurate clinical and histological diagnosis of cancer is essential for the adequate management of patients and prognosis assessment. The report issued by the pathologist should not only provide useful diagnostic information but several parameters that might be important in assessing prognosis and guiding management plan of the patients should be documented accurately due to the fact that histological report should be as accurate and thorough as possible (35).Traditionally, narrative report had been reported in paragraphic format of the following three paragraphs macroscopy, microscopy and conclusion all completed with free text and without any further guidelines to essential histological features that guides management plan to the patient. Narrative reports are still the standard in most jurisdictions; even though they are prone to misinterpretation and do not always contain all mandatory information. In synoptic-like structured format with this method, the pathologist follows a checklist per cancer type to ensure that all mandatory parameters are reported (37,38).

2.1 The essential features in the narrative gross sheets of malignant melanoma.

The study done by Thompson et al at Queensland hospital revealed that documentation of the most important features of melanoma was high in which clear diagnosis (99.8%; 95% CI 98.6-100), thickness (99.8%; 95% CI 98.6-100), comment on adequacy of excision (87.9%; 95% CI 84.9-91.0) and measurement of margins (91.9%; 95% CI 88.8-91.4

Another study done by Vuhahula et al which involved 28cases of MM showed that malignant melanomas in black Africans are predominantly located on the lower extremities. Since their biological features have not been well focused and findings indicate that melanomas located on the lower extremities in black Africans show several features of aggressiveness (14).

The study done in Brazil showed that these were located on the upper limbs (n = 22, 11.1%), lower limbs (n = 67, 33.7%), trunk (n = 62, 31.2%) and head and neck (n = 40, 20.1%). The women predominantly presented tumors on the trunk (28.7%), lower limbs (27.7%) and head/neck (25.7%) while the men had tumors mainly on their lower limbs (40.2%) and trunk (34.0%). These differences were statistically significant (24).

Another study done in India by Panda et al, which involved 182cases of MM, out of all the cutaneous melanomas, majority of cases were found in lower extremity, out of which sole of the foot was found to be the most common site (78.2%). Next in order did dorsum of foot comprise 30 cases (17.47%). One case each was seen in scalp and vulva. Twelve non-cutaneous melanomas were found, out of which 6 cases were seen in anorectal, 2 were conjunctiva melanoma, and 1 case each in nasal cavity, palate, gingivo-buccal sulcus, and vagina. Of all the cases, 85 cases (46.7%) showed metastasis. Majority [79 (92.94%)] of these metastatic lesions were from lower limbs to inguinal nodes. The primary site was known for 106 cases (20).

The study done by Mulenga et al in Malawi (2019) which involved 103 cutaneous melanomas and 3 mucosal melanomas 98 cases (95%) of cutaneous melanomas were located on acral sites, and 90 cases (87%) were on the foot. Only 8cases presented on the hands, with 7 of 8 occurring in females. Twenty-six cases (20%) had metastatic melanoma in the lymph nodes with no known primary sites. 24 cases with lymph node metastasis (92%) were in the inguinal region, suggesting the lower extremity as the possible primary site (26). The study done in Nepal by Thapa S, et al the study included 35 cases of malignant melanoma the predominant site was lower extremities. Most cases were less than 3 cm (18).

2.2 Essential diagnostic and prognostic histormophological features of malignant melanoma that should be included in narrative report system

Histomorphological features of malignant melanoma plays important role in determining the treatment outcome of the patients such as risk of progression to metastatic disease, disease relapse due to incomplete excision of the tumor and treatment resistance due to unresectable tumor. Malignant melanoma histormophological features exists in different forms Malignant melanoma can be pigmented (melanotic) and non pigmented (amelanotic) and the common histomorphological features are epitheliod, spindle, bizarre and mixed (6,7, 23).

The study done Vuhahula et al in Tanzania involved 28 cases of MM showed that the findings related to clinico-pathological characteristics in which the tumors had a median thickness of 6.4 mm, ulceration was present in 71%, and vascular invasion in 36%, indicating the presence of advanced and aggressive melanoma (14).

The study done by Ibrahim M et al in Sudan 2015 showed that Pigmentation was found in 83.7% of all cases while others showed epithelioid cell type (61.2%), spindle cell type (32.7%), extremely bizarre cells (4.1%), and mixed cell types (spindle and epithelioid) in 2.0% of all cases. Also another study done by Thompson B at Queensland revealed that the most predominant cell types of malignant melanoma were epitheliod, spindle and nevoid (18).

The study done Haydu L.E et al showed that Less than half (n = 672, 48%) of the excision specimen reports of primary invasive cutaneous melanomas included all seven essential histopathological features. Of the 317 partial biopsy reports, 190(60%) contained the four essential features that should be measurable in these reports. Some partial biopsy reports also recorded the three margins (17%). Breslow thickness and level of invasion (Clark) were the best recorded features: they were specified in >90% of reports on both excision specimens and partial biopsies. Margins were the most incompletely reported features (30).

The study done by Monshizadeh L et al, at Western Australian Melanoma Services(WAMA), revealed that the malignant melanoma cases had important histological features assessed as follows, ulceration in 42.5% and mitotic rate in 40.8% of tumors. Breslow thickness and the Clark level were always assessed and excision margins were reported in 98% of cases. In addition, there was no documentation on the presence or absence of microsatellites in 79.1%, growth phase in 76.4%, perineural invasion in 64.1%, predominant cell type in 58.2%, tumor infiltrating lymphocytes in 51%, regression in 49%, and vascular invasion in 37.9% of cases of malignant melanoma (30).

2.3 Comparison of the completeness of diagnostic and prognostic histomorphological features of malignant melanoma cases between synoptic versus narrative reporting format

The study done in Brazil among the patients identified with nodular melanoma, the majority was Clark IV (46.3%) or Clark V (33.7%). Regarding acral lentiginous melanoma, the Clark V was predominant (52.4%). Of those who had lentigomaligna melanoma, the Clark level predominant was II (76.5%). The superficial spreading melanoma group had a mean age of 65.3 years and 48.6% were Clark III. Of the total sample of invasive CM, there was a predominance of cases with Clark IV (n = 65, 32.7%) and V (n = 52, 26.1%) levels. Tumor-

infiltrating lymphocytes, ulceration and histopathological regression were found in 76.9%, 54.8% and 17.1% of the invasive tumors respectively. The mitotic index was ≥ 6 mitoses/HPF (high-power field) in 37.2%. This value was statistically significantly higher (p = 0.011) in men (n = 45, 46.4%) than in women (n = 29, 28.7%), (24).

The study done by Karim et al, has shown that the differences in the frequency with which the most important histopathological features for management, staging and prognosis were mentioned were as follows. Breslow thickness was reported in 100% of synoptic and 97.3% of non-synoptic SMU reports (P < 0.001) and in 99.6% of synoptic and 91.1% of non-synoptic non-SMU reports (P < 0.001). Clark level was reported in 100% of synoptic and 90.2% of non-synoptic SMU reports (P < 0.001) and in 100% of synoptic and 83.6% of non-synoptic non-SMU reports (P < 0.001). (32)

The presence or absence of ulceration was reported in 99.6% of synoptic and 67.4% of non synoptic SMU reports (P < 0.001) and in 99.6% of synoptic and 53.5% of non-synoptic non-SMU reports (P < 0.001). Mitotic index was reported in 100% of synoptic and 79.3% of non-synoptic SMU reports (P < 0.001) and in 99.6% of synoptic and 78.1% of non-synoptic non-SMU reports (P < 0.001). The distance from the nearest lateral ⁄ peripheral margin was reported in 99.7% of synoptic and 60.9% of non synoptic SMU reports (P < 0.001) and in 98.7% of synoptic and 65.4% of non-synoptic non-SMU reports (P < 0.001). The distance from the deep margin was reported in 99.7% of synoptic and 47.8% of non synoptic SMU reports (P < 0.001) and in 94.8% of synoptic and 38.7% of non-synoptic non-SMU reports (P < 0.001). (32)

The study done by Maley A et al to assess the documentation of important elements in reporting malignant melanoma as suggested by college of American pathologists, elements critical to staging, such as ulceration, mitotic rate, and microsatellitosis, were omitted in more than 50% of non-synoptic reports, with tumor thickness absent in 10%. The comparison between synoptic versus narrative pathological in reporting essential parameters were as follows procedure type 64 (77%) vs 26 (67%) p value.22,specimen laterality 76 (92%) versus 35 (90%) p value.74 tumor site 83 (100%) versus 38 (97%) p value.14,histologic type 54 (65%) versus13 (33%) p value.001, maximum tumor thickness 83 (100%)versus 35 (90%) p

value.003,Ulceration 80 (96%) versus 19 (49%) p value.001,margins 67 (81%) versus 18 (46%) p value.001, mitotic rate (index) 81 (98%) versus 24 (62%) p value .001 ,Microsatellitosis 56 (67%) versus 6 (15%) p value .001,Lymph-vascular invasion 71 (86%) versus 14 (36%) p value.001,Pathologic staging 38 (46%) versus 3 (8%) p value .001.the overall percent for inclusion of essential parameters between synoptic and narrative report format all elements included 17 (20%)versus 0 (0%) p value .002 (31)

Also another study done by Murali R et al on comparison of intraobserver variability of histopathological reporting of key prognostic parameters revealed that, there was excellent agreement between SMU and non-SMU pathologists in the assessment of Breslow thickness (kappa = 0.883; intra class correlation coefficient = 0.968) and ulceration (kappa = 0.832), while that for Clark level was only moderate (kappa = 0.627). The degree of concordance in the assessment of overall pathologic stage was excellent (96.2%), but there was poor agreement for the evaluation of sub stages IB (77.3%) and IIB (72.4%), due largely to poor Clark level concordance. Although there was a high level of agreement between pathologists in the assessment of overall pathologic stage, the significant level of discordance in pathologic sub staging suggests that accurate and reliable sub stage-based prognostic prediction may not be possible in all cases (37).

CHAPTER THREE

3.0 METHODOLOGY

3. 1.Study design

Hospital based, retrospective descriptive cross sectional study

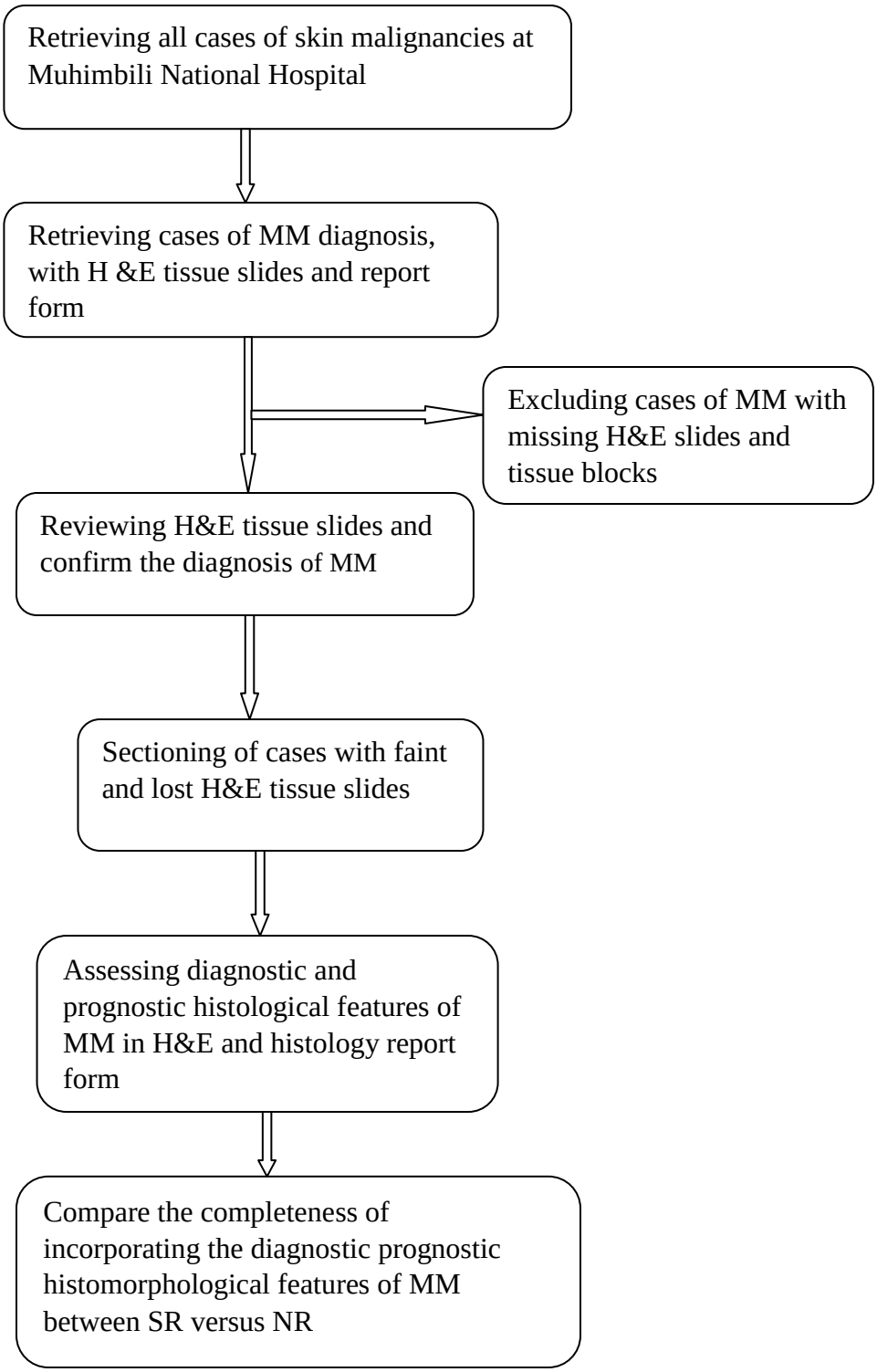
3.2 Study duration

This study will be conducted for 5 years' duration of confirmed cases of malignant melanoma at Muhimbili National Hospital from January 2015 to December 2019.

3.3 Study area

The study will be conducted at Muhimbili National Hospital, located at East Upanga Ilala district the region of Dar es Salaam the largest teaching and referral hospital. The hospital has 1,500 bed facility, attending 1,000 to 1,200 outpatients per day, admitting 1,000 to 1,200 inpatients per week. The patients' population are coming from Dar es Salaam and within coastal regions and a small proportion of patients coming from other regions of Tanzania, most of them are referral cases. Specifically, the study will be conducted at the histopathology unit of Central Pathology Laboratory (CPL) of Muhimbili National Hospital. The histopathology unit receives about 10000 to 120000 biopsies per year and about 1000 biopsies per month.

3.4 Work flow


Retrieving all cases of skin malignancies at Muhimbili National Hospital
Retrieving cases of MM diagnosis, with H &E tissue slides and report form
Excluding cases of MM with missing H&E slides and tissue blocks
Reviewing H&E tissue slides and confirm the diagnosis of MM
Sectioning of cases with faint and lost H&E tissue slides
Assessing diagnostic and prognostic histological features of MM in H&E and histology report form
Compare the completeness of incorporating the diagnostic prognostic histomorphological features of MM between SR versus NR

3.5 Study population

All confirmed cases of malignant melanoma whose biopsies were submitted to histopathology laboratory unit at Muhimbili National Hospital from January 2015 to December 2019 will be enrolled.

3.6 Inclusion criteria

All cases diagnosed and confirmed with malignant melanoma during mentioned study period above will be included and should have request forms with relevant clinical information such as age, sex and site of the biopsy taken, histology report form, tissues blocks and H&E tissue slides. All the cases will be read by the investigator and reviewed by supervisor for final diagnosis confirmation.

3.7 Exclusion criteria

Cases with unavailable H &E tissue slides and tissue blocks and histology report form will be excluded from the study.

3.8 Sample size estimation and selection.

The true prevalence of malignant melanoma in Tanzania is still not known but there is increasing trend of malignant melanoma cases. However, the study done by Chalya PL *et al* (2012) showed that prevalence of malignant melanoma was 8.6% where as it constituted 104 cases of malignant melanoma out of 1203 cases of malignancies reported in the period of 3 years at University teaching Hospital in north-western Tanzania

The sample size will be obtained by Kish and Leslie formula $n = Z^2*p*(1 -p)/e^2$

Whereby;

n=represents the number of malignant melanoma required

Z =represents the number of standard errors away from the mean = 1.96

p = represents the estimated proportion = 8.6%= 0.086

e= represents the estimated level of precision= 5% = 0.05

Thus**,** the sample size for 95% confidence level is; n=99

3.9 Sampling method

The random sampling of malignant melanoma cases will be obtained from the histopathology reports archive, jeeva system, tumor board book as well as register book within study period. All malignant melanoma cases will be retrieved and reviewed to confirm the diagnosis and assessment of essential histological features to be included in the histopathology report. All the cases of malignant melanoma with request forms and Hand E slides will be given serial number and selected by simple random sampling to attain the required sample size, for the lost and faint H&E slides, available tissue blocks will be retrieved, recut and stain with hematoxylin and eosin.

3.10 Data variables

Independent variable; Essential histomorphological features such as Breslow thickness, mitoses, ulceration margin status and histopathological reports.

Dependent variable; malignant melanoma, age and sex

3.11 Data collection techniques.

The special designed standard template with all required essential histormorphological features set as gold standard for the quality synoptic reporting of malignant melanoma will be used to collect data from the selected cases of malignant melanoma which will meet the inclusion **criteria then assessment of documentation of the essential histological features in narrative report versus synoptic report then review the H and E tissue slides to look whether some of the essential features not reported due to lack of synoptic guiding tool to include all essential histomorphological features present in H and E tissue slides** . Socio demographic characteristics and essential histomorphological features of malignant melanoma will be filled in and will be pretested at MNH before actual commencement of the study to ensure validity and reliability of the data that will be collected.

Also slides will be reviewed to confirm diagnosis of malignant melanoma and evaluating the presence of essential histomorphological features required in the prepared synoptic template

form of malignant melanoma. In case of lost H and E tissue slide the tissue block will be sectioned and staining will be carried out as explained below

H&E staining

1. This will be done to all selected cases with lost slides or deleted slides
2. Tissue blocks will be retrieved for sectioning. Sections of 3 micrometers will be cut from iced paraffin embedded blocks using rotary microtome (Sakura model) SRM200 CW, mounted on slide labeled with ID number and will be allowed to drain before being put on hot plate at 60 degrees centigrade for 15-30 minutes.
3. Sections will be dewaxed in two changes of xylene ten dips each and brought down to water through descending grades of ethyl alcohol (absolute, 95%, 80%, 70%),
4. The section will be stain with Harris hematoxylin for ten minutes, washed in tap water until water is clear, and then will be differentiated in 1% HCL in 70% ethanol for three seconds.
5. Thereafter tissue sections will be washed in running tap water and subsequently blued in running tap water for ten minutes.
6. The sections will be counter stained in 1% aqueous eosin for three minutes, and will be washed in tap water until water is clear, then they will be dehydrated in ascending grades of ethanol (70%, 80%, 95% and absolute) 10 dips each,
7. Clearing of the section in two changes of xylene and then mounted in DPX and labeled.
8. Interpretation of results

H&E slides will be read by the investigator to confirm the diagnosis of malignant melanoma and assessing the essential histomorphological features then will be compared to the prepared template set as gold standard for quality synoptic reporting of malignant melanoma and then reviewed by the senior pathologist.

3.12 Investigation tools, validity and reliability

Data captured sheet standard synoptic template with all the essential histomorphological features required in quality report of malignant melanoma will be used as investigation tool. In case of the staining of slides will follow the hospital and manufacturer SOP, and prior to the staining of the of study slides, there will be a pretest of staining so as to assure validity and reliability of the stains. Data will be managed electronically using SPSS computer software version 20 (IBM, USA) computerized software program after cross checking the filled checklists for quality control of the data which was obtained from the selected cases which met the inclusion criteria. The obtained data will be analyzed using Statistical Package for Social Sciences (SPSS) version 20 whereas correlation of important pathological features will be analyzed using Spearman's rank correlation coefficient.

3.13 Data analysis

Data of essential histomorphological features of malignant melanoma assessed and described in H and E slides then confirmed in the prepared template (set as gold standard for quality report) will be recorded and analyzed by SPSS version 20 and demographic data will be analyzed by frequency and percentages. P value will be computed for categorical variables using chi-square test and fisher exact test. P value of less than 0.05 will be considered statistically significant. Correlation of important pathological features will be analysed using Pearson’s correlation coefficient.

3.14 Ethical considerations and approval

The proposal will be presented to the department of Pathology of Muhimbili University of Health and Allied Sciences. Ethical clearance will be sought from the Research and Publication Committee of the School of Medicine and from the Senate Research and Publications Committee of Muhimbili University of Health and Allied Sciences. Administrative permission to conduct the study will be obtained from Muhimbili National Hospital as per the hospital management protocols. **This retrospective study will use histopathology reports, tissue blocks and H and E tissues slides from archives of MNH-CPL, however ethically the special permission will be asked from higher authority of**

MNH as per hospital management protocols to utilize archives materials for data collection also the confidentiality of the data will be highly observed in the such a way that the identity of the patient will not be disclosed I will identify the patients with code number

3.15 Study limitations

Some cases of malignant melanoma may be missed due to loss of reports form and tissue blocks as well as H and E slides

3.16 Study mitigation

The protocols will be strictly followed to have a thorough searching of available request form report form and H and E slides even precutting tissue blocks and staining tissue slides. The tissue slides will be examined by the investigator and re-examined by the supervisor.

CHAPTER FOUR

4.0 BUDGET AND WORK PLAN

4.1 Budget

4.1.1 Summary of the budget and allocation

The estimated budget for this study will be 2,305,000 Tanzanian shillings this will be funded by Ministry of Health, Community, Development, Gender, Elderly and Children (MoHCDEC) of Tanzania.

ITEM CATEGORY	UNIT OF ITEMS	UNIT COST (Tshs)	QUANTITY NEEDED	TOTAL COST(TSH)
Hematoxylin for slide staining for histopathological evaluation	12.5 gm/ box	92,500	4 boxes	370,000
Eosin for slide staining for histopathological evaluation	12.5gm/ box	37500	4 boxes	150,000
Xylene as clearing reagent	0.5 liter	55,000	1l	110,000
Absolute alcohol for rehydration during staining procedure	2.5 liter	95,000	5l	170,000
Super frosted glass charged slides	75pcs/box	85,000	3	255,000
Mounting media DPX	100mls	100,000	2	200,000
Marking and labelling pens for slide	1 pack	280,000	1	280,000
Slide coverslip	100pcs	60,000	2	120,000
Stationery services	N/A	250,000		250,000
Assistant technologists for block sectioning and staining procedure	N/A	200,000	2	400,000
GRAND TOTAL				2,305,000

4.2 Budget justification

Funds are required to purchase hematoxylin and eosin stain, absolute alcohol, xylene, Marking and labelling pens, glass slides, coverslips, mounting media and stationery because these will not be freely available during the study.

4.3 Work plan

	JAN-MARCH 2020	APRIL-JUNE 2020	JULY - SEPT 2020	AUG-DEC 2020	JAN-MARCH 2021	APRIL-JULY 2020
Proposal development and ethical clearance						
Data collection and laboratory work						
Data entry and analysis						
Report writing and submission						
Manual script development						

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APPENDICES

Appendix i: English questionnaire

TEMPLATE FOR ESSENTIAL HISTOMORPHOLOGICAL FEATURES IN REPORT OF MALIGNANT MELANOMA.

Date …….

1. Patient hospital registration number……..
2. Patient HP number……..
3. Study number…….
4. Age: 5-20
    - 21-35,
    - 36-50,
    - 51-65,
    - >66
5. Sex …….
6. Site of the biopsy…….
7. Type of biopsy…….
8. . Histopathological subtypes
    - Lentigomaligna melanoma …… Superficial spreading …….Nodular Melanoma…….
    - Acral lentiginous ……. Desmoplastic ……..

Not otherwise classified……Other (specify) ……

9. Breslow thickness (depth) ..... mm

10. Ulceration: Not identified……. Present……. Cannot be assessed ……

11. Mitotic count ……per 10HPF

12. .Lymph vascular invasion: Not identified……. Present…… Cannot be assessed……

13. .Microsatellite/in-transit metastasis: Not identified…… Present……. Cannot be assessed……..

14. .Margin: Involved……. Not involved…… Not applicable…….

15. Neurotropic/perineural invasion: Not identified…….. Present……. Cannot be assessed…….

16. Anatomic (Clark) Level (applicable to invasive tumor only)

At least level……(explain)……. ___ II (melanoma present in but does not fill and expand papillary dermis) ___ III (melanoma fills and expands papillary dermis)___ IV (melanoma invades reticular dermis) ___ V (melanoma invades subcutis) ___ Cannot be determined

17.Tumor-Infiltrating Lymphocytes (applicable to invasive tumor only)

___ Not identified, ___ Present, nonbrisk, ___ Present, brisk, ___ Cannot be determined

Appendix ii: Procedures for Section cutting

This procedure is applicable during section cutting at Muhimbili National Hospital, Central Pathology Laboratory

1. Insert a microtome blade in the knife holder of the Microtome machine and screw it tightly in position.
2. Set the Microtome to trim at 15-20 µ
3. Cover the blade and fix tissue block in the Microtome's block holder.
4. Adjust the feed mechanism until the wax block touches the knife by moving the block holder forward and upward.
5. Ensure that the whole surface of the block will move parallel to the edge of the trimming blade in order to ensure a straight ribbon of section.
6. Tighten all adjusting screws on the Microtome as faults on sectioning are most frequently due to looseness of the block or knife.
7. Trim the block at 15-20µ until full surface of the tissue is exposed.
8. Remove the trimmed tissue block from the Microtome and place it face down on Tissue-Tek's Cooling part for at ≈15 minutes.
9. Replace the fixed microtome blade with the new one and check that it is screwed tightly in the position.
10. Set the Microtome's thickness gauge to 3 µ for section cutting.
11. Add 2-3 drops of 70% alcohol on a clean glass slide.
12. Wipe the surface of the cooled tissue block free of water and place it into the block holder.
13. Operate the Microtome until complete sections have been cut.

14. Pick the cut section and place it on a glass slide containing 70% alcohol.
15. Float the cut section onto the water bath (at 45°C).
16. Label another clean frosted glass slide with same PID as on the block and use it to pick the floating cut section.
17. Stand the slide on a grooved wooden rack and leave for 10- 15 minutes to dry.
18. Place the dried slide with cut section onto hot plate (600C) to melt the wax.
19. Arrange in a staining rack and send for staining procedure immediately after removing from the hot place.

Appendix iii: Procedures for Manual Hematoxylin and Eosin staining

This procedure is applicable during manual Hematoxylin and eosin staining at Muhimbili National Hospital, Central Pathology Laboratory.

1. Dewax sections and bring down to water.
2. Stain in Harris hematoxylin for 10 minutes.
3. Rinse in running tape water.
4. Differentiate in 1% acid alcohol for 5-10 seconds.
5. Rinse in tap water
6. Blue the sections in tap water for 10 minutes.
7. Counter stain in 1% aqueous eosin for 3 minutes.
8. Rinse in tap water.
9. Dehydrate the sections through ascending grades of alcohols.
10. Clear in two changes of xylene (3 minutes in each)
11. Mount with DPX or mounting machine.