



Original Article

## Histopathological Evaluation of the Microtomy Artefacts on Haematoxylin and Eosin Section; Hospital Based Cross-Sectional Study

Oscar Ottoman<sup>1, 2\*</sup>, Shaban Urassa<sup>1</sup>, Edrick Elias<sup>1, 2</sup>, Jeffer Bhuko<sup>1</sup> & Aron O. Isaya<sup>2</sup>

<sup>1</sup>Catholic University of Health and Allied Sciences, P. O. Box 1464, Mwanza, Tanzania.

<sup>2</sup>Central Pathology Laboratory, Bugando Medical Centre, P. O. Box 1370 Mwanza, Tanzania.

\*Author for correspondence: ORCID ID: <https://orcid.org/0000-0002-2929-3834>; email: [drosarcarmuhini1988@gmail.com](mailto:drosarcarmuhini1988@gmail.com)

Article DOI: <https://doi.org/10.37284/eajhs.5.1.848>

Date Published: **ABSTRACT**

27 September 2022

**Keywords:**

*Histopathology,*

*Microtomy,*

*Artefacts.*

Background information: Microtomy artefacts are abnormal structures or features in histological slides resulting from tissue sectioning by microtome. Objective: To determine the type and prevalence of microtomy artefacts found in histopathological tissue sections slides at Bugando Medical Centre (BMC). Methodology: This was a cross-sectional observational study that involved 547 consecutive hematoxylin and eosin (H&E) stained sections of histological archived tissue slides of January 2021. The slides were retrieved from the archives of the histopathology laboratory at BMC, Mwanza Tanzania and analysed for artefacts under a light microscope. Results: A total number of 547 histopathological slides were retrieved for the study and 412 (75.3%) slides had microtomy artefacts present while the remaining 135 (24.7%) histopathological slides had no microtomy artefacts. Of 412 slides with microtomy artefacts, 204(49.5%) slides had only one type of microtomy artefacts while the remaining 208 (50.5%) slides had more than one type of microtomy artefacts. There was a total of 672 microtomy artefacts, and the majority 576 (85.7%) were due to section cutting, followed by trimming artefacts in 92 (13.69%) of the slides. The least artefact was floatation which was seen in 4 (0.6%) of the slides. For the floatation artefact, the folding artefact was the most commonly seen in 300(54.8%) of the slides. Conclusion: Higher prevalence of microtomy artefacts at BMC reflects the problem of interpretation of histopathological slides in our setting. Section folding artefacts were the most prevalent pattern of artefact observed in this study.

#### APA CITATION

Ottoman, O., Urassa, S., Elias, E., Bhuko, J., & Isaya, A. O. (2022). Histopathological Evaluation of the Microtomy Artifacts on Haematoxylin and Eosin Section; Hospital Based Cross-Sectional Study. *East African Journal of Health and Science*, 5(1), 318-325. <https://doi.org/10.37284/eajhs.5.1.848>.

#### CHICAGO CITATION

Ottoman, Oscar, Shaban Urassa, Edrick Elias, Jeffer Bhuko and Aron O. Isaya. 2022. "Histopathological Evaluation of the Microtomy Artifacts on Haematoxylin and Eosin Section; Hospital Based Cross-Sectional Study." *East African Journal of Health and Science* 5 (1), 318-325. <https://doi.org/10.37284/eajhs.5.1.848>.

#### HARVARD CITATION

Ottoman, O., Urassa, S., Elias, E., Bhuko, J., & Isaya, A. O. (2022) "Histopathological Evaluation of the Microtomy Artifacts on Haematoxylin and Eosin Section; Hospital Based Cross-Sectional Study." *East African Journal of Health and Science*, 5(1), pp. 318-325. doi: 10.37284/eajhs.5.1.848.

#### IEEE CITATION

O. Ottoman, S. Urassa, E. Elias, J. Bhuko, & A. O. Isaya, "Histopathological Evaluation of the Microtomy Artifacts on Haematoxylin and Eosin Section; Hospital Based Cross-Sectional Study.", *EAJHS*, vol. 5, no. 1, pp. 318-325, Sep. 2022.

#### MLA CITATION

Ottoman, Oscar, Shaban Urassa, Edrick Elias, Jeffer Bhuko & Aron O. Isaya. "Histopathological Evaluation of the Microtomy Artifacts on Haematoxylin and Eosin Section; Hospital Based Cross-Sectional Study." *East African Journal of Health and Science*, Vol. 5, no. 1, Sep. 2022, pp. 318-325, doi:10.37284/eajhs.5.1.848.

## INTRODUCTION

Artefacts are errors observed in scientific investigation or experiment that is naturally present but occurs as a result of the preparative or investigative procedure [1]. Artefacts are classified into three major groups and these are pre-analytic artefacts, analytical artefacts, and post-analytic artefacts where artefacts appear in section [2]. In histopathology, types of artefacts can occur from the point of collection, reception, and grossing, up to the final process of mounting [3].

The accurate diagnosis of pathological conditions under a microscope requires the preparation of tissue sections with the application of stains, in the end, such sections represent as closely as possible a tissue structure in life-like form [4]. The preparation of high-quality sections requires skills and experience in the field of laboratory discipline. Most pathologists encounter slides that are either improperly fixed or mishandled during tissue processing, resulting in alteration in tissue details [5].

Histopathological artefacts are classified as pre-analytical and analytical. The pre-analytical phase includes pre-fixation artefacts and fixation artefacts while analytical phase artefacts occur during tissue grossing, tissue processing, embedding, microtomy, floatation, staining, and mounting [6]. Microtomy

artefacts are abnormal structures or features in histological slides resulting from tissue sectioning by microtome [7]. Routinely, rotary microtome is used in histopathology laboratory for tissue sectioning. Tissue sectioning involves different steps which are: cooling of the tissue block, trimming, sectioning, and floatation process where after, the tissue section is picked up into the slide. Microtomy artefacts are folding, knife marks, indulation, coarse chatter, fine chatter, scores, thin section, thick section, holes, and floater [6].

In microtomy, each artefact is caused by one or combined factors. Scores and tearing sections are caused by a nick or blemish in the knife edge and when sectioning hard particles such as foci of calcification and debris within the block [6]. Chatters may occur due to tiny vibrations in the knife edge, excessive hardness, and brittleness of the block, excessive steep knife angle [6]. Holes may occur due to excessively rough trimming of the paraffin blocks with greater thickness. Floater artefacts may appear due to improper using a dirty towel, knife, or gloves and improper water bath cleaning [6]. Thick and thin sections may occur when the wax is too soft for tissue, the block or blade is loose, the clearance angle is insufficient, or the mechanism of the microtome is faulty [6]. Folds artefact may occur when very thin paraffin sections are forced to stretch unevenly around other structures which have different consistencies [6].

In our setting and Tanzania in general, there is no published data on histopathological evaluation of the microtomy artefact on haematoxylin and eosin sections. Also, the quality section among histopathological slides in our setting showed to have an error section and this may reflect a prolonged Turnaround Time (TAT) on the pathological report. Due to this, the study aimed to determine the type and frequency of microtomy artefacts that occur in the histopathology laboratory at BMC and to create awareness and desire to determine the magnitude of error in histopathology laboratory practices.

## METHODS

This was a cross-sectional study design involving the retrospective archived tissue slide of January 2021 from the histopathology laboratory at Bugando Medical Centre (BMC), Mwanza Tanzania. A total number of 547 Haematoxylin and Eosin (H&E) stained tissue slides were retrieved from the Central Pathology Laboratory (CPL) archive.

A consecutive sampling technique was used in which all H&E histopathological slides registered in January 2021 were recruited into the study. Any slide with no tissue section or broken was excluded from the study. Tissue sections were examined microscopically by the researcher and verified by

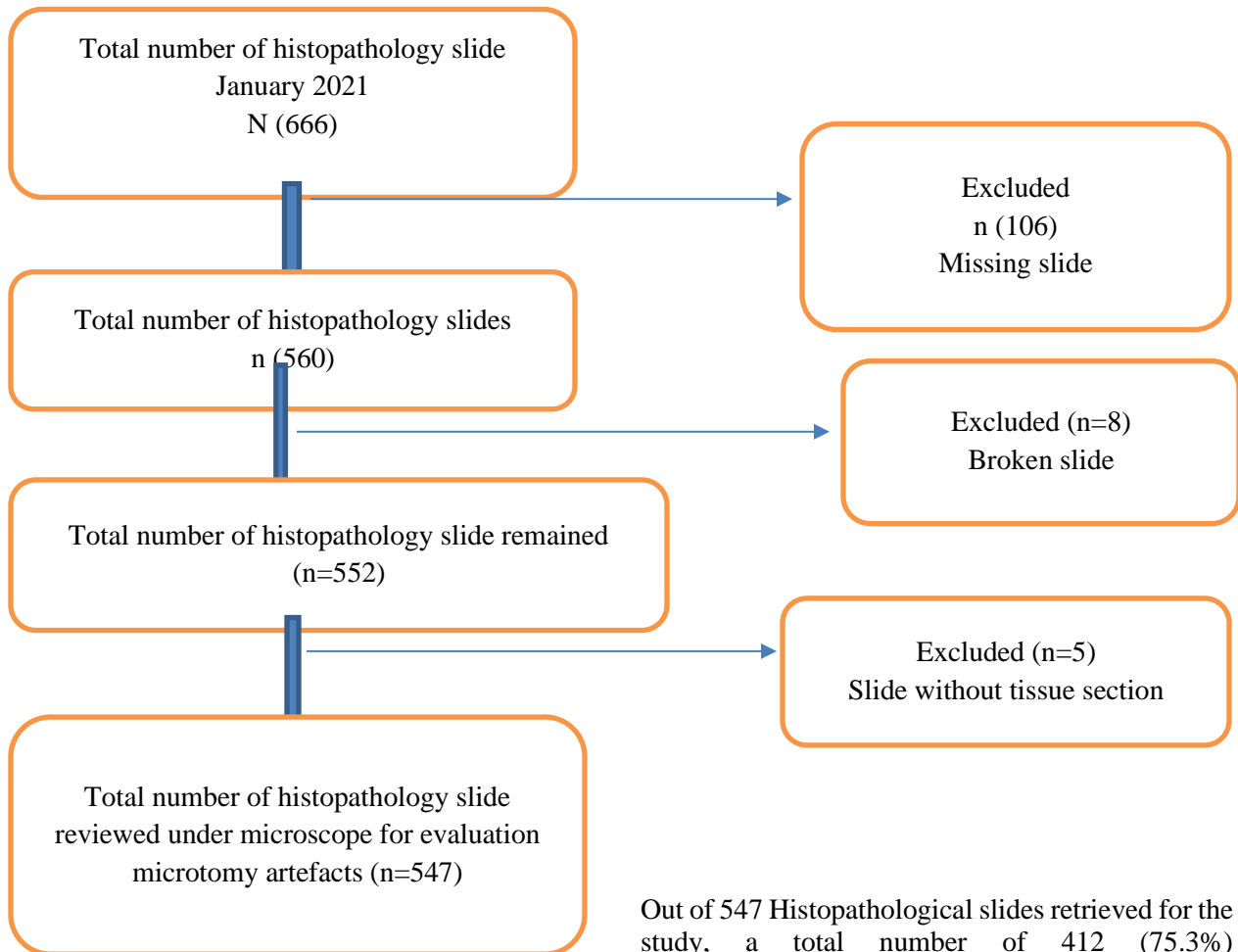
two registered Anatomical pathologists using a light microscope (OLYMPUS CX21) to establish the type of microtomy artefact present based on microtomy steps. All microtomy artefacts observed were categorized into 3 groups, artefacts due to sectioning, artefacts due to floatation, and artefacts due to trimming.

Data collected were entered into Microsoft excel 2016 for data cleaning and finally transferred to Statistical Package for Social Science software (SPSS) version 20 for analysis of prevalence, frequencies, and types of microtomy artefacts. The results were presented by using tables and pie charts. Ethical approval to conduct the study was obtained from the joint CUHAS and BMC research ethical and review committee.

## RESULTS

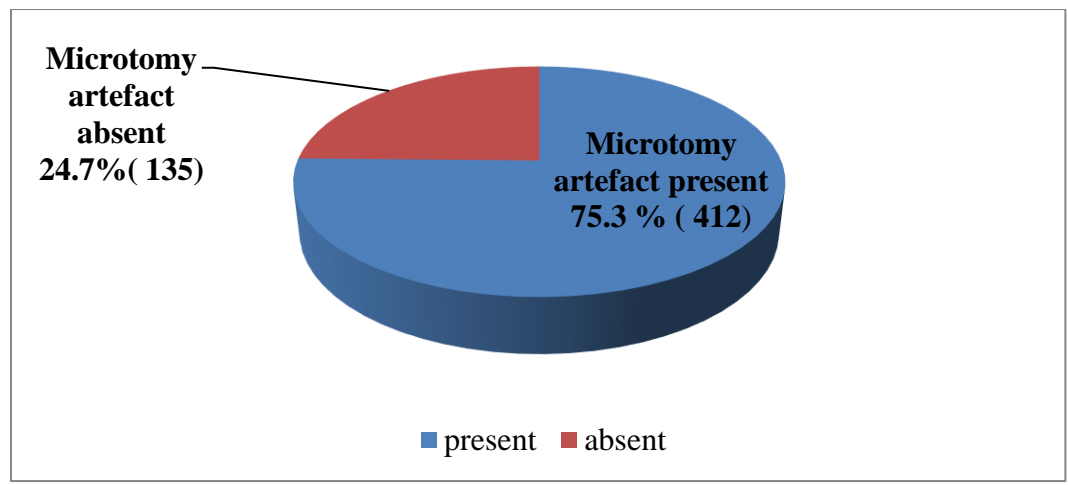
A total number of 666 histopathological slides were registered in January 2021 at the archives of Central Pathology Laboratory (CPL), Bugando Medical Centre in Mwanza, Tanzania. Of all registered slides, a total number of 560(84.1%) H & E-stained slides were retrieved. Among 560 retrieved slides, 547 slides were recruited for assessment of microtomy artefacts (See *Figure 1*), and for final analysis. The microtomy artefacts assessed were those resulting from trimming, sectioning, and floatation.

**Figure 1: Study flow chart**

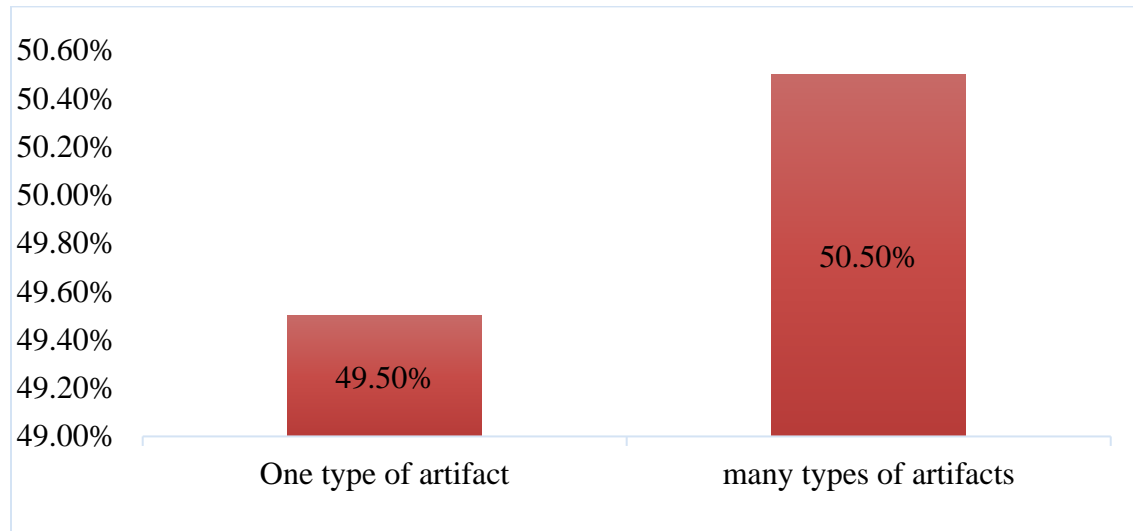


Out of 547 Histopathological slides retrieved for the study, a total number of 412 (75.3%) histopathological slides had microtomy artefacts, and the remaining 135 (24.7%) had no Microtomy artefacts (See *Figure 2*).

**Figure 2: Prevalence of Microtomy artefacts**



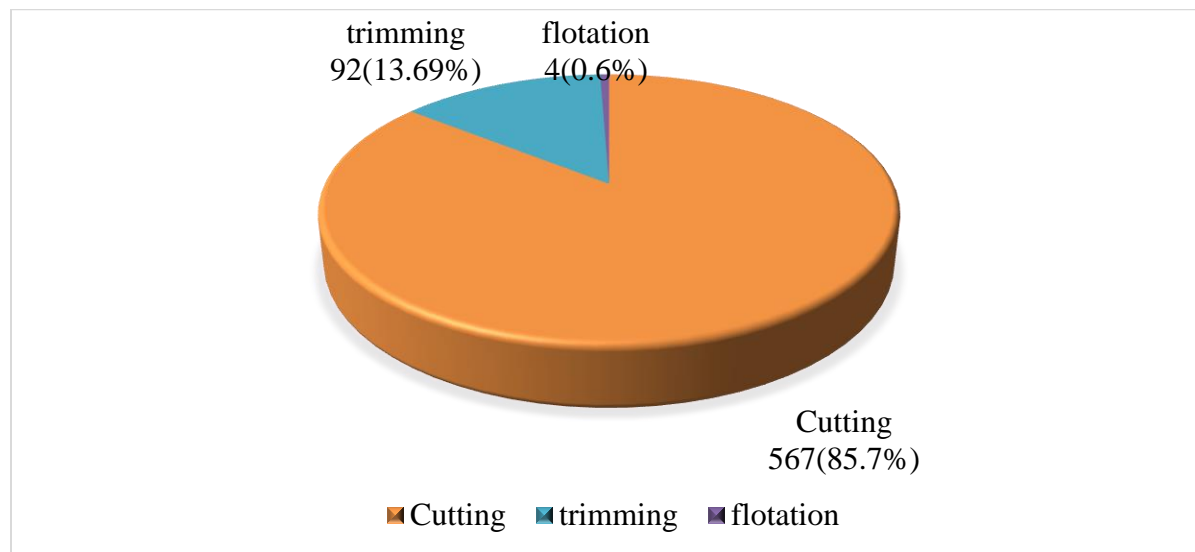
**Figure 3: Prevalence of Microtomy artefacts**



For all 547 slides which were evaluated for artefacts 50.5% had more than one type of artefact while 49.5% had only one type of artefact (see *Figure 3*). Also, a total number of 672 artefacts were identified in all 547 slides, and the commonest 576(85.7%)

were due to section cutting, followed by trimming artefacts seen in 92 (13.69%) slides. Floatation artefacts were present in 4 (0.6%) of the slides. (See *Figure 4*).

**Figure 4: A Pie chart showing the frequency of microtomy artefacts according to different microtomy steps**



In 576 overall microtomy artefacts due to cutting assessed, folds were the most to occur and account for 300 (44.64%), followed by knife mark 177 (26.33%), undulation 59 (8.78%), coarse chatters 31 (4.61%), fine chatters 3 (0.45%), and least was due to scores, tears, thick section each occurred in 2

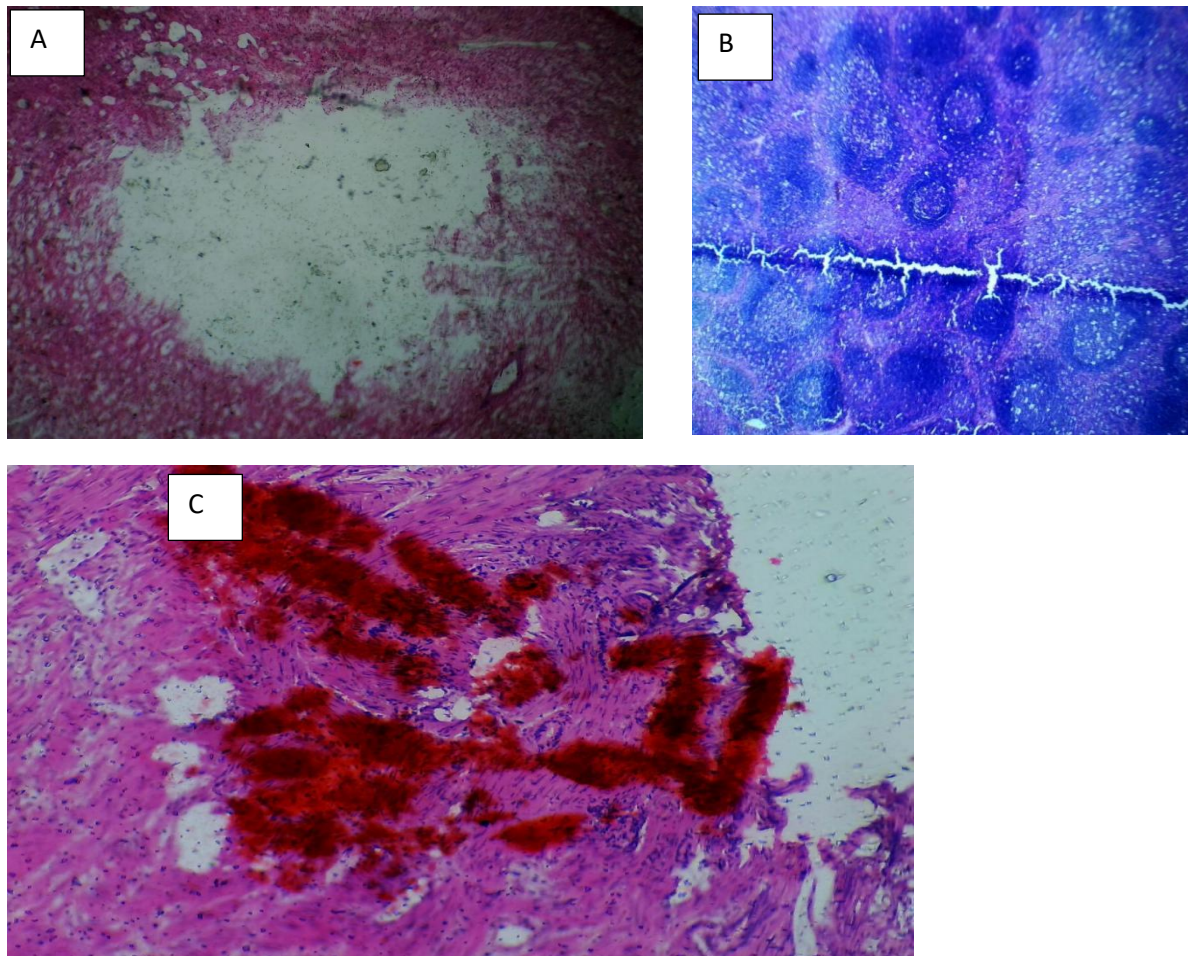
(0.3%) histopathological slides (See *Table 1*). Artefacts due to trimming occurred in 92 (13.6%) slides and all were due to holes artefacts (See *Table 1*). The least artefact based on microtomy steps were artefacts due to flotation and accounts 4 (0.6%) were due to Floaters (See *Table 1*). Histological

section tissue slides with holes, folds, thick sections, and knife scores microtomy errors were taken (see *Figure 5*).

**Table 1: Frequency of microtomy artefacts due to cutting, trimming, and flotation microtomy steps.**

Type Of Artefact	Frequency (N)	Percentage (%)
Folds	300	44.64
Knife Mark	177	26.33
Indulation	59	8.78
Coarse Chatters	31	4.61
Fine Chatters	3	0.45
Scores	2	0.30
Tears	2	0.30
Thick Section	2	0.30
Holes	92	13.69
Floaters	4	0.60

**Figure 5: Microscopic views (x10 HPF) under the H and E sections showed microtomy artifacts. (a) Hole (b) Thick section and Knife scores (c) Folds**



## DISCUSSION

Recognition of microtomy artefacts and taking a measure to overcome them is the biggest challenge in histopathology laboratories in limited-resource countries [6, 8]. Microtomy artefacts were introduced into the tissue during the techniques of tissue sectioning, trimming, and tissue flotation [6,9]. The commonest reason for microtomy artefacts to occur in histopathology laboratories were lack of regular training for technical staff, overuse of microtomy machines without maintenance, lack of supervision in the analytical phase of histopathology laboratory, and a low number of technical staff, and overwork [8,10]. Microtomy artefacts varied from the smaller ones to the largest ones, due to this some artefacts may be able to make the tissue architecture non-diagnostic and finally difficult to the interpretation of slides result and delay in turnaround time [4]. This may lead to poor management of the patient and finally increase the mortality rate [4, 9]. Despite having experienced technicians still, the prevalence of microtomy artefacts is raising [9].

In this study, the majority of histopathological H&E-stained sections had more than one type of microtomy artefacts. This finding differs from a study done by Igho et al. in Nigeria which showed that the majority of H&E-stained slides had one kind of artefacts [10]. The probable reason for the low number of histopathological sections to have more than one type of artefacts compared to our study was due to low number of tissue slides assessed (388 histological tissue slides)

The prevalence of microtomy artefacts was 75.3%, and the majority were due to the tissue sectioning step. Some studies done in developing countries showed the same result of tissue sectioning step was also the leading cause of artefacts [8, 10], and the reasons have been linked to poor tissue fixation, processing, and poor microtomy techniques. The same reasons have been observed in studies done in India and Iran [8, 11].

In developed countries studies done in Italy showed a low prevalence of microtomy artefacts about 23%. The reasons were attributed to good device maintenance, quality assurance, calibration, and function performance checking for microtome,

water batch, hot plates, and other devices used during microtomy [12]. Also, proper techniques for microtomy and being experienced and expert in microtomy may help to reduce the prevalence of artefacts in developed countries [12].

In this study, section cutting was the common cause of microtomy artefacts followed by trimming and very few cases were associated with flotation. In tissue sectioning, the common artefacts was folding. These findings coincide with other studies done in India which showed that folds were the most common artefacts during section followed by scoring and split section [8]. Also, a study done in Nigeria by Igho et al. showed the same findings of the highest number of microtomy artefacts were due to folds followed by tearing and knife edge [10]. The probable reason for the highest number of folds artefacts is due to warm block, section too thin, clearance angle too great, water bath, poor flotation techniques, and poor fixation and processing techniques.

Artefacts due to trimming were associated with hole formation in tissue sections, and this has been observed in Iran and Nigeria [10, 11]. Excessive rough trimming of paraffin blocks with a greater thickness causes tissue fragments from block to face, thus appearing as a void space [13].

In this study, artefacts due to flotation occurred less frequently, and these findings have been observed in one study in India. The reason for the low number of floaters was due to the proper use of water baths, cleaning, and use of distilled water rather than tap water in the most histopathological laboratory [8].

## CONCLUSION

The prevalence of microtomy artefacts in the histopathological section done at the Central Pathology Laboratory in BMC was high. The majority of histological sections present more than one type of microtomy artefacts. Section folding artefacts were the most prevalent pattern of artefacts observed in this study.

## LIMITATIONS

Being a retrospective study, we could not establish how these errors affect Turnaround Time (TAT) and the quality of the results

**ACKNOWLEDGMENT**

We would like to thank Bugando Medical Centre (BMC) which permitted us to conduct this study at their hospital. Our sincere appreciation goes to Central Pathology Laboratory (CPL) histopathology unit for providing us with archived histological slides and extreme support during data collection. We would like to express sincere thanks to all staff of the Histopathology Unit at BMC and the CUHAS pathology department.

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