AUDIT OF HISTOPATHOLOGICAL PROCESSES OF RETINOBLASTOMA COLLABORATIVE LABORATORY AT THE DENTAL SCHOOL UNIVERSITY OF NAIROBI

BY

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DECLARATION

I certify that this dissertation is my original work and has not been submitted for a degree award in any other University.

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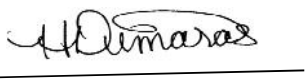
   Signature  Date _____

DEDICATION

This work is dedicated to my loving family.

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ABBREVIATIONS

AFRO	African Regional Office of World Health Organization
CAP	College of American Pathologists
DECF	Daisy's Eye Cancer Fund
EUA	Examination under Anaesthesia
FNA	Fine Needle Aspirate
KNH	Kenyatta National Hospital
MOH	Ministry of Health
RbCoLab	Retinoblastoma Collaborative Laboratory
SDS	School of Dental Sciences
SEER	Surveillance, Epidemiology and End Results data base (National Cancer Institute)
SOP	Standard Operating Procedure
SPSS	Software Package for Social Sciences
TNM	Tumor, Nodes, Metastases
UON	University of Nairobi
WHO	World Health Organization
CEV	Carboplantin, Etoposide, Vincristine
RBI	Tumour suppressor gene
ERC	Ethics Research Committee
USA	United States of America
pTNM	Tumour, Nodal status and Metastasis (staging system)

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ABSTRACT

Background

Retinoblastoma is the most common primary malignant intraocular tumor of childhood presenting before age of 3 years. In Africa and other developing countries, mortality rates for retinoblastoma patients are high (over 40%) due to diagnostic inefficiency and insufficiency. Histopathology laboratories required to report on retinoblastoma pathology do not have sufficient quality assurance measures which result in delayed diagnosis and therefore late treatment of these patients in Africa. Although laboratories within the public health care systems in Africa have adopted the World Health Organization AFRO (regional office of Africa) step wise quality assurance program, its implementation in surgical pathology is deficient. There is lack of data on quality assurance and therefore constraints in evidence based laboratory medicine practice in Africa. Innovative implementation science initiatives on quality assurance in African laboratories are therefore required. These would lead to improvement of histopathology practice resulting in quality retinoblastoma diagnostics which can be implemented in laboratories within the region.

Objectives

The main objective of this study was to conduct an audit of histopathology laboratory processes of the Retinoblastoma Collaborative Laboratory at the University of Nairobi Dental School.

Methodology

This was a retrospective study conducted at the Retinoblastoma Collaborative Laboratory at the University of Nairobi School of Dental Sciences. Formalin fixed and paraffin embedded tissues submitted for evaluation of suspected or confirmed diagnosis of retinoblastoma over a period of three years (2012-2014) were included in the study. The requisition forms were retrieved and examined for appropriate entries and gross descriptions. The corresponding histopathological slides were retrieved from the archives and examined for integrity. They were examined by two blinded pathologists. Data was recorded in proformas and analysed using SPSS version 20. Demographic data was presented in tables, while dependent variables was presented in charts. All statistical tests were performed at 5% level of significance (95 % confidence level).

Results

One hundred and thirteen (113) cases of retinoblastoma were analyzed. Clinical history was indicated in 101(89.4%) cases. Age in months was indicated in only 7 (6.2%) cases the rest was indicated in years, mean age at presentation was 3 years. Date of surgery was indicated in 108 (95.6%) of the cases. Documentation of the gross findings like specimen dimensions was indicated in all cases. Documentation of important prognostic histopathologic features (optic nerve involvement) was omitted in 16 cases (14.2%) initial reports. Ninety six cases (85%) had adhered to College of American Pathologists Standard CAP) both in reporting and processing.

Conclusion

Most of the ophthalmologists provide adequate clinical information for retinoblastoma specimens. Gross examination by the pathologist is satisfactory. Reporting of prognostically important histopathological features of retinoblastoma by pathologists was complete in the majority of cases. Level of agreement between the initial histopatholgical findings and audited reports was present (this was established using Kappa value), and agreement by chance excluded. The laboratory has not fully adhered to college of American Pathologist (CAP) standards recommended for eye processing specifically for retinoblastoma especially in reporting tumor size and location after resection.

Recommendations

The laboratory should participate in external quality control program. Periodic audits should also be integrated as part of the routine system to enable them maintain quality. The tool that has been used to audit this laboratory can be used to audit other histopathology laboratories. Continue use of Proforma to achieve 100% capture of clinical information, and 100% adherence to CAP standards.

1.0 INTRODUCTION

Retinoblastoma is the most common intraocular cancer of childhood. It is an aggressive tumor of the eye that is initiated by mutation of the *RB1* gene which is the first described tumor suppressor gene[1]. Constitutional loss of one *RB1* allele predisposes an individual to this cancer. Loss of the other allele from a developing retinal cell initiates development of retinoblastoma tumors.

Incidence of retinoblastoma is constant worldwide at one case per 15000-20000 live births, which corresponds to about 9000 new cases every year worldwide[1]. The disorder has no validated geographic or population hotspots, but the greatest disease burden is recorded in large populations that have high birth rates such as in Asia and Africa. Regions with greatest prevalence also have the highest mortality. More than 40% of children with retinoblastoma in Asia and Africa die compared to over 90% survival rates in Europe, Canada and the USA[1]. In India studies have shown that there is a 2-3 times reported increase in the incidence of tumors of the eye (majority of which are retinoblastoma in children<15 years of age[2]. In Nigeria retinoblastoma is the most common eye tumor and it tops the record in childhood malignancies[1], while in Kenya the incidence of retinoblastoma is 1:17,030 live births[3].

In Canada mean age of retinoblastoma at diagnosis is 27 months for unilateral retinoblastoma and 15 months for bilateral disease[1]. In India, the mean age at diagnosis is 36 months for unilateral and 24 months for bilateral retinoblastoma[2], In Kenya mean age at diagnosis is 36 months for unilateral retinoblastoma, and 25 months for bilateral disease[1]. Retinoblastoma tends to be diagnosed later in developing countries.

Early diagnosis of the disease leads to effective management with sight saving options[4]. A delay of more than 6 months from the first clinical sign to diagnosis is associated with (70%) mortality recorded in developing countries[1]. In Canada and developed countries early diagnosis and genome counseling has led to effective diagnosis and management of the disease[4]. In vitro fertilization and genetic studies have also been suggested as a way of reducing the rate of retinoblastoma in families with RB1 mutation[4].

Retinoblastoma prevalence is still high in developing countries including Kenya, due to late diagnosis as a result of poor access to appropriate health care services equipped with adequate histopathology laboratory services[4].

The Retinoblastoma Collaborative Laboratory (RbCoLab) was established in Kenya in July 2012. Its aim was to improve retinoblastoma care, in terms of facilitating early diagnosis which leads to early treatment and possible preservation of the eye. This was to reduce the turnaround time since specimen were taking too long to be reported in the general laboratory hence delay in management of patients.

CAP standards have been chosen in this study because it is currently in use in this laboratory and it is an internationally recognized and accredited body that assesses laboratories.

The aim of this study is to assess the performance of this laboratory, also to assess the performance against College of American Pathologists' (CAP) standards and to give recommendations on areas that need improvement.

2.0 LITERATURE REVIEW

Retinoblastoma is an eye tumor of infancy and childhood. It has a characteristic aggressive growth and a genetic aetiology[1]. It arises from the retina of the eye and in rare case from the pineal gland[5].It has been reported to be approximately 11% of all cancers occurring in the first year of life, and 3% of the cancers developing among children younger than 15 years of age, according to the SEER registries studies done in the period between1975-1995[5]. In United States of America approximately 300 children and adolescents younger than 20 years are diagnosed with retinoblastoma annually. Majority of the cases occur in young children, with two-thirds (63%) of all retinoblastoma's occurring before the age of two years, and 95% occurring before the age of 5 years. Studies by Young et al[5] showed that bilateral tumors were age dependent with most cases occurring in children less than one year, with no gender bias[5].

Retinoblastoma has a favorable prognosis in developed countries, with more than 93% surviving at five years. This has been heightened by early diagnosis (in utero) where facilities are available[6]. However, in less economically developed countries mortality is high due to late diagnosis and poor socioeconomic status, since early diagnosis of retinoblastoma is influenced by socioeconomic and maternal educational factors [6,7]. This is further worsened by poor awareness of retinoblastoma by both the public and healthcare professionals. Poor access to appropriate healthcare facilities is another reason for increased mortality in retinoblastoma in less economically developed countries, Kenya included [3,6]. Delayed diagnosis leads to tumor spread beyond the confines of the eye which is almost impossible to cure.

Lack of standardization of laboratory processes can lead to incomplete reports being released from the laboratory hence resulting in poor patient management in terms of choosing appropriate treatment plan for the patient[8] which can lead to increased mortality rate. Improvement of lab processes in conjunction with clinical management is expected to reduce the mortality rates.

2.2 Aetiology

Retinoblastoma is caused by mutation of the RB1 gene (the first described tumor-suppressor gene). Constitutional loss of one RB1 allele predisposes an individual to cancer, loss of the allele from a developing retinal cell starts off the development of retinoblastoma tumours[1] (Alfred Knudsons two hit hypothesis).

Retinoblastomas are classified into two types: those linked to genetic mutations and sporadic retinoblastomas [5]. The malignancies that are genetically linked are further classified into two groups: Those occurring in children who carry retinoblastoma gene inherited from one parent which is also known as familial retinoblastomas, and the ones in which the disease occurs as a result of a new mutation, usually in the father's sperm but sometimes also can occur in the mother's egg, this is also known as a sporadic heritable retinoblastoma. Hereditary retinoblastomas are likely to be bilateral and to occur most likely during the first year of life. Sporadic retinoblastomas mostly are unilateral and occur after the first year of life[5].

2.3 Clinical presentation.

1. Leukocoria (white pupil)
2. Strabismus (which can be either exotropia-eye turned outward/temporal or esotropia- eye turned inward/nasal)
3. Change in eye appearance: heterochromia or red, painful or watery eyes.
4. Reduced visual acuity (evidenced by change in child's behavior; failure to fix or following in infants, clumsiness in ambulatory children).
5. Others: glaucoma, inflammation and swelling. [4]

2.3.1 Leukocoria

Leukocoria which signifies white pupil is the most common initial presentation of retinoblastoma. It is first apparent when the tumour is still contained within the eye. The white tumour reflects light and blocks view of the red retina. Usually, it is noticed by parents in dim light or after taking a photograph[1].

Other causes of leukocoria include: Coats' disease, cataract, toxocariasis, and retinopathy of prematurity[1].

The following table summarizes the difference in presentation between sporadic and familial retinoblastoma [9].

Forms of presentation:

Sporadic(non-hereditary)	**Familial(hereditary)**
Unilateral, Unifocal	85% bilateral, multifocal
60% of all cases	40% of all cases.
Present later	Present earlier
Children of the affected are normal	Children of the affected have 45% chance of inheritance
Chromosomal anomaly is a somatic mutation	Chromosomal anomaly is a germline mutation
Relatives have a low risk of retinoblastoma development	Relatives have a high risk of retinoblastoma development
	Autosomal dominant with high penetrance.

2.4 Diagnosis

The key element in diagnosis of retinoblastoma lies in early detection of the disease and immediate referral to a bigger facility equipped with specialists (ophthalmologists, pathologists, oculists, radiologists among others) who will aid in the management of the patient. This will help in saving the life of the child and can also facilitate preservation of vision that could be lost otherwise[4]. Methods of diagnosis include:-Flash photograph, Ophthalmoscopic examination under anaesthesia (EUA), ocular ultrasound, computer tomography, magnetic resonance imaging, genetic counseling and testing, histopathology of enucleated eyes. Fine needle aspiration (FNA) (tissue biopsy for confirmation of diagnosis is not necessary to reduce the risk of seeding[4].

2.4.1 Flash photography

Flash photography aids in early detection of retinoblastoma, if the significance of white reflex is well known in the population. It is the easiest form of detection since parents notice the white–eye reflex in photographs which will propel them to seek medical attention[1].

2.4.2 Red reflex test.

The test is carried out in a dimly lit room. The pupil is observed through the ophthalmoscope held about two feet from the patient. Broad beam is used so that both eyes are illuminated at the same time. Normally a red reflex that fills the pupil is observed, they should be symmetric

in character, and white light reflex that appears to reflect off the cornea (corneal light reflex or Hirschberg reflex). An absent or reduced red reflex indicates an opacity of the cornea, which can be due to infection or scar, Lens; which can be due to cataract or tumor, or vitreous due to hemorrhage. In retinoblastoma yellow reflex is produced due to its yellowish white colour[10].

2.4.3 Ocular ultrasound

Used to assess tumor size, also detects areas of calcification within the tumor. Obstetric ultrasound has also been used to facilitate early management of retinoblastoma; prenatal intervention. Tumors can be detected as early as 33 weeks of gestation using obstetric ultrasound; used to visualize large intraocular retinoblastoma in the foetus[4].

2.4.4 Computer tomography and magnetic resonance imaging

Computer tomography is used to show tumor extent, areas of calcification and presence or absence of pineal lesions in trilateral disease. Magnetic resonance imaging assists in the evaluation of optic nerve involvement, and detection of extra ocular extension or a pinealoblastoma[10].

2.4.5 Genetic testing

Retinoblastoma is a genetic disease characterized by RB1 mutation, molecular genetic testing is done to determine if a heritable RBI mutation is present[4].

2.4.6 Histopathology.

Histopathology is the mainstay of diagnosis of Retinoblastoma especially in the late stage disease. Not only does it confirm the presence of retinoblastoma but also determines the way forward in terms of management. It also helps in the grading of the tumor and can also be used in the subsequent follow up of the patient after surgery[11]. Risk factors identified during histopathological examination of enucleated eyes allows for staging and is an important indicator for adjuvant chemotherapy[11].

Microscopically tumors are composed of dense masses of small round cells with hyperchromatic nuclei and scanty cytoplasm, with trabecular and nesting formations. Hematoxyphilic deposits in and around blood vessel walls are often seen in necrotic areas. Another feature characteristic of retinoblastoma is presence of “Flexner-Wintersteiner rosettes” and fleurettes which are also a sign of differentiation toward retinal structures. Tumors that are well differentiated are known as retinocytomas and are considered to be

benign. They present as small placoid, noninvasive lesions composed entirely of benign-appearing cells with numerous fleurettes, lacking necrosis or mitotic activity[12].

Tumor staging

Pretreatment clinical classification (cTNM) is an essential tool in selecting and evaluating therapy. It is based on evidence acquired before treatment that is from physical examination, Imaging, endoscopy, biopsy, surgical exploration and other relevant examinations. Post-surgical histopathological staging on the other hand is used to guide adjuvant therapy and provides additional data, to estimate prognosis and evaluate end results, pTNM is made on the basis of evidence acquired before treatment and is supplemented by additional evidence acquired from surgery and from pathological examination[13](Appendix D).

The pathologic assessment of the primary tumor (pT) involves resection of the primary tumor or biopsy adequate to evaluate the highest pT category. The pathological assessment of regional lymph nodes (pN) on the other hand involves removal of the lymph nodes adequate to validate the absence of regional lymph node metastasis or sufficient to evaluate the highest pN category. Lastly the pathological assessment of distant metastasis (pM) entails microscopic examination[13].

Spread and metastases: Retinoblastoma can invade the optic nerve, from which it can extend to the brain or be carried there by the subarachnoid fluid. It can also invade the uveal tract. Distant metastases may be to the cranial vault or involve distant sites particularly the skeletal system[12].

High risk features in retinoblastoma include**;** postlaminar optic nerve spread of tumor, massive choroidal invasion and extrascleral extension[11]. Choroidal massive involvement occurs when invasion of the tumor is 3mm or more.

Retinoblastoma treatment

1. Enucleation

Enucleation involves the removal of the involved eye before it spreads to other parts of the eyes. This is done in tumors that show high risk signs of potential tumor spread (e.g. orbital cellulitis, poor view of the inside of the eye, bleeding inside the eye, neovascular

glaucoma, tumor anterior to the retina, suspicious optic nerve or suspected extraocular disease on imaging). This is a definitive cure for intraocular retinoblastoma[4].

2. **Focal therapy (Laser therapy or cryotherapy):**

These methods physically destroy both dividing and non-dividing tumor cells and surrounding tissues. They are effective in treating small retinoblastoma tumors and residual intraocular tumor after chemotherapy. In cryotherapy a trans-scleral cryoprobe cooled by nitrous oxide is used to double or triple freeze-thaw and destroy the tumor and underlying choroid. Ice crystals are used to lyse the tumor cell membranes. Cryotherapy is used to treat peripheral retinoblastoma, and for peripheral recurrences after chemotherapy. Laser coagulation physically destroys viable tissue and tumor with heat. It is used for small tumors, residual tumor after chemotherapy and recurrences after chemotherapy particularly for lesions posterior to the equator[4].

3. **Chemotherapy (Local chemotherapy or systemic chemotherapy):**

Local chemotherapy involves the use of local carboplantin, applied on the subconjuctival or subtenon membrane singly or as adjuvant to systemic chemotherapy, to increase the intravitreal carboplantin concentration. Systemic chemotherapy involves the use of combination of carboplantin, etoposide and vincristine (CEV) in differing doses at three weeks Intervals. Chemotherapy reduces tumor size, and promotes resolution of retinal detachment and regression of vitreous seeds. Chemotherapy rarely cures Retinoblastoma, and requires consolidation of chemotherapy by focal therapy[4].In Kenyatta four courses of carboplantin, etoposide and vincristine are given at three week interval.

4. **Radiotherapy**

It involves the use of external beam radiation. It is rarely given for primary retinoblastoma due to the high risk of inducing secondary non-retinoblastoma cancers, cosmetic side effects and the fact that excellent results are achievable with chemotherapy/ focal combination therapy, however when chemotherapy or focal therapy fails, to control the tumor then external beam radiation is recommended[4].

5. Supportive care.

Support programs are put in place to provide assistance and help families cope with the many stresses associated with retinoblastoma. This will help curb problems associated with abandonment of therapy especially in countries of low and middle income where these support groups are not yet strong[1].

2.5 AUDIT PROCESS IN HISTOPATHOLOGY LABORATORY

Medical laboratory audits are carried out with the aim of assessing standards of practice and improving laboratory systems as well. Audits have been carried out successfully in Britain by National Laboratory Quality Control Scheme which confirmed that audits improve the work of individual laboratories. Studies have demonstrated how histopathological reports could benefit from audit for instance report of the Large Bowel Cancer Project organized in St. Mary's Hospital, London. 2046 histopathological reports of patients were reviewed from 22 histopathology departments and considerable observer variation in histological grading was found[14]. In Kenya an audit on histopathologic reporting of mastectomy specimens for breast cancer at Kenyatta National Hospital in 2006; report on tumor margins, showed that there were omissions in reporting in 25% of cases. The author recommended the introduction of standard proforma for reporting mastectomy specimens[15].

In 2009, another audit was done targeting histopathologic reporting of Retinoblastoma specimens at Kenyatta National Hospital[16]. This study by Maingi reported interobserver variations among the pathologist reports (extent of tumour \spread 46.8%). The author reiterated the need for synoptic reporting; proforma/formatted results [16]. Other findings from the same study included: Inadequate clinical information provided by the ophthalmologists in histopathological request forms which was found in 18.3% hence the need for "easy to fill" request forms. The pathologist reports were inadequate missing out vital information e.g. optic nerve invasion in 8.6% of the study cases, hence the need for issue of formatted pathology reports recommended. Lastly periodic audits of histopathologic reporting of retinoblastoma were recommended as a means of quality assurance[17].

Histopathologic audits play an important role in quality control and quality assurance in surgical pathology. They ensure accuracy and completeness of reports, and timeliness of all reports generated in the laboratory. These goals are partly achieved by using a checklist in

reporting cases and by carrying out interdepartmental consultations for difficult, controversial or problematic cases, which are reviewed and discussed before they are signed out [14,17,].

Quality control programs are carried out through random review of cases, intradepartmental and interdepartmental conferences, quality control technical aspects within histopathology laboratory, inter-institutional assessment review, examining specimen records for adequacy, and assessment of turnaround times to monitor the timely reporting of surgical pathology specimens and correct any deficiencies detected[16]. Diagnostic accuracy in histopathology laboratory can be assessed by looking at the level of agreement often from interdepartmental consultation. This has been used as a measure of accuracy[17].

Pathology reports are affected by intra- and inter-observer variations which have been documented amongst pathologist for a wide range of surgical specimens. Histopathologic audit can be used as a tool to examine consensus in these variations in diagnosis and to check diagnostic accuracy[14, 18]. One method of determining the level of agreement between two pathologists is to calculate overall or effective percentage of agreement[19].These calculations provide a measure of agreement but does not take into consideration the agreement that would be expected purely by chance. If pathologists agree purely by chance, they are not really "agreeing" at all; only agreement beyond that expected by chance can be considered "true" agreement. Kappa value is such a measure of "true" agreement[20]. It indicates the proportion of agreement beyond that expected by chance, that is the achieved beyond-chance agreement as a proportion of the possible beyond-chance agreement, a Kappa value of zero means no agreement(0%) while a value of 1.00 means 100% agreement[21].

2.5.1 Complete Histopathology report.

Providing a completed histopathology report is an important professional responsibility of a pathologist. Assessment of the quality of the diagnostic report is a critical component of quality assurance program[18]. For the histopathology report to be complete, adequate clinical information should be provided. A Q-Probes study done revealed that about 40% of deficiencies in specimen accession was related to missing or inaccurate clinical information[18].

Maingi's study done at KNH revealed that vital clinical information needed for making diagnosis were left out and could have caused the gross interobserver variation[16]. In 93 request forms that were submitted for reporting 17% had no clinical history, 7% had no

clinical diagnosis, 7% laterality of the eye involved was not indicated and 3% of the reports had no date of procedure indicated. The quality of submitted clinical information can be used as an important indicator in quality assurance program in the histopathology laboratory[18].

2.5.2 Timeliness

Timeliness is also a key factor in the quality assurance program. Pathology reports should be available in a timely manner, so as to facilitate prompt management of patient. Different bodies have come up with guidelines on how long a histopathology report should be released. The CAP Laboratory Accreditation Program recommends that results of "routine" cases be completed within two working days. The Association of Directors of Anatomic and Surgical Pathology recommends that written reports be available in 2 days for urgent cases and 3 days for "biopsies" and for "surgicals" verbal reports to be available within 1 day or earlier[18]. This took into consideration the additional time required for prolonged fixation (decalcification), additional sectioning or recuts, staining protocols and consultations[18].Worldwide, turnaround time recommended from receipt of specimen to reporting is 2 weeks to allow for proper tissue processing and thorough analysis of structures[18].

2.5.3 Adequacy of diagnostic information.

Another key element in quality assurance program is the adequacy of diagnostic information. The diagnostic information provided should be complete. This can be achieved by standardization of the reporting systems. As early as 1983, Hutter and Rickert conducted a study that emphasized on the value of standardized reporting and the use of a checklist format to ensure the completeness of the surgical pathology report[18].

The Association of Directors of Anatomic and Surgical Pathology have recommended that adoption of standardized reporting scheme will facilitate the transfer of diagnostic information to clinical colleagues and improve communication among surgical pathology laboratories[16]. It has also been documented that the use of standardized reporting or a checklist system is associated with complete diagnostic information[18]. This helps in prevention of omission of information that can be significant to patient management.

From January 1, 2004, the commission of cancer of American College of Surgeons mandated use of the checklist elements of the CAP protocols as part of its cancer program standards for approved cancer programs[16]. The Association of Directors of Anatomic and Surgical Pathology have developed a set of recommendations of the reporting of common malignant

tumors[18]. The purpose of these guidelines is to provide informative reports for physicians and to serve as a valuable educational resource for pathology services[18].

From the pathologist's point of view the use of proforma forms for histopathology reporting of cancer specimen's has the following advantage[16]:

1. They ensure that a standard of care exists in all cancer reports, by including all prognostic and predictive factors useful in cancer therapy.
2. They ensure that time is saved since the surgical pathologist has a checklist of all gross and microscopic features.
3. Typographical errors are avoided since these forms are set out in an easy to tick format.
4. They are relatively easy to transcribe as format can be computerized and downloaded.
5. The clinician can extract from them relevant data with ease and cancer registries can be facilitated / supplemented by automated download if the database is suitably computerized.

Laboratory Audit process is a continuous process and takes into consideration the following aspects:

- That quality manuals or SOPs are provided for in the laboratory; as an index to documentation.
- That there is an appointment of a quality manager who ensures the implementation and maintenance of the quality system;
- That there is a systematic approach to document control;
- And finally an annual management review is in place to ensure the continuation of the service at a level that meets the needs and requirements of users.
- All steps of quality assurance are assessed; that is preanalytical, analytical and post analytical[22].

Assessment of histopathological specimens is subjective and may be determined by factors such as the technical quality of the microscopic sections and provision of adequate clinical and radiological findings by the requesting physician. The histopathological report is a diagnostic decision made by the diagnostic surgical pathologist and is committed to paper and stored indefinitely for future reference. Since the specimen blocks and slides are also stored, it has been a departmental policy in many institutions to carry out random reviews or audits on this reports[16]. Reporting histopathologist should ensure that a standard protocol is used so that all details concerning the specimen is documented in the report[18].

2.6 The College of American Pathologists (CAP)

The College of American Pathologist (CAP) is a leading organization of board certified pathologists, serving patients, physicians, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine. It is internationally recognized for its Laboratory Accreditation and Proficiency Testing Programs, and its mission to provide quality resources for CAP members[23].

CAP Cancer Committee has developed tumor site specific checklists for pathologists to use as a common framework for cancer reporting, this was as a result of variation in reporting by pathologists hence the need for standardization of reporting histopathological specimens[6].
Checklists are available in CAP Web site (www.cap.org)[24].

2.7 Rationale of the study.

Mortality due to retinoblastoma is still high in developing countries, Kenya included, due to late diagnosis or misdiagnosis as a result of poor access to appropriate health care services[4] including poor pathology laboratory services. The number of laboratories that are well equipped and staffed with pathologists is minimal, hence delay in diagnosis, increase in mortality, and difficulty in maintaining International Standards (including College of American Pathologists (CAP) standards).

Introduction of well-equipped laboratories, staffed with pathologists and also by maintaining International standards the mortality rate is expected to decrease due to reduction in turnaround time, improvement of the staging system aiding in effective management of the patient.

For these laboratories to provide optimum services, a good histopathologic Quality Assurance program should be put in place. This should take into consideration pre-analytic, analytic and post analytic processes in handling of surgical specimens. The histopathologic Quality Assurance program also should include standardization of laboratory's activities that is laboratory should design documents that help in the reporting of retinoblastoma so that all information required in the management of patient is included. This will also guide clinicians and enable them submit adequate demographic and clinical information which is essential for pathologists to make correct diagnosis, Grading and staging.

No audit has ever been undertaken in Retinoblastoma Collaborative Laboratory in the University of Nairobi, School of Dental Sciences to assess the adequacy of clinical information, documentation of gross examination findings and completeness of pathology reports for retinoblastoma, and whether or not CAP standards are followed.

This study will enable us evaluate all these parameters, and make recommendations to improve the work of the laboratory. It will also offer a platform whereby in future audits can be carried out to evaluate the success of changes made in accordance with the recommendation given in the study.

This study was also a continuation of Maingi's study done at KNH (An audit and review of the histopathologic reporting of retinoblastoma specimens at Kenyatta National Hospital) whereby progress in reporting of retinoblastoma cases was assessed against improved technical processing of eyes submitted to the RbColab, with an aim of constantly improving standards in this critical area.

2.8 Research question

1. What is the level of agreement between study review reports and the primary reports in the Retinoblastoma Collaborative Laboratory?
2. Do the laboratory processes in Retinoblastoma Collaborative laboratory meet CAP standards recommendations for retinoblastoma?

2.9 Broad objective

To conduct a retrospective audit of histopathology laboratory processes of the Retinoblastoma Collaborative Laboratory of the University of Nairobi

2.10 Specific objectives

1. To determine the completeness and clarity of the request forms.
2. To determine the quality of technical preparation of histological slides.
3. To determine the level of agreement between study review reports and the primary reports in diagnosis of retinoblastoma.
4. To determine the facility's adherence to the college of American pathologists (CAP) standards recommended for eye processing specifically for retinoblastoma.

3.0 Materials and methods

3.1 Study design

This was a retrospective study.

3.2 Study area.

The study was conducted at Retinoblastoma Collaborative Laboratory in the University Of Nairobi School Of Dental Sciences (UON-SDS). The Retinoblastoma Collaborative Laboratory (RbCoLab) Project was initiated in 2008 by means of a memorandum of understanding between Daisy Eye Cancer Fund (DECF) and University of Nairobi School of Dental Sciences. It is a histopathology laboratory and a national training and referral center for head and neck pathologies.

3.3 Study population.

One hundred and thirteen surgical ocular specimens slides from all patients who were clinically diagnosed and histologically confirmed to have retinoblastoma received in retinoblastoma collaborative laboratory from January 2012 to December 2014.

3.3.1 Inclusion criteria

- Histologically confirmed retinoblastoma specimens slides which were available for evaluation, received in retinoblastoma collaborative laboratory between November 2012 and December 2014

3.3.2 Exclusion criteria

- Ocular specimens that were initially reported as retinoblastoma then after review their diagnosis changed to other diseases were excluded.

3.4 Sample size.

The sample size was calculated using the disagreement rate of 5.9%. This is calculated from Renshaw and Gould in the study: Comparison of disagreement and error rates for three types of interdepartmental consultations[17] where they found the disagreement level to be between 5.9 % and 10.7%.

The Fisher's[17] formula was used for calculating the sample size using the prevalence of 5.9%.

$$n = \frac{Z^2 P (1-P)}$$

d^2

Where:

n is the minimum sample size for proposed study

Z is the normal standard deviate corresponding to 95% confidence interval

P is the known prevalence

D is the margin of error of precision set at ± 5%

$$n = \frac{1.96^2 \times 0.059 \times 0.941}{0.05^2}$$

= 85.3127

=86

A sample size of 113 cases was studied.

3.5 Sampling method.

All retinoblastoma histopathological reports and their corresponding slides were systematically retrieved from the archives until the sample size was achieved.

3.6 DATA COLLECTION

3.6.1 Enrolment

The principal investigator systematically retrieved pathology request forms and histopathology reports for retinoblastoma from the archives at retinoblastoma collaborative laboratory from November 2012 till the sample size was achieved.

Corresponding histological specimen slides were retrieved from the laboratory store by the principal investigator.

Corresponding request forms, reports, and histological specimen slides were assigned study numbers by the principal investigator and relevant information contained in them were recorded in the predesigned audit proforma.

Laboratory processes involved in the preparation of retinoblastoma specimen were assessed by means of audit of the slide preparation quality and peer review of pathologist report as per recommendation of CAP.

3.6.2 Microscopic examination of tissue sections

Systemic microscopic examination of the retrieved histological slides was done. Morphological features of the tumour cells were documented to confirm the presence of retinoblastoma. Anatomic structures of the eye were also examined for tumor involvement. This included: the choroid, sclera, iris, anterior chamber, optic nerve and extrascleral tissue. This enabled the principal investigator assign a pathological stage using the pathologic pTNM staging system.

3.6.3 Data entry into the audit proforma.

Patient`s demographic data, relevant clinical information, macroscopic description of the ocular specimens and microscopic findings recorded in the pathology request forms and the initial pathology report forms were entered into the audit proforma (Appendix A).

Histopathological findings obtained after review of sections from the retrieved specimen blocks were recorded in the audit proforma (Appendix A).

Appendix B is the form routinely used for retinoblastoma pathology and appendix C is the CAP standard form (this is where information was extracted to complete the study proforma).

3.7 Quality Assurance

Requisition forms, pathology reports and specimen slides were checked to confirm whether they correspond with each other. Two blinded supervisors and the principal investigator independently reviewed the work of the primary pathologist then compared it to see the level of agreement. Where there was disagreement a tie breaker was involved. In the event of lack of consensus between the four pathologists, then the diagnosis with majority consensus was accepted. The study proforma and initial report were compared to obtain the level of agreement. Data was entered into the audit proforma, avoiding mix-ups and transcription errors.

The principal investigator checked the process of receiving ocular specimens on arrival to the laboratory for correct labeling and assessed them for adequate fixation, the process of trimming, whether or not they are following College of American Pathologists standards

recommended for processing retinoblastoma specimen, if SOP's are in place, all this was recorded in the study proforma, as this was the routine in the laboratory.

3.8 Data analysis

Data obtained from the audit of request forms and pathology reports, and that obtained following histopathological review was recorded in audit proforma. These data was then coded, entered and organized in a pre-designed Microsoft Access database. Data cleaning was performed at the end of data entry and analysis conducted using SPSS version 20 software. The number of specimen with adequate/inadequate clinical information, adequate/inadequate microscopic information and complete/incomplete pathology reports was described and analyzed.

Descriptive summary statistics were performed and presented as proportions and percentages in form of tables, charts and narratives. The level of agreement between histopathological findings recorded in the initial pathology reports and in the audit reports after review was calculated using the Chi-square test and kappa statistics. All statistical tests were performed at 5% level of significance (95% confidence interval).Association between dependent variable (outcome-agreement) and independent variables (predictors) was analysed using Kappa Value.

Dissemination of results

Presentations will be done in local and international conferences. Publication of the results will be done in peer reviewed journals.

3.9 Ethical considerations

Permission to conduct this study was sought from Kenyatta National Hospital and University of Nairobi Ethical Research Committee (KNH/UON-ERC) and study undertaken after formal approval.

New findings that arose during the course of study that was beneficial to the patient management e.g. change in diagnosis in terms of tumor staging or a different diagnosis, or newly identified adverse prognostic factors was communicated to the clinician involved in the patient follow-up through the supervising ophthalmologist. Patient confidentiality was adhered to in all stages of the study. No extra costs were incurred by the patients.

4.0 RESULTS

This audit study was conducted between January 2012 to December 2014.One hundred and thirteen (100 %) cases of retinoblastoma were analysed. The diagnoses for excluded specimens were Staphiloma, coats disease, heamangioma and squamous cell carcinoma. New diagnosis that came up was osteoma. Table 1 below shows the distribution of the cases studied by year.

Table 1: Distribution of cases studied per year (n=113)

Year	**Frequency**	**Percentage (%)**
2012	35	31
2013	51	45.1
2014	27	23.9
TOTAL	**113**	**100**

The highest number of the specimens studied was delivered to the laboratory during the year 2013 (45.1%).

Clinical information

Demographic characteristics of patients with retinoblastoma: Age, Gender,

Of the 113 cases analyzed, only 7 (6.2%) cases had age indicated in months. These were for children who were under the age of one year. The rest of the cases 106 (93.8%) had ages indicated in years. The mean age at presentation was 3 years. Sex was indicated in 90 (80.4%) of the cases. Only 22 (19.6%) cases had no sex indicated. Of these 62 (55.4%) were male and 50 (44.6%) were female. Male to female ratio was 1.2:1.

Table 2: Information on date specimen was received in the laboratory, Date of surgical procedure, clinical history contained on the request form.

	Indicated	%	**Not indicated**	%	**Total**	%
Date specimen received in the laboratory	79	69.9	34	30.1	113	100
Date of surgery	108	95.6	5	4.4	113	100
Clinical history	101	89.4	12	10.6	113	100

It is in 79 cases (69.9%) out of 113 that the date received in the laboratory was indicated. In 34 cases this was not indicated. In 108 cases (95.6%) had the date of surgery indicated, while in 5 cases the date was not indicated. Clinical history was indicated in 101 cases (89.4%), while in 12 cases (10.6) it was not indicated.

Laterality of eye involved (that is laterality of the specimen submitted): The left eye was involved in 57 cases (50.4%) and the right eye in 41cases (36.3%). Both eyes were involved in 2 cases (1.8%) and in 13 cases (11.5%) bilateral involvement of the eye was not indicated. Enucleations accounted for 17 of the specimens (15%) while in 96 cases (85%) enucleations were not indicated.

Clinical diagnosis and presence of name and signature of requesting physician:

Diagnosis was included in 31cases (28.7%) and in 77 cases (71.3%) was not included. The name and signature of requesting physician was included in 110 cases (98.2%), and was not included in only 2cases (1.8%).

Gross examination of retinoblastoma specimens

Specimen dimension was indicated in all the specimen presented. Number of tumour present; single or many, appearance of tumour and tumour consistency was not included in any of the reports. Optic nerve thickness; distal end had 108 (95.6%) cases indicated and only 5 (4.4%) cases not indicated, proximal end had 93 (82.3%) indicated and in 20 (17.7%) was not indicated.

Growth pattern before resection was indicated in 92 (81.4%) and was not indicated in only 21 (18.6%). The Growth pattern was further classified into endophytic, exophytic and diffuse.

Majority of the cases had no tumour location indicated. Only 45 (39.8%) cases had growth pattern after resection indicated. The rest i.e. 68(60.2) cases was not indicated.

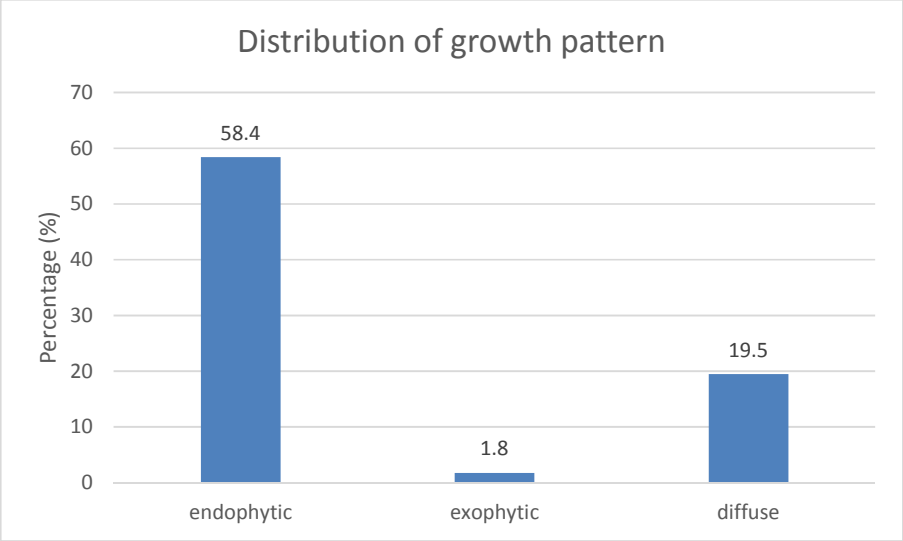

Figure 1: Distribution of growth pattern.

Technical preparation

During assessment of technical preparation a number of things were considered: fixation of the specimen whether satisfactory or not, dehydration process whether was adequate or not, clearing solution used in the laboratory, the embedding of blocks whether it was satisfactory or not, the staining, and documentation of the laboratory procedures with SOP's. All these were assessed by looking at the quality of the slides. All the histopathological slides (100%)

were classified as satisfactory for examination. The documentation of the laboratory procedures with SOP's was also satisfactory.

Histomorphology

Table 4 below illustrates the completeness of documenting important histopathological features as recorded in the initial pathology reports and after histopathologic review (audit reports).

Table 3: Comparison of documented histopathological features between initial reports and audit reports

	Initial report				Audit report			
	Indicated	%	Not indicated	%	Indicated	%	Not indicated	%
Percentage of retinal involvement	95	84.8	17	15.2	98	99.0	1	1.0
Choroidal invasion	80	70.8	32	28.3	98	99.0	1	1.0
Histologic grade	77	69.0	35	39.0	96	97.0	3	3.0
Optic nerve involvement	83	73.5	16	14.2	43	38.1	36	31.9
Retinoblastoma staging	96	85.0	15	13.3	46	40.7	53	46.9

Percentage of retinal involvement was indicated in 95 (84.8%) cases in initial report and was not indicated in only 17 (15.2%) cases. In audit report 98 (99%) cases had retinal involvement indicated and only in 1(1%) case it was not indicated. This was due to fading of the specimen slide hence poor vision. Choroidal invasion was indicated in 80 (70.8%) cases in initial report and in 98 (99%) cases in audited results. It was not indicated in 32 (28.3%) cases in the initial report and only in 1 (1%) case in the audit report. Histological grade was indicated in 77 (69%) cases of the initial report and 96 (97%) cases in the audited reports. It was not indicated in 35 (39%) cases in the initial report and in 3 (3%) cases in the audited report. Optic nerve involvement was indicated in 83(73.5%) of initial report and in 43 (38.1)

of the audited reports. It was not indicated in 16 (14.2%) of initial report and 36(31.9%) of audited report. The cases that were not indicated in the audit report was due to incompleteness of the specimen slides available. Some slides were missing. Retinoblastoma staging was indicated in 96 (85.0%) of the initial report and 46(40.7%) in the audited report and was not indicated in 15(13.3%) of the initial report and 53(46.9%) of the audited report. Staging in the audited report was difficult due to the poor quality of the slides, that is some slides had stains faded hence some features were not seen hence not staged to avoid errors in staging. The observed agreement between the initial and the audit reports after chance was excluded was as follows: Choroidal invasion Kappa (N=113) = 0.23, (p = 0.91), For Retinol invasion Kappa (N =113) = 0.88, (p = 0.98), for Optic nerve invasion Kappa (N=113) = 0.34, (p = 0.65).

Resection margin

Also assessed was presence of tumour at the resection margin. In the initial report 12(10.6%) had tumour present in the resection margin. In the audited results 18(15.9%) had tumour present in the resection margin. In the initial report 81(71.7%) had tumour absent in the resection margin while in the audited report it was 61(54%)

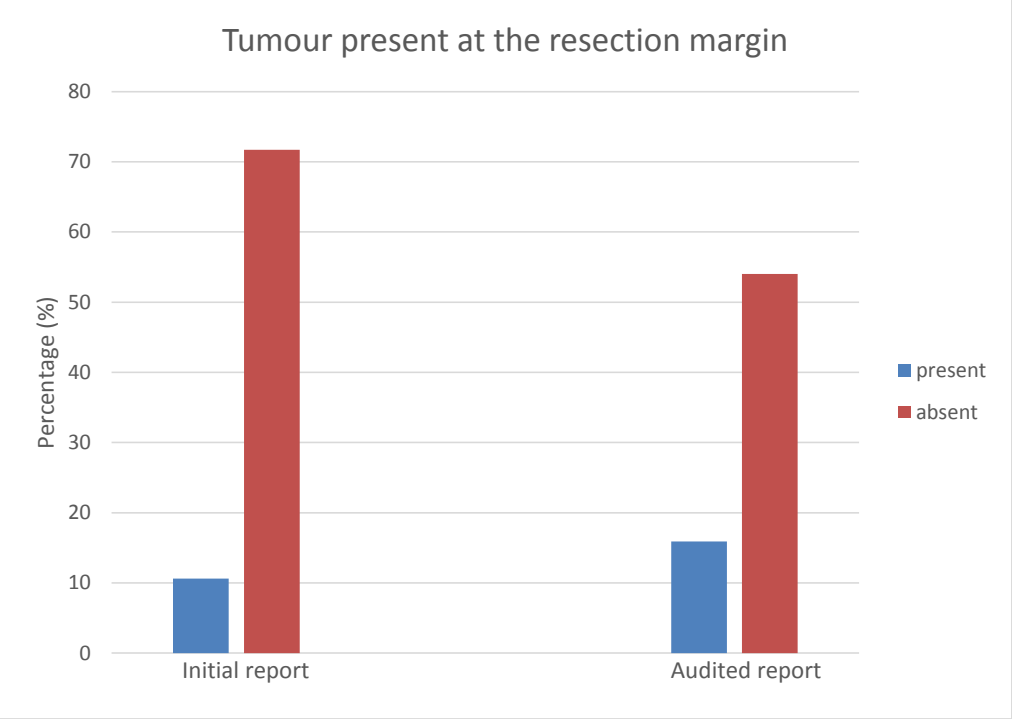

Figure 2: The resection margin: Initial report n=93 Final report n=79.

Final diagnosis

Final diagnosis made by the initial pathologist and the reviewed reports were also examined and inter observer variation was calculated using the Kappa value. The observed agreement between the initial and the audit reports after chance was excluded was: Kappa (n =113) = 0.15, (p = 0.91).

Histologic grading

Table 5 below shows the degree of differentiation both in the initial report and in the audited report. After histopathologic review, majority of the tumours were found to be poorly differentiated (48%).

Table 4: Histologic grading (degree of differentiation) (n=102)

	Initial report		**Audit report**	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Well differentiated	42	41.2	37	36.3
Moderately differentiated	2	2.0	15	14.7
Poorly differentiated	31	30.4	49	48.0
Cannot be graded	2	2	1	1.0
Not graded	25	24.5	0	0
Total	**102**	**100**	**102**	**100**

After histopathologic review 37 specimen were found to be well differentiated, 15 were moderately differentiated 49 were poorly differentiated and 1 could not be graded due to extensive necrosis. In the initial report 42 specimens were graded as well differentiated, but after the review 5 cases were reclassified as moderately differentiated. All the specimens that

were reported as moderately differentiated (2) were confirmed to be moderately differentiated, and 13 more cases reclassified as moderately differentiated, previously they had been classified as well differentiated. All the 31 specimen that were classified as poorly differentiated were confirmed and additional 18 specimen added to the group. Two cases could not be classified in the initial report but after the review 1 case was reclassified as poorly differentiated. Twenty five cases were not graded in the initial report.

Adherence to CAP standards

In the cases that were reviewed, the assessment of CAP standards were measured by assessing the following parameters which are recommended by the College of American pathologist concerning the reporting of retinoblastoma tumours: type of procedure indicated, specimen size, specimen laterality among others as shown on the table 5 below.

Table 5: Assessment of adherence to CAP standards (n=110) by assessing presence or absence of different parameters included in the reporting of retinoblastoma as per CAP recommendation.

	Indicated		**Not indicated**			**Indicated**		**Not indicated**	
	Freq.	%	Freq.	%.		Freq.	%	Freq.	%
Type of surgical procedure	16	14.5	94	85.5	Tumour inv. other struct.	107	97.3	3	2.7
Specimen size	110	100	0	0.0	Histologic features	96	87.3	14	12.7
Specimen laterality	103	93.6	7	6.4	Growth pattern	94	85.5	16	14.5
Tumour site	33	30.0	77	70.0	Extent of o.n invasion	99	90.0	1	10.0
Tumour basal size	110	100	0	0.0	Histology grade	88	80.0	22	20.0
Tumour size post resection	63	57.3	47	42.7	Tumour margins	97	88.2	13	11.8
Tumour location post resection	45	40.9	65	59.0	TNM staging	94	85.5	16	14.5

From table 5 we found that some areas were well presented, for instance in the indication of specimen size where all 110 (100%) cases were indicated, tumour basal size 110 (100%) had been indicated, tumour involving other structures 107 (97.3%) had been indicated, specimen laterality 103 (93.6) were indicated, extent of optic nerve invasion 99 (90%) had been indicated. However there are some areas that were poorly presented, this include; type of procedure where 94 (85.5%) had not been indicated, tumour size was not indicated in 77 (70%) cases, tumour location was not indicated in 65 (59.0%) cases. The rest were fairly represented.

Turnaround Time

Majority of the specimen 52 (64.2%), were reported between 1-2 weeks. Eight (9.9%) were reported within a week,11 (13.6%) were reported between 2-3 weeks,7(8.6%) were reported between 3-4 weeks and 3(3.7%) were unreported for more than 4weeks.The shortest time taken was 3 days and the longest time taken was 6weeks 4days.The median duration of reporting was 1-2weeks and the mode was also 1-2 weeks.

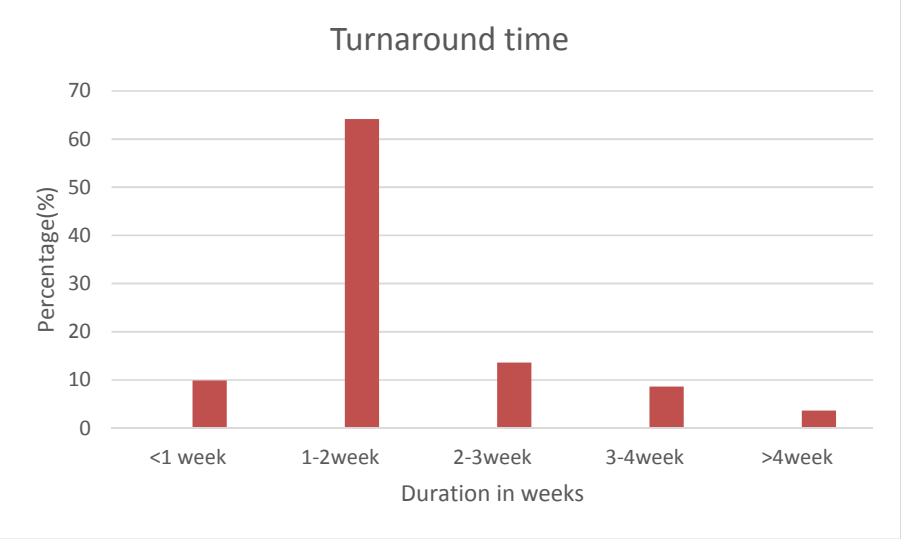

Figure 3: Turnaround time for specimen reporting.

5.0 DISCUSSION

Clinical information provided on requisition forms for histopathology examination is very important in the sense that it gives the pathologist vital information on the specimen being presented for histopathological examination and also a guidance in making the right diagnosis[15]. Lack of clinical diagnosis may lead to errors made by pathologist when making diagnosis or may lead to issuing of incomplete reports[15].Clinical diagnosis given in the request forms helps the pathologist correlate his pathological findings with the clinical diagnosis the clinician had given[15].

A total of 113 ocular specimens were included in this study. Only seven cases (6.2%) had age indicated in months. This could have been due to clinicians not being aware of the importance of reporting age in months. Age should be indicated in months for uniformity of reporting data and easy comparison of data around the world[3].

Gender was not indicated in 22 cases (19.6%). This may be due to oversight from the clinicians. Clinicians should take their time when completing the request form to avoid oversight errors. Studies have shown that oversight errors can cause omissions of important parameters from a report[32]. For example in a study done by Bull et al[32] where they discussed that omissions can lead to pathologist giving misleading information to the clinician, they went ahead and gave an example of lack of reporting lymph node metastasis in colorectal carcinoma can lead to a patient missing chemotherapy.

It is important to indicate in the request form the date that the specimen is received in the laboratory. This helps in the calculation of turnaround time which in turn can be used to monitor the performance of the department and improvements made if necessary[15, 44] .Thirty four specimen (30.1%) had no date indicated. This may have been due to the fact that there was no provision for dates in the request forms. Request forms should be structured in a way that allows this information to be captured. Date of surgery was indicated in most of the request forms. Only 4.4% (5) of the cases had not been indicated. This is important as this information helps in calculation on the overall turnaround time.

Clinical history is important in helping the pathologist make a definitive diagnosis. Lack of clinical history may result in erroneous diagnosis[34]. Clinical history and diagnosis also enables the pathologist compare his or her findings with that of the clinician. In this study most (89.4%) of the request forms had clinical history given.

In this study, the left eye was more affected with the tumour than the right eye. The ratio of left: right involvement was 1.3:1. 51.3% had unilateral disease while 23% had bilateral disease. The rest had no information given. This compared well with a study done by Akhiwu et al[41] where he found that the prevalence of bilateral retinoblastoma in the U.S.A was 27%, in Great Britain 36%, in South Africa 18%. In this same study he found that the unilateral cases were more than the bilateral just as in my case, and that the incidence of bilateral disease reduced with increasing age. 13 cases had no information given.

Enucleation was indicated in 17 cases (Exenteration is no longer performed as a procedure). This is due to the clinician assuming that since exenteration is no longer been performed then there was no need for indication. Procedures undertaken during surgery should always be indicated, this can help in future references should the case be revisited[15].

The name and signature of the requesting physician was indicated in 98.2% of the cases that were reviewed.

Specimen dimension was indicated in all the specimen received. This is an improvement in comparison with the study done by Maingi et al in 2009[16], whereby the specimen dimension was frequently missing. This may be due to the introduction of the structured request forms. However number of tumour present, appearance of tumour and tumour consistency was not included in any of the reports reviewed. This compared well with Maingi's study[16].Pathologist should be encouraged to give comprehensive details on gross examination as this helps in the determination of the extent of tumor spread. This information can also play an important role should the specimen be reviewed years later. This has also been supported by a study done by Morson[14] where he stressed on the fact that avoidable errors largely lay in the gross and macroscopic assessment of specimen.

Optic nerve invasion is an important prognostic factor for metastasis and survival[35, 36]. The diameter of the nerve at the proximal and distal end gives information as to whether the tumour has spread to the nerves or not. Most of the cases that were reviewed had optic nerve dimensions taken; only five cases had not been reported. This was an improvement from Maingi's study where optic nerve measurement had not been indicated in most of the cases[16]. This was due to the introduction of structured request forms.

Growth pattern was indicated in most (81.4%) of the request forms. It was further classified as endophytic, exophytic and diffuse. Majority of the tumours examined had endophytic growth pattern.

Majority of the cases had no tumour location indicated. It is important to describe tumour location as this will go a long way in assisting the pathologist when reporting the tumour. This helps the pathologist be able to orientate himself or herself with the tumour in relation to the other surrounding normal tissues[32].

All the retinoblastoma specimen slides that were reviewed were classified as satisfactory in terms of staining, showing good technical preparation of the histological specimen in that laboratory.

Generally microscopic examination improved with the introduction of standard report forms. Most of the information required by the clinician were included in the standard report forms. Vital information like percentage of retinal involvement, choroidal invasion, histologic grade, optic nerve involvement, retinoblastoma staging and presence or absence of tumour at the resection margin were included. This compared well with a study done by Mathers et al in the use of a standard proforma in breast cancer reporting whereby information like microcalcification, tumor grade, tumor size and hormone receptor status were documented more frequently in the proforma. They concluded that the introduction of a standard proforma significantly improved the completeness of reporting breast cancer specimen[45].

Kappa value is a quantitative measure of the magnitude of agreement between observers [37]. It is the agreement beyond that expected by chance. If pathologists agree purely by chance, they are not really “agreeing at all”. Only agreement beyond that expected by chance can be considered “true” agreement. Vierra and Garrett[37] in their paper: “understanding interobserver agreement: The Kappa value” gave the interpretation of Kappa value using a scale of 0.0 to 1.0.In this scale 0.0 is poor agreement, 0.6 is moderate agreement, and 1.0 is almost perfect agreement.

In this study the kappa value for final diagnosis made by the pathologist was 0.15.This is slight agreement according to the above scale, meaning the pathologist had a “true” agreement and were not just agreeing by chance. This also applied in assessing the following parameters: Retinal invasion (0.88- almost perfect agreement) and Choroidal invasion (0.23- Fair agreement).

It was challenging to assess the presence of tumour at the resection margin in the audited reports since some slides had some sections missing, however after the review tumour was found in five more histopathology slides that were initially reported as absent. This was due to the presence of tumour necrosis which needed keen examination.

Histologic grading was also assessed. In the initial report majority of the cases were reported as well differentiated, and only two cases as moderately differentiated. In the audited report thirteen more cases were reclassified as moderately differentiated. This compared well with a study done by Tosoni et al[46] whereby a significant interobserver differences was observed in both grading and staging of tumours. Out of the 235 tumours that were included in the study and were previously reported as pT1 after review 35% were reclassified as pTa, 56% as pT1 and 32% as pT2-4. Pathologists should come up with a standardized clear cut method of determining the grade of the tumour. Tosoni[46] recommended that at least two different pathologist should independently grade a tumour before radical therapy is initiated.

In the audited report majority fell on the category of poorly differentiated (48%), this compares well with a study done by Maingi whereby after histopathology review most of the tumours were found to be poorly differentiated (48.4%). This also compared well with other studies done elsewhere. A study by Van Meetreen et al[38] whereby they looked at 44 retinoblastoma cases, of which 24 (54.5%) were poorly differentiated, 31.8% were moderately differentiated and 13.6% well differentiated. Another study done by Owoeye et al[39]; reported poorly differentiated to be (82.6%), and moderately differentiated to be (17.4%).However a study done by Ajaiyeoba et al[40] showed that there is no relation between the level of differentiation of tumour and the prognosis of the patient. The prognosis of the disease is widely based on retrolaminar nerve involvement and presence of perivascular tumour cuffing.

In the initial reports 25 cases had not been graded. Most of the cases that had no level of differentiation were reports that had been reported earlier in the studies, when the lab had just started operating. Meaning there was no interdepartmental consultations. Later in the study, the pathologist was keen to give the histologic grading. The introduction of regular interdepartmental consultations led to the improvement of reports by the pathologist.

In this study it was difficult to assess the TNM staging since some slides had their original stain fading away, and some slides had some parts of the specimen for instance optic nerve

missing, or incontinous choroid, or prelarminar part not connected to the laminar part of the optic nerve. To avoid errors in staging the slides that were incomplete were not staged.

Facility's adherence to the College of American Pathologists (CAP.)

The college of American pathologist (CAP) [23] is a leading organization in performing laboratory accreditation and proficiency testing programs. They have developed checklist whereby pathologists can use in comprehensive reporting of a specimen. This is aimed at reducing variation in reporting and enhancing standardization in reporting.

Generally the laboratory has attempted to adhere to CAP standards. This is seen by the good representation of some parameters, for instance; in the indication of specimen size whereby 100% of the cases were indicated, tumour basal size indicated in 100%, tumour involving other structures indicated in 97.3%. With the introduction of new request form with structured reporting system, CAP checklist was integrated hence the use in the reporting. This has enabled the trimming, processing and reporting of specimen be comprehensive and standardized as recommended by the college of American pathologist. This also compares well with a study done by Biffin and Mella et al[32] where they recommended the usage of template proforma reporting that agrees with national standards aimed at improving the quality of information obtained from pathologist that will aid in patient management. In this study only 51.6% of rectal cancer reports had circumferential resection margin reported, only 30% had the number of lymph nodes involved indicated and only 46.6% of rectal carcinoma reports had adhered to the minimum standard.

However some areas were poorly presented, for instance; type of surgical procedure undertaken, whereby 85.5 % had not been indicated. One of the reasons why this may have happened is because the clinicians assume that since they only did enucleations that there was no need to include this information in the requisition forms. Other areas that were equally poorly presented were in indication of tumour size whereby 70% of the cases had not been indicated and tumour location after resection, where 59% of the cases had not been indicated. This compared well with a study done by Maingi[16] whereby he found that tumour size had not been indicated in 59% of the cases studied and tumour location after resection had not been described adequately in all the cases studied. This may have been due to the fact that the pathologist was not using the proforma guideline during the trimming process.

Worldwide turnaround time recommended from receipt of specimen to reporting is two weeks, to allow for proper tissue processing and thorough analysis of the structures .In this laboratory majority of the cases (64.2%) met this threshold. This compared well with a study done in Nigeria by Malami et al[42] where he found that the turnaround time in Aminu Kano Teaching hospital was 2-16 days. However this did not compare well with studies done in Spain by Ribe[43] et al where they found that the turnaround was much shorter; 6.24 days. This may have been due to the improved working condition in Spain, whereby there are more staff and more sophisticated equipment as compared to the African countries.

6.0 CONCLUSIONS

From this audit, most of the Ophthalmologists, provide adequate clinical information for retinoblastoma specimens as seen in data obtained from the request forms. Technical preparation of histopathological slides were satisfactory in all the histopathological specimens examined. Level of agreement between the initial histopathological findings and audited reports was slight 0.15; however almost perfect agreement was observed in reporting retinal invasion 0.88, fair agreement was observed in reporting optic nerve invasion 0.34, there was no “Chance agreement”, hence excluded in the study. The laboratory has not fully adhered to College of American Pathologists (CAP) standards recommended for eye processing specifically for retinoblastoma. Both the clinicians and pathologist need to adhere to the CAP standards checklist in handling retinoblastoma cases.

Study limitations

The staining on the histopathological slides was fading away on some slides hence difficulty in assessing some features.

Some slides were missing hence doing complete analysis of a case in some cases proved challenging.

7.0 RECOMMENDATIONS

1. The laboratory should participate in external quality control program.
2. Periodic audits should also be integrated as part of the routine system to enable them maintain quality.
3. The tool that has been used to audit this laboratory can be used to audit other histopathology laboratories.
4. Continue use of Proforma to achieve 100% capture of clinical information, and 100% adherence to CAP standards.

REFERENCES

1. Dimaras H, Kimani K, Dimba E et al, Retinoblatoma, [Internet] [2012 cited 2014 Jan 11th] Available from www.thelancet.com.
2. Meel R, Radhakrishnan V, Bakhshi S, Current therapy and recent advances in the management of retinoblastoma: Indian Journal of Medical and Peadiatric Oncology.2012:33:2:80-89
3. Nyamori J, Kimani K, Njunguna M et al The incidence and distribution of retinoblastoma in Kenya Br. J Ophthalmol 2011:10;1136
4. Dimaras H, Dimba E et al, Guidelines from the Canadian Retinoblastoma society,Br J Ophthalmol 2010:14;1415-1416
5. Young J, Smith A, Steven D et al National Cancer Institute, SEER Pediatric Monograph.NIH Pub. No 99-4649. Bethesda, MD, 1999. Arch Pathol lab Med. Vol 133 August 2009.
6. Dimaras H, Dimba E, Gallie B, Challenging the global retinoblastoma survival disparity through a collaborative research effort. Br.J Ophthalmol 2010:94:11:1415-1416
7. Canturk S, Qadduomi I, Khetan V et al, Survival of retinoblastoma in less-developed countries impact of socioeconomic and health-related indicators Br J Ophthalmol 2010:94:1432-1436.
8. Programs T, Terms C, An Overview of the college of American Pathologist Cancer Checklist 2009.
9. IAEA Pediatric Radiation Oncology Training Dr Lasker Version: 2009:1;5-8
10. Harbour J, Murphree A, Karcioglu Z et al Retinoblastoma, [Internet] 2009 cited 2013 June 25th Available from www.springerlink.com
11. Eagle C, High Risk Features and Tumour differentiation in Retinoblastoma. Arch Pathol Lab Med vol 133 August 2009.
12. Juan R, Rosai and Ackerman's Surgical Pathology 10th Ed Elsevier In vol 2 Philadelphia 2011
13. Sobin L, Wittekind C, Classification of Malignant Tumors 7th Ed. Willey-Liss New York:2002
14. Morson B: Histopathology reporting in large-bowel cancer. British Medical J. 1981; 283:1493-94.
15. Macharia B, A review and audit of the histopathology reporting of mastectomy specimens in Kenyatta National Hospital MMed. Dissertation 2006.

16. Maingi S, An audit and review of the histopathologic reporting of retinoblastoma specimens at Kenyatta National Hospital MMed. Dissertation 2009.
17. Renshaw A, and Gould E, Comparison of disagreement and Error rates for three types of Interdepartmental consultations. America Society for clinical pathology 2005:124,878-882
18. Owen D, Tighe J, Quality evaluation in histopathology. Br. Med. J. 1981, 283; 1493-94.
19. Sim J, Wright C: Research in Health Care: Concepts, Designs and Methods. Cheltenham England: Nelson Thorne, 2000
20. Cohan J: Coefficient of agreement for nominal scales. Educ Psychol Meas.1960:20:37-46.
21. Daly L, Bourke G, Interpretation and uses of medical statistics. 5th ed. Oxford, England; Blackwell Science,2000
22. Burnett D, Blair C, Haeney M, et al; Clinical Pathology accreditation; Standards for the medical laboratory. J Clin Oathol. Oct 2002:55(10):729-733.
23. Overview of the CAP Cancer Checklist January 2009
24. CAP FACT SHEET. Protocol revision date January 2005 Based on AJCC/UICC TNM, 6th edition.
25. Virasadki C, Analysis of Epidemiological Data Using R and Epicalc 1st Ed Chanmaung Press Thailand 2008.
26. Vinay K, Abul K, Nelson F et al, Robbins and Cotran Pathologic Basis of Disease 8th Elsevier Philadelphia 2010.
27. Abramson D, Schefler A, Continuing Medical Education Update on retinoblastoma. The Journal of retinal and vitreous diseases 2004:24:6:828-848
28. Grossniklaus E, Kivella T, Harbour W et al Protocol for the examination of specimens from patients with retinoblastoma [Internet] 2011 [cited 2013 June 25th] Available from www.cap.org/cancerprotocols
29. Deegan W, Retinoblastoma: A Review of current Treatment Strategies. Journal of Ophthalmic Prosthetics 1990:1:1-6
30. Wippold F, Perry A, Review Neuropathology for the Neurroradiologist: Rossettes and Pseudorosettes, AJNR 2006:27
31. Sanmugasunderum S,Olson R, Holland P et al National Retinoblastoma strategy Canadian Guidelines for care.Can J Ophthalmology: 2009:44:2:1-45
32. Bull A, Biffin A, Mella J: Colorectal cancer pathology reporting: a regional audit. J.Clin.Pathol.1997; 50, 138-142.

33. Miller J, Slater D: Do histopathology reports of primary cutaneous melanoma contain enough essential information? J. Clin. Pathol. 1996; 49; 202-204.
34. Ramsay A. Errors in histopathology reporting: detection and avoidance. Histopathology. 1999; 34:481-490.
35. Kimani K.: A review of retinoblastoma: Presentation. Diagnosis and management at Kenyatta National Hospital. Thesis, 2000
36. Ajaiyeoba I., Komolafe O.Olusanya B., et al: Histological determinant of mortality in retinoblastoma in Ibadan, Br J Ophthalmol, 2008; 92: 1297-1298.
37. Viera J, Garret M: Understanding Interobserver Agreement: The Kappa Statistic.Fam Med 2005; 37 (5):360-3.
38. Van Meeteren N., Van der Valk P., Van der Linden H. et al: Histopathologic features of retinoblastoma and their relation with in vitro drug resistance measured by means of the MTT assay. Cancer 2001; 92:2933-40.
39. Owoeye J., Afolayan. Ademola-Popoola D.: Retinoblastoma: A clinico-pathological study of retinoblastoma in Ilorin Nigeria. African Journal of Health Sciences, 2006; 13:117-123.
40. Ajaiyeoba I., Komolafe O., Olusanya B.,et al: Histological determinant of mortality in retinoblastoma in Ibadan.Br J Ophthalmol.2008;92:1297-1298
41. Akhiwu W, Igbe A: Epidemiology of Retinoblatoma, Retinoblastoma: An update of clinical, Genetic cancelling, Epidemiology and Molecular Tumour Biology, Prof Govindesamy Kumaramanickavel (ED), ISBN: 978-953-51-0435-3 InTech.
42. Malami S, Uiyasu Y: Local audit of diagnostic surgical pathology as a tool of quality assurance, Nigerian journal of medicine 2008; 17(2):188-192.
43. Ribe A, Ribalta T,Lledo, et al; Evaluation of turnaround times as a component of quality assurance in surgical pathology, Int J Qual Health care1998;10(3):241-5.
44. Erasmus R, Zemlin A: Areview of Clinical audit in the laboratory J Clin Pathol 2009; 62:593-597.
45. M Mathers, J Shrimanker, D Scott et al; The use of a standard proforma in breast cancer reporting: J Clin Pathol. 2001 Oct; 54(10):809-811
46. Tosoni I, Wagner U, Sauter G; Clinical significance of interobserver differences in the staging and grading of superficial bladder cancer BJU Int. 2000 Jan;85(1):483-530

APPENDICES

Appendix A: Proforma (audit)

Study number _____

Pathology number _____

1.0 Demographic and clinical information

- Form has name of patient : Yes ☐ No ☐
- Form has hospital and ward number (OP/IP Number) included: Yes ☐ No ☐
- Is gender indicated or not: Yes ☐ No ☐
- Has age(in months) been included in the form Yes ☐ No ☐
- Date of procedure included or not included Yes ☐ No ☐
- Date Received indicated or not indicated: Yes ☐ No ☐
- Clinical information(Previous treatment, chemotherapy, or none) has it been included: Yes ☐ No ☐
- Tumor involvement: (1)Is it unilateral ☐ (2) Is it bilateral ☐ (3) or Not indicated ☐ (Tick one)
- Laterality of eye involved: (1)Is it Right eye involved ☐ (2)Left eye involved ☐ (3) Both eyes involved ☐ (4) Or Not indicated.(Tick one)
- Has Clinical diagnosis been included: Yes ☐ No ☐
- Indicate which type of procedure was undertaken (tick one): (1)Enucleation ☐ (2) Exenteration ☐
  (3)Other (specify ☐
- Has the name and signature of requesting physician been included Yes ☐ No ☐

2.0 Macroscopic examination(Indicate/ Tick as appropriate)

- Which type of specimen has been submitted (1)Enucleation ☐ (2) Exenteration ☐ (3) Biopsy ☐ (4) Others (specify) _____
- Which side of the eye has been presented for examination? (tick one)(1) Right eye ☐ (2) Left eye ☐ (3) Not indicated. ☐
- Has the specimen dimensions been included (in cm)(tick one):-
  - ☐ Anteroposterior
  - ☐ Horizontal
  - ☐ Vertical
  - ☐ Optic nerve length

- Has the optic nerve thickness/ been indicated in diameter(mm)(tick one)

  -Distal end ☐

  -Distal end not determined ☐

  -Proximal end ☐

  -Proximal end not determined ☐

- Tumor description: Has the growth pattern been indicated, Yes ☐ No ☐
- if yes indicate by ticking one :

(1) Endophytic ☐ (2) exophytic ☐ (3) diffuse ☐ (4) cannot determine ☐

-Has the number of tumor present been indicated: Yes ☐ No ☐

-Has the site/ location of tumors been indicated: Yes ☐ No ☐

-Has the appearance of tumor been described (cut surface): Yes ☐ No ☐

-Has the tumor consistency (cut surface) been indicated: Yes ☐ No ☐

3.0 Technical preparation

- Was the fixation of the specimen satisfactory: Yes ☐ No ☐
- Was the dehydration process adequate: Yes ☐ No ☐
- Was the clearing solution used in the laboratory clear: Yes ☐ No ☐
- Are the Embedded blocks satisfactory: Yes ☐ No ☐
- Are the sectioned slides satisfactory : Yes ☐ No ☐
- Is the Staining of specimen slides satisfactory: Yes ☐ No ☐
- Is the documentation of the laboratory procedures (SOP, References, Quality manual, Records, Labels) Adequate: Yes ☐ No ☐

4.0 Microscopic examination

- Has the Percentage of retinal involvement been included: Yes ☐ No ☐
- Has the microscopic involvement of ocular structures been included: Yes ☐ No ☐ : If yes tick the structures that have been included: ☐

  (1) Sclera ☐ (2) Optic disc ☐ (3) Vitreous

  (4) Extrascleral extension ☐ (5) Vortex veins ☐ (6) Ciliary body ☐

  (7) Iris ☐ (8) Anterior chamber ☐ (9) Angle Schlemm's canal ☐

  (10) Cornea ☐ (11) Lens ☐ (12) other (specify) ☐

  (13) Choroidal invasion ☐

- Has the histologic type (Histogenesis) of tumor been determined:

  Yes ☐ No ☐
- Has the histological grade been indicated: Yes ☐ No ☐
- Has the optic nerve been involved or not (tick one) (1) Involved ☐

  (2) Not involved ☐
- Status of tumor at resection margin; is the tumor present at resection margin or not (tick one) (1) Present ☐ (2) Absent. ☐
- Is the staging of retinoblastoma present: Yes ☐ No ☐
- Has the laboratory Adhered to CAP standard (1) Yes ☐ (2) No ☐

Investigator……..

Signed ……..

Date …….

Supervisor……. Supervisor……..

Signed …… Signed ……

Date ……. Date ……

Appendix B: Processing of Surgical ocular specimen

1. Surgical Specimen are received at the laboratory reception desk, the following are crosschecked before the specimen is accepted into the laboratory;
   -Whether specimen is accompanied with the requisition forms
   - Whether the name on the requisition form and the name on the specimen are marching
   -Whether the correct fixative has been used, that is (10%) formal saline.

2. A laboratory number then is issued; both the specimen and the requisition form are labeled with the unique laboratory number. The patient's names, age, sex, specimen type are entered against accession number in the histology registry.
3. The globe specimens are allowed to fix in 10% formalin solution for a minimum of 48 hours before sectioning.
4. The gross examination is done by the resident and the supervising pathologist and the findings are detected to the assisting histotechnologist.
5. After examination of the specimen the optic nerve is removed before opening the globe to prevent the nerve from accidental contamination with artifactual clumps.
   - ✓ During sectioning, a section is made that extends from pupil through the optic nerve.
   - ✓ The globe is then sectioned in either horizontal or vertical plane with pupil and optic nerve included in the cassette.
   - ✓ The interior of the globe is then examined for the presence or absence of tumor.
   - ✓ Sections that represent the tumor are then put in labeled cassettes which are then immersed in a fixative for processing.

6 Next is automated processing of tissues.

7. The blocked sections are then embedded with paraffin wax.
8. Sectioning using a microtome is done and the sections floated in warm water to remove wrinkles.
9. The sections are then picked on a glass slide and placed in a warm oven for 15 minutes to enable them adhere to the slide.
10. The tissues are then deparaffinized by dipping them in xylene to alcohol to water, then standard Heamatoxylin and Eosin are used for staining.
11. The stained microscopic sections are then covered with a cover slip and are left to dry.
12. Microscopic examination of the processed tissue is then done.

Appendix C: RETINOBLASTOMA PATHOLOGY REPORT. (PROFORMA)

Patient name: Lab specimen number:

Date of birth (dd/mmm/yyyy): / / Sex: ☐ Female ☐ Male

Hospital: Ward: OP/IP number:

Date of procedure: / / Date received: / /

Time of collection: ☐ am ☐ pm

Doctor's name:

CLINICAL INFORMATION PROVIDED BY DOCTOR (as per request form)

Laterality: ☐ Unilateral ☐ Bilateral ☐ Trilateral

Previous treatment: ☐ None ☐ Chemotherapy ☐ Other (specify):

Clinical assessment: ☐ Optic nerve involvement ☐ Extra-orbital involvement

☐ Recurrence (specify): ☐ Metastasis (specify):

Other notes (e.g. nodal involvement, etc.):

Family history of retinoblastoma? ☐ Yes ☐ No ☐ Unknown

MACROSCOPIC EXAMINATION

Type of specimen: ☐ Eye ☐ Orbital biopsy ☐ other (specify):

Side: ☐ Left ☐ Right

Structures included: ☐ Medial rectus ☐ other:

Extra-ocular muscle marked for orientation: ☐ Medial rectus ☐ other: ☐ None

Specimen dimensions: Anteroposterior: cm horizontal: cm

Vertical: cm Optic nerve length: cm

Optic nerve thickness/diameter:

Distal end: mm ☐ cannot determine (specify):

Proximal end: mm ☐ cannot determine (specify):

Tumour dimensions after grossing: Base at cut edge: mm Height at cut edge: cm

☐ Cannot determine (specify):

Growth pattern: ☐ Endophytic ☐ Exophytic ☐ Diffuse

☐ Cannot determine (specify):

RETINOBLASTOMA PATHOLOGY REPORT

MICROSCOPIC EXAMINATION

Percentage of retinal involvement: %

Microscopic involvement of ocular structures.

☐ None ☐ Sclera ☐ Optic disc

☐ Vitreous ☐ Extrascleral extension ☐ Vortex veins

☐ Ciliary body ☐ Iris ☐ Anterior chamber

☐ Angle/Schlemm's canal ☐ Cornea ☐ Lens

☐ Other (specify):

☐ Choroid; maximum extent of choroidal invasion: mm Notes:

Optic Nerve ☐ within lamina cribrosa

☐ prelaminar

☐ retrolaminar; specify extent of involvement: mm

Status of tumour at resection margin: ☐ Present ☐ Absent

Surgical margins ☐ cannot be assessed

☐ Tumour at margins.

☐ None

pT STAGING (EYE)

☐ pTX Primary tumor cannot be assessed

☐ pT0 No evidence of primary tumor

☐ pT1 Tumor confined to eye with no optic nerve or choroidal invasion

☐ pT2a Tumor superficially invades optic nerve head but does not extend past lamina cribrosa *or* tumor exhibits focal choroidal invasion.

☐ pT2b Tumor superficially invades optic nerve head but does not extend past lamina cribrosa *and* exhibits focal choroidal invasion

☐ pT3a Tumor invades optic nerve past lamina cribrosa but not to surgical resection line *or* tumor exhibits massive choroidal invasion

☐ pT3b Tumor invades optic nerve past lamina cribrosa but not to surgical resection line *and* exhibits massive choroidal invasion

☐ pT4a Tumor invades optic nerve to resection line but no extra-ocular extension identified

☐ pT4b Tumor invades optic nerve to resection line a*nd* extra-ocular extension identified

FINAL REPORT

Name of Pathologist: Date (dd/mmm/yyyy): / /

Signature:

Appendix D: COLLEGE OF AMERICAN PATHOLOGISTS (CAP)
Protocol for the Examination of Specimens from Patients with Retinoblastoma

Protocol applies to retinoblastoma only.

Surgical Pathology Cancer Case Summary

RETINOBLASTOMA: Enucleation, Partial or Complete Exenteration (Notes A, B, C)

Select a single response unless otherwise indicated.

Procedure

___ Enucleation

___ Partial exenteration

___ Complete exenteration

___ Other (specify): _____

___ Not specified

Specimen Size

For Enucleation

Anteroposterior diameter: ___ mm

Horizontal diameter: ___ mm

Vertical diameter: ___ mm

Length of optic nerve: ___ mm

Diameter of optic nerve: ___ mm

___ cannot be determined (see Comment)

For Exenteration

Greatest dimension: ___ cm

+ Additional dimensions: ___ x ___ cm

___ cannot be determined (see Comment)

Specimen Laterality

___ Right

___ Left

___ not specified

Tumor Site (macroscopic examination/transillumination) (select all that apply) (Notes D, E)

___ Cannot be determined

___ Superotemporal quadrant of globe

___ Superonasal quadrant of globe

___ Inferotemporal quadrant of globe

___ Inferonasal quadrant of globe

___ Other (specify): _____

Tumor Basal Size on Transillumination

___ Cannot be determined

Anterior-posterior length: ___ x ___ mm

Transverse length: ___ x ___ mm

Tumor Size after Sectioning (Note F)

___ Cannot be determined

Base at cut edge: ___ mm

Height at cut edge: ___ mm

Greatest height: ___ mm

Tumor Location After Sectioning

___ Cannot be determined

Distance from anterior edge of tumor to limbus at cut edge: ___ mm

Distance of posterior margin of tumor base from edge of optic disc: ___ mm

Tumor Involvement of Other Ocular Structures (select all that apply) (Note I)

___ cannot be determined

___ Cornea

___ Anterior chamber

___ Iris

___ Angle

___ Lens

___ Ciliary body

___ Vitreous

___ Retinal detachment

___ Optic disc

___ Choroid, minimal (solid tumor nest less than 3 mm in maximum diameter [width or thickness])

___ Choroid, massive (solid tumor nest 3 mm or more in maximum diameter [width or thickness])

___ Sclera

___ Vortex vein

___ Orbit

Histologic Features (select all that apply)

___ cannot be determined

___ Undifferentiated

___ Differentiated

+ ___ Homer Wright rosettes

+ ___ Flexner-Wintersteiner rosettes

+ ___ Fleurettes

___ Necrotic

Growth Pattern (Note L)

___ Cannot be determined

___ Endophytic

___ Exophytic

___ Combined endophytic/exophytic

___ Diffuse

Extent of Optic Nerve Invasion

___ Cannot be determined

___ None

___ Anterior to lamina cribrosa

___ At lamina cribrosa

___ Posterior to lamina cribrosa but not to end of nerve

___ To cut end of optic nerve

Histologic Grade

___ pGX: Grade cannot be assessed

___ pG1: Well differentiated

___ pG2: Moderately differentiated

___ pG3: Poorly differentiated

___ pG4: Undifferentiated

Margins (select all that apply)

___ Cannot be assessed

___ No tumor at margins

___ Tumor present at surgical margin of optic nerve

___ Extrascleral extension (for enucleation specimens)

___ Other margin(s) involved (specify): _____

Pathologic Staging (pTNM) (Note M)

TNM Descriptors (required only if applicable) (select all that apply)

___ m (multiple primary tumors)

___ r (recurrent)

___ y (post-treatment).,

Primary Tumor (pT)

___ pTX: Primary tumor cannot be assessed

___ pT0: No evidence of primary tumor

___ pT1: Tumor confined to the eye with no optic nerve or choroidal invasion

pT2: Tumor with minimal optic nerve and/or choroidal invasion:

___ pT2a: Tumor superficially invades optic nerve head but does not extend past lamina cribrosa *or* tumor exhibits focal choroidal invasion

___ pT2b: Tumor superficially invades optic nerve head but does not extend past lamina cribrosa *and* exhibits focal choroidal invasion

pT3: Tumor with significant optic nerve and/or choroidal invasion:

___ pT3a: Tumor invades optic nerve past lamina cribrosa but not to surgical resection line *or* tumor exhibits massive choroidal invasion

___ pT3b: Tumor invades optic nerve past lamina cribrosa but not to surgical resection line *and* exhibits massive choroidal invasion

pT4: Tumor invades optic nerve to resection line or exhibits extra-ocular extension elsewhere:

___ pT4a: Tumor invades optic nerve to resection line but no extra-ocular extension identified

___ pT4b: Tumor invades optic nerve to resection line and extra-ocular extension identified

Regional Lymph Nodes (pN)

___ pNX: Regional lymph nodes cannot be assessed

___ pN0: No regional lymph node involvement

___ pN1: Regional lymph node involvement (preauricular, cervical, submandibular)

___ pN2: Distant lymph node involvement

Distant Metastasis (pM)

___ Not applicable

___ pM1: Metastasis to sites other than CNS

___ pM1a: Single lesion

___ pM1b: Multiple lesions

___ pM1c: CNS metastasis

___ pM1d: Discrete mass(es) without leptomeningeal and/or CSF involvement

___ pM1e: Leptomeningeal and/or CSF involvement

Additional Pathologic Findings (select all that apply)

+ ___ None identified

+ ___ Calcifications

+ ___ Mitotic rate: Number of mitoses per 40x objective with a field area of 0.152 mm^2 (specify): ___

+ ___ Apoptosis

+ ___ Basophilic vascular deposits

+ ___ Inflammatory cells

+ ___ Hemorrhage

+ ___ Neovascularization (specify site): _____

+ ___ Other (specify): _____

+ Comment(s)

Appendix E: The International Retinoblastoma Staging System.

Stage 0: Patients treated conservatively.

Stage I: Eye enucleated, completely resected histologically

Stage II: Eye enucleated, microscopic residual tumor

Stage III: Regional extension (a) overt orbital disease

(b) Preauricular or cervical lymph node extension

Stage IV: Metastatic disease

(a) Heamatogenous metastasis (1) Single lesion (2) Multiple lesions

(b) CNS extension: (1) Prechiasmatic lesion

(2) CNS mass

(3) Leptomeningeal disease.

Appendix F: Photomicrograph of microscopic appearance of retinoblastoma

Plate1: Well differentiated retinoblastoma (x10).This is characterized by numerous true rosettes (Flexner-Wintersteiner rossetes) appearing in most of the tumour sections as shown by the arrows.

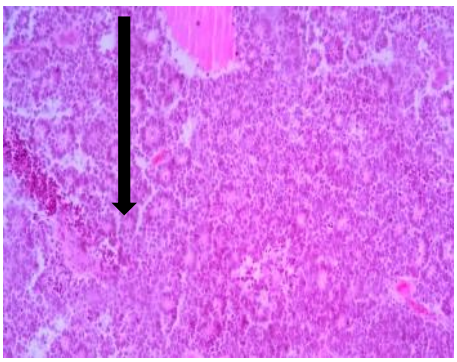

Plate 2: Moderately differentiated retinoblastoma(x10) which is characterized by a moderate number of true rosettes.

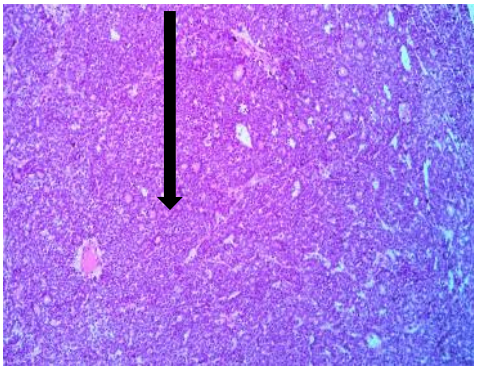

Plate 3: Poorly differentiated retinoblastoma(x10). Characterized by sheets of polygonal round blue cells with no rosette formation.

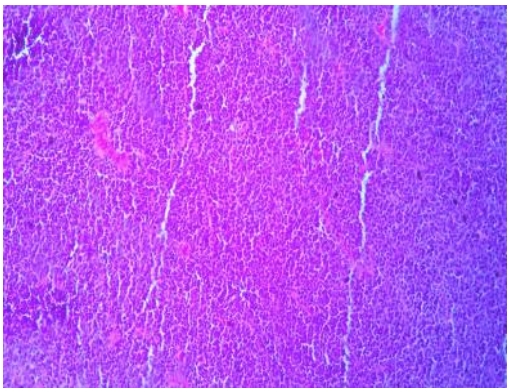

Plate 4: Shows necrotic retinoblastoma. Sometimes the tumour is characterized by vast necrosis with little areas of viable cells. The arrow is pointing at the necrotic area.

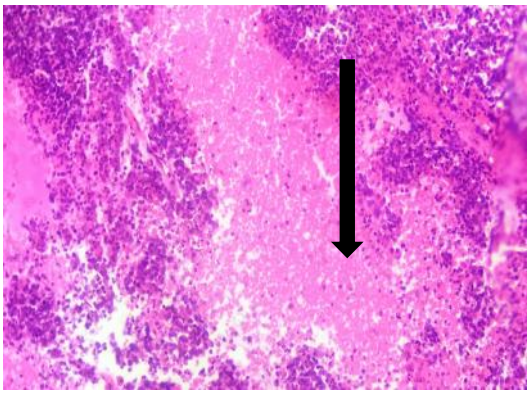

Plate 5: Shows massive choroidal invasion with the tumour cells.

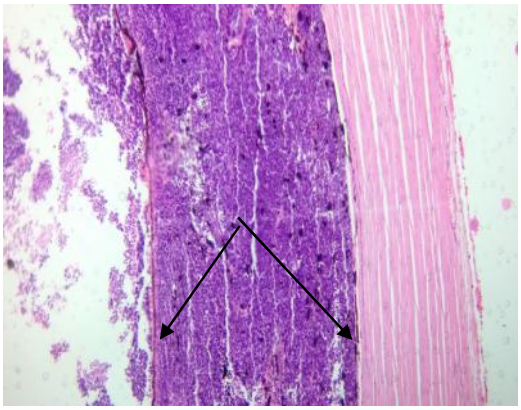

Plate 6: Tumour involving the cilliary body as shown below.

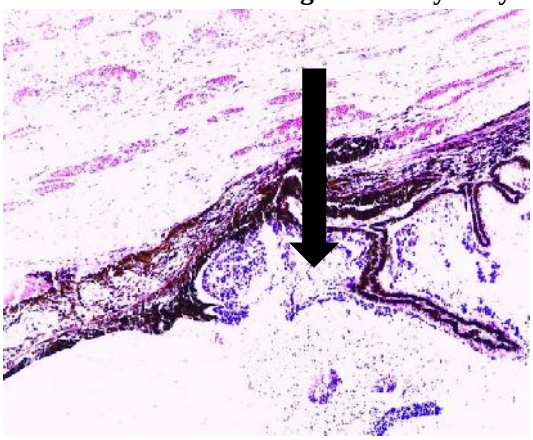

Plate 7:Tumour involving the anterior chamber

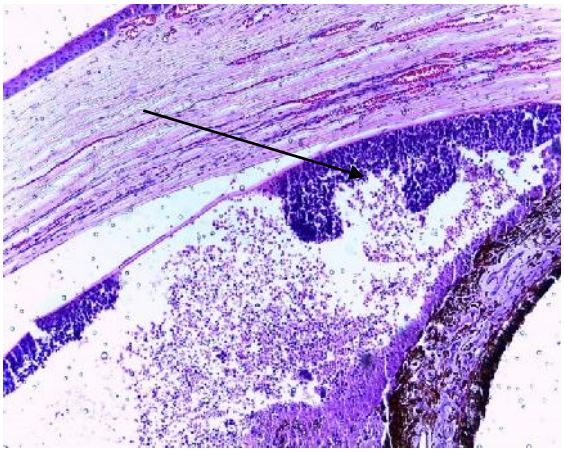

Plate 8: Optic nerve invasion by retinoblastoma; the arrow shows aggragates of tumour cells.