

DISSERTATION

**TUMOUR DIFFERENTIATION AND HIGH RISK HISTOLOGY FEATURES AS
PROGNOSTIC FACTORS AMONG PATIENTS WITH RETINOBLASTOMA AT
KENYATTA NATIONAL HOSPITAL AND PRESBYTERIAN CHURCH OF
EAST AFRICA KIKUYU HOSPITAL.**

BY

DR JOHN MUTHURI M.B.Ch.B (University of Nairobi)

**A dissertation submitted as part fulfillment of the requirement for the award of degree of
Masters of Medicine in Human Pathology, University of Nairobi.**

2016

DECLARATION

I declare that this dissertation is my original work under the guidance of my supervisors and has not been submitted for a degree at any other university.

CANDIDATE: DR JOHN MUTHURI (M.B.Ch.B UON)

Signature. _____ Date. _____

SUPERVISORS APPROVAL

This dissertation has been submitted for examination with our approval as University supervisors.

1. PROF. J. GITHANGA

Associate Professor, Hematology Thematic Unit,

Department of Human Pathology,

University of Nairobi Kenya.

Signature. _____ Date. _____

2. DR. W. WAWERU

Senior Lecturer, Anatomic Pathology Thematic Unit,

Department of Human Pathology,

University of Nairobi, Kenya.

Signature. _____ Date. _____

3. DR. E. DIMBA

Senior Lecturer,

Department of Oral and Maxillofacial Surgery, Oral Pathology and Oral Medicine,

University of Nairobi, Kenya.

Signature. _____ Date. _____

4. DR. K. KIMANI

Senior Lecturer,

Department of Ophthalmology,

University of Nairobi, Kenya.

Signature. _____ Date. _____

5. DR. H. DIMARAS, PhD

Assistant Professor,

Department of Ophthalmology & Vision Sciences,

University of Toronto, Canada.

Honorary Lecturer,

Department of Human Pathology,

University of Nairobi, Kenya

Signature.  _____ Date. _____

DEDICATION

This book is dedicated to all retinoblastoma patients and their parents/guardians.

ACKNOWLEDGEMENTS

The support from the following institutions and individuals made this study possible:

1. Grand Challenges Canada, Cancer Pathology in Africa grant, administered through Daisy's Eye Cancer Fund-Kenya for sponsoring this study.
2. Ministry of Health- Government of Kenya for sponsoring my post graduate studies.
3. My Supervisors Prof. J. Githanga, Dr. W. Waweru, Dr. E. Dimba, Dr. K. Kimani and Dr. H. Dimaras for their guidance and support throughout the study period.
4. Dr. M. Mungania availability and willingness to assist as the tie breaker in case of histopathology discrepancy.
5. My family for standing with me throughout my life.
6. The staff of the Presbyterian Church of East Africa Kikuyu Hospital Eye Unit (especially Dr. J. Kabiru and Dr. A. M'bongo) and M.P. SHAH Hospital histology laboratory led by Dr. T. Onyuma for their facilitation during data collection.
7. Dr. E. Munyoro for her input in developing the telephone interview manuscript/document.
8. Mr. J. Gichana of Retinoblastoma Collaborative Laboratory University of Nairobi Dental School for preparing good histopathology slides.
9. Mr. J. King'ori and Mr. D. Wainaina of Kenyatta National Hospital and Presbyterian Church of East Africa Kikuyu Hospital Eye Unit registries respectively for their support in retrieval of medical records.
10. Dr. P. Wanzala and Mr. D. k'Owino for their assistance in data analysis.
11. My fellow postgraduate students, for your friendship and encouragement.

ABBREVIATIONS

CAP	College of American Pathologists.
CSF	Cerebrospinal Fluid.
CT	Computed Tomography.
EBRT	External-Beam Radiation Therapy.
ERC	Ethical Research Committee.
H &E	Haematoxylin and Eosin.
ICD-O	International Classification of Diseases-Oncology.
I/P no	In-Patient number.
KNH	Kenyatta National Hospital.
MRI	Magnetic Resonance Imaging
MYCN	v-MYC avian myelocytomatosis viral-related oncogene, Neuroblastoma-derived.
RB	Retinoblastoma.
RBCOLAB	Retinoblastoma Collaborative Laboratory.
RB1	Retinoblastoma Tumour Suppressor gene.
RB1-/-	Bi-allelic inactivation of the RB1 gene.
RB1+/+ MYCN ^A	Amplification of MYCN gene associated with normal RB1 gene alleles
RCP	Royal College of Pathologists.
SOP	Standard Operating Procedures.
SPSS	Statistical Package for Social Sciences.
PCEA-KH	Presbyterian church of East Africa Kikuyu Hospital.
TNM	Tumour, Nodes and Metastasis.
U.K	United Kingdom.
U.O.N	University of Nairobi.
U.S.A	United States of America.

LIST OF FIGURES

Figure 1: Illustration of the study design.....	16
Figure 2: flow chart illustrating patients identification, medical records, specimen block retrieval, data collection and entry.....	21
Figure 3 Overview of the Cohort Cases.....	24
Figure 4: Distribution of Hospital where enucleation was done:	25
Figure 5: Distribution of reasons for loss of follow-up for those contacted:.....	26
Figure 6: Distribution of participants by Sex:.....	26
Figure 7: Distribution of participants by Laterality	26
Figure 8: Distribution of participants by Age at presentation:	27
Figure 9: Age at presentation of participants in months vs. laterality	28
Figure 10: Over view of participants outcome:	29
Figure 11: Distribution of the presenting Complaints.	31
Figure 12: Distribution of Adjuvant Chemotherapy Regimens	31
Figure 13: Distribution of completed Adjuvant Chemotherapy	32
Figure 14: Kaplan Meier Overall Survival probability curve:.....	36
Figure 15: Comparison of the survival according to the degree of differentiation.....	36
Figure 16: Comparison of the survival according to the degree of choroidal Invasion:.....	37
Figure 17: Comparison of the survival according to the degree of sclera Invasion:	37
Figure 18: Comparison of the survival according to the degree of Optic nerve Invasion:	38
Figure 19: Comparison of the survival according to TNM staging:.....	39

LIST OF TABLES

Table 1: Association among Laterality, Family history, Leukocoria, Proptosis and Outcome of participants:	30
Table 2: Association among Adjuvant Chemotherapy and Outcome of participants	32
Table 3: Association among Metastasis, Recurrence and outcome of participants	33
Table 4: Summary of Characteristic Histopathologic Features of enucleated eyes.....	34
Table 5: Association between Degree of differentiation, choroidal Invasion, Scleral invasion, Optic nerve invasion and Outcome of participants:.....	35
Table 6: Association between TNM Staging and Outcome of participants:.....	36
Table 7: Multivariate Analysis:	40

TABLE OF CONTENTS

DECLARATION.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS:.....	v
ABBREVIATIONS.....	vi
LIST OF FIGURES:.....	vii
LIST OF TABLES:.....	viii
TABLE OF CONTENTS.....	ix
ABSTRACT.....	xii
1.0 INTRODUCTION:.....	1
2.0 LITERATURE REVIEW	2
2.1 Epidemiology of Retinoblastoma:	2
2.2. Etiology of RB:.....	2
2.2.1 Genetic Etiology [RB1-/-]:.....	2
2.2.2 Genetic RB1+/+ MYCN ^A RB:	4
2.3 Clinical presentation of RB:.....	4
2.4. Differential diagnosis of RB:	5
2.5. Investigations of RB:	5
2.6. Histological features of RB:.....	6
2.6.1 RB -/- Microscopic: Histiogenesis and Degree of tumour differentiation.	6
2.6.2 Microscopic: Extent of tumour spread.....	7
2.6.3. Pathological staging of RB:	8
2. 6.4 Histology of RB1+/+ MYCN ^A RB:.....	9
2.7. Management of RB:.....	9
2.7.1 Enucleation:	9
2.7.2 Systemic chemotherapy:	9
2.7.3 External-beam radiation therapy (EBRT):	10
2.8. Patient outcome:.....	10
2.8.1 Mortality:	11
2.9. Retinoma:.....	11
2.9.1. Retinoma genetics:.....	12

2.9.2. Retinoma diagnostic clinical features:	12
2.9.4. Retinoma prognosis and follow-up:	13
3.0. JUSTIFICATION:	14
4.0. RESEARCH QUESTIONS:.....	15
5.0. HYPOTHESIS:.....	15
6.0. BROAD OBJECTIVE:	15
7.0. SPECIFIC OBJECTIVES:	15
7.1. Primary Objectives:	15
7.2. Secondary Objectives:	15
8.0 METHODOLOGY:	16
8.1 Study Design:.....	16
8.2 Study Area:	17
8.3.1 Inclusion Criteria:	17
8.3.2Exclusion Criteria:	17
8.4 Sample size determination:	18
8.5. Data Collection: Medical records retrieval, review and phone interview:.....	19
8.8. Examination and reporting:.....	20
8.9 Quality Assurance:.....	22
8. 10. Ethical consideration:.....	22
8.11. Data collecting instruments:.....	22
8.12. Variables:	23
8.13. Data management and statistical analysis plan:	23
9.0 RESULTS:.....	24
10.0 HISTOPATHOLOGY APPERANCES (PHOTOMICROGRAPHS);	41
11.0 DISCUSSION:.....	44
12.0 STUDY LIMITATIONS:	48
13.0 CONCLUSIONS:.....	49
14.0 RECOMMENDATIONS:	50
15.0 REFERENCES:	51
APPENDIX I: RBCOLAB RB PROFORMA:	56
APPENDIX II: TNM PATHOLOGICAL CLASSIFICATION OF OCCULAR RB.....	58
APPENDIX III: KNH/UON ETHICAL APPROVAL LETTER:	60

APPENDIX IV: PCEA-KH APPROVAL LETTER:..... 62
APPENDIX V: TELEPHONE INTERVIEWS MANUCRIPT: 63
APPENDIX VI: QUESTIONNAIRE:..... 66
APPENDIX VII: M.P. SHAH APPROVAL LETTER: 70
APPENDIX VIII: SOP FOR RETRIEVED SPECIMEN BLOCKS HANDLING AND PROCESSING: 71
APPENDIX IX. STUDY LABORATORY PROFORMA. 72

ABSTRACT

1. BACKGROUND

The management protocol for retinoblastoma (RB) post-enucleation, recommends adjuvant treatment for patients exhibiting high risk histopathology features. The degree of tumour differentiation in RB has not been shown to have significant prognostic association in most studies.

2. OBJECTIVES

To determine the prognostic value of the degree of tumour differentiation and high risk histopathologic features, and the frequencies of retinoma and histomorphology consistent RB+/⁺ MYCN^A among primarily enucleated RB cases at the KNH and PCEA-KH.

3. DESIGN

A retrospective cohort clinical-pathological review of primarily enucleated RB patient from January 2005 to June 2012 at KNH and PCEA-KH.

4. METHODS AND MATERIALS

One hundred and forty (140) patients' medical records were retrieved from KNH and PCEA-KH. The clinical data obtained included: demographic data, surgical procedure, chemotherapy treatment, and patient outcome which were recorded in a structured questionnaire. Those whose outcome was unknown due loss to follow-up, their next of kin were contacted after seeking verbal consent.

Archived specimen eye blocks of these patients were retrieved, processed and microscopically assessed for: retinoma, RB-/-, RB1+/⁺ MYCN^A RB consistent histomorphological features, degree of tumour differentiation, choroidal invasion, scleral invasion and optic nerve invasion which were recording in a structured proforma. Data was then entered into an Access computer program, cleaned and analysed using Statistical Package for Social Scientists (SPSS) version 20.0 Software.

5. RESULTS

Of the 140 patients eligible for this study, 106 had a known outcome, 76 (71.7%) being alive while 30 (28.3%) were deceased. The Kaplan-Meier survival probability of the 140 patients was 0.85 at 12 months, 0.78 at 36 months and 0.65 at 60 months.

Poorer outcome were noted in patients with bilateral disease ($p=0.016$), proptosis ($p=0.039$), not completing adjuvant chemotherapy ($p=0.042$) and metastasis or recurrence ($p<0.001$). Patients with poorly differentiated tumours had also a significantly poorer outcome compared to those with well or moderately differentiated tumours ($p=0.037$), while the high risk histopathology features were confirmed to confer a significantly poorer outcome; massive choroidal invasion ($p=0.002$), scleral invasion ($p=0.006$) and post-laminar optic nerve invasion ($p<0.001$).

Multivariate analysis showed a significant association with poor outcome with: proptosis, metastasis, recurrence, poorly differentiated tumours, massive choroidal, sclera and post-laminar optic nerve invasion.

The frequency of retinoma was 2.8% among enucleated RB specimens, while no case histomorphologically consistent with RB $+/+$ MYCN^A was noted.

6. CONCLUSION

Patients with poorly differentiated tumour were associated with a poorer survival. High risk histopathology features, were confirmed to having a significant poorer survival. The frequency of retinoma in eyes primarily enucleated for RB was low compared to published figures, while no histomorphological consistent RB $+/+$ MYCN^A subtype was identified in this study.

1.0 INTRODUCTION

Retinoblastoma (RB) is a primitive embryonal tumour arising in the retina and it is the most common intraocular malignancy of childhood occurring before the age of five¹. Its prognosis has tremendously improved over the years especially in developed countries with cure rates of more than 90% being achieved². In the developing countries however, poor health care infrastructure and late presentation have resulted to 5 year survival of less than 50%^(3, 4). In Kenya a 3 year survival rate of 26.6% was reported by Gichigo et al⁵.

Current treatment protocols recommend adjuvant therapy post-enucleation for patient exhibiting the following high risk histopathology features: post-laminar optic nerve invasion, massive choroid invasion and sclera invasion⁶ that have been associated with significant poor prognosis. The degree of tumour differentiation has been determined as a prognostic factor in most cancers such as pancreatic adenocarcinoma and colorectal carcinoma^(7, 8). In case of RB, few studies have shown some prognostic association for tumour differentiation, though not statistically significant^(9, 10) while other studies have shown no prognostic association^(11, 12). Due to this controversy it's not a feature used to direct adjuvant chemotherapy and therefore not routinely included in RB pathology reports.

The histologic features of retinoma the benign variant of RB are distinct from RB, with its frequency among enucleated RB cases being described in some studies ranging from 6%¹³ to 20.4%¹⁴. However no such data is locally available. It has long been thought that RB only occurred following RB1^{-/-} mutation however, advances in RB1 molecular testing has recently demonstrated that approximately 1.4% of unilateral non-familial RB cases have undetectable RB1 gene mutation (RB1^{+/+}) and are induced by amplification of MYCN gene (MYCN^A)¹⁵. These RB ^{+/+} MYCN^A tumours have distinct histomorphologic feature from those of RB1^{-/-} tumour resembling neuroblastoma. In view of its recent discovery few if any cases have been reported locally hence its frequency is also unknown in the Kenyan RB population.

This study aims to determine the prognostic value of the degree of tumour differentiation and high risk histology features with the frequencies of both retinoma and histomorphology consistent RB1^{+/+} MYCN^A RB among primary enucleated RB cases locally.

2.0 LITERATURE REVIEW

2.1 Epidemiology of Retinoblastoma

Retinoblastoma (RB) accounts for 33% to 55% of ocular and orbital malignancies in children. In Nigeria Owoeye¹⁶ found 33% of ocular tumours was RB. In the U.S.A RB accounts for 6% of all pediatric malignancies under the age of 5 years (Broaddus et al)¹⁷ while in Kano (Nigeria) it accounts for 30% of all pediatric cancers¹⁸. The worldwide incidence of RB for children aged 0-4 years varies from 3.4 per million in Bulgaria¹⁹ to a very high 42.5 per million in Mali²⁰. In the U.S.A it ranges from 1: 15,000 to 1:20,000 live births per year¹ while in Kenya the incidence is 1:17000 live births per year²¹.

There is no sex difference noted in most of the studies. In the U.S.A Eagle et al found the ratio between male and female to be 1.02:1¹⁴ and in Nigeria Owoeye et al found male to female ratio of 1:1.2¹⁶. However in Kenya, Kimani²² and Gichigo⁵ found a slight male predominance with no statistical significant difference, the male to female ratio was 1.26:1 and 1.16:1 respectively. While Nyamori²¹ and Maingi²³ found a statistically significant male preponderance with a ratio of 1.49:1 and 1.5:1 respectively. The latter two studies speculated that either the findings were coincidental or boys are taken to hospital more preferentially than the girls.

2.2. Etiology of RB

RB was the first disease where a genetic etiology of cancer was described and the first tumour suppressor gene RB1 identified. It has therefore been long thought that RB only occurred following gene mutation RB1^{-/-}, however advances in RB molecular testing have demonstrated cases with RB1^{+/+} MYCN^{A15}.

2.2.1 Genetic Etiology [RB1^{-/-}]

Knudson²⁴ in 1971 developed the hypothesis that RB is caused by two mutational events whereby there is loss or mutation of both alleles of the RB gene [RB1^{-/-}], localized at chromosome 13q1.4 which is required for disease development. The RB1 gene encodes a 110 KDa RB protein (pRB) which regulates the cell-cycle at the checkpoint between G1 and entry into the S-phase.

Numerous studies however have indicated that other molecular events, in addition to the loss of pRB, are necessary for tumorigenesis. A study by Dimaras et al on retinoma clarified that the two hits in RB1 (M1-M2) only lead to genomic instability with up regulation of the senescence-associated proteins p16INK4a and p130, suggesting that tumour progression occurs when there is further genomic rearrangement (M3-Mn)²⁵. There are two forms of RB1-/- associated RB; germline (heritable) and somatic.

2.2.1.1 Germinal RB1-/-

This form accounts for 40% of all RB-/-, with the affected patients having a germline inactivated RB1 allele present in all body cells and a somatic loss of the second allele in retinal cells. They may have a family history of the disease, and are at risk of passing on the mutated RB1 gene to their offspring's. They usually present with bilaterally disease, but 10% - 15% are unilateral. Identification of this mutation in a family should prompt follow-up of all young children in that family by an ophthalmologist in order to diagnose RB early. Patients with germline mutations are also at risk for developing trilateral RB² and second non-ocular cancers²⁶.

Trilateral RB refers to bilateral RB associated with an intracranial primitive neuroectodermal tumour in the pineal or suprasellar region. It usually occurs in the first 5 years and is found in approximately 3% of all children with RB and in 10% for those having bilateral or familial disease². It has a dismal prognosis hence; patients with bilateral or familial RB are advised to have screening for pineoblastoma at least twice yearly for the first 5 years of life²⁷.

The risk of developing second non ocular tumours is higher in patients with germline mutation with a 5% chance of developing them during the first 10 years of follow-up, 18% during the first 20 years, and 26% within 30 years while the 30-year cumulative incidence is approximately 35% or even higher for those patients who received radiation therapy²⁸. Most second malignancies are high-grade tumours having poor prognosis, they include: osteogenic sarcoma, neuroblastoma, chondrosarcoma, rhabdomyosarcoma, glioma, leukemia, squamous cell carcinoma and cutaneous melanoma.

2.2.1.2 Somatic RB1-/-

Somatic RB -/-accounts for 60% of cases whereby the affected individuals are born with two normal copies of the RB1 gene. Both copies of the RB1 gene are then inactivated somatically in a single developing retinal progenitor cell in early childhood. About 75% of the sporadic tumours are caused by this mechanism and are usually unilateral, unifocal and not heritable.

2.2.2 Genetic RB1+/+ MYCN^A RB

This form of RB has the following distinct genetic characteristic compared to classical RB-/- tumour; has no mutation at RB1 (RB +/+), expression of an intact functioning RB protein (pRB) and amplified 28 – 121 copies of MYCN gene (MYCN^A)¹⁵. MYCN is a member of the basic helix-loop-helix (bHLH) family of transcription factors involved in cell proliferation. It has therefore been postulated that; children having this type of RB may benefit from anti MYCN treatment, however further studies are necessary. Detection of histomorphologically consistent RB1+/+ MYCN^A RB in Kenya would therefore identify children who might benefit from such future anti-MYCN therapy.

2.3 Clinical presentation of RB

The majority of RB -/- cases are diagnosed by 3 years of age and 90% by the age of 5years. In Kenya Gichigo found 63% presented by the age of 3 years at KNH⁵. Children with bilateral RB constitute about 30-40% and unilateral 60-70%. In Kenya, Nyamori found 25.8% bilateral cases and 74.2% unilateral cases countrywide²¹ while Gichigo found 28 % of cases to be bilateral and 72% to be unilateral at KNH⁵. Patients with bilateral RB present earlier than unilateral RB Nyamori found bilateral cases to have a mean age of 26 months and unilateral cases 35.9 months²¹ while Gichigo found bilateral cases to have a mean age of 24.3 months and unilateral cases 39.8 months⁵.

The most common presentation of RB-/- in children is leukocoria²⁹; other presentations are strabismus, glaucoma, hyphema. Proptosis although rare in developed countries is still a frequent presentation in developing countries depicting late disease presentation³⁰. In Kenya Gichigo observed that 43% presented with leukocoria, 27% with ocular inflammation and 18% with proptosis⁵.

Children with RB1^{+/+} MYCN^A tumours present at an earlier age of 3.5 to 10 months compared to RB -/- tumours that present at 15 to 37 months. The RB1^{+/+} MYCN^A tumours are usually unilateral, presenting with large masses with often optic invasion depicting its aggressive nature¹⁵.

2.4. Differential diagnosis of RB

There are many diseases that clinically mimic RB. Shield et al found the three most common conditions to be persistent fetal vasculature (28%), Coats disease (16%), and ocular toxocariasis (16%)³¹. Other less common conditions that may resemble RB include congenital cataract, retinopathy of prematurity, familial exudative vitreoretinopathy, Norrie disease, incontinentia pigmenti, and advanced rhabdomyosarcoma.

2.5. Investigations of RB

Patients suspected to have RB usually undergo indirect ophthalmoscopy and fundus photography. In young children, these examinations are typically done under general anaesthesia. Needle biopsies are rarely, if ever, indicated in RB, as puncturing the eye can lead to tumour seeding and orbital invasion because the tumour is loosely cohesive and friable³². Ultrasonography is useful as it demonstrates masses with high reflectivity that block sound, causing characteristic shadowing behind the tumor, False-positive results are however common. CT scan is more widely used in developing countries because its easily available and more affordable compared to MRI. CT scan shows calcification, with tumour extent or pineal lesion. Since a recent analysis has demonstrated an increased lifetime risk of leukemia and brain tumours in paediatric patients subjected to this imaging modality, MRI is now the preferred modality for imaging³³. MRI has excellent resolution in the diagnosis of extraocular soft-tissue disease and can readily distinguish between RB and Coats disease. One weakness of MRI is that calcification, a key feature of RB, is less readily demonstrable than with CT.

Cytological examinations of CSF is indicated when there is gross evidence of involvement of the optic nerve by imaging studies or histopathology involvement beyond the lamina cribrosa of the enucleated eye. A bone marrow examination and a bone scan are indicated only when the clinical examination is suggestive of metastases or when a blood count abnormality is present³⁴.

2.6. Histological features of RB

RB is ultimately confirmed by histology. The College of American Pathologist (CAP) and Royal College of Pathologist (RCP) guidelines on RB recommends that a total four cassettes composed of: the optic nerve stump, the Pupil-Orbital section, and the two minor calottes are sampled from the enucleated eyes and processed^(35, 36). From this the tumour histogenesis, grade and extent of spread are determined.

In-order to standardize RB histopathology reporting, a structured proforma capturing RB histopathology features, is recommended. An example (Appendix I) is currently being used at the retinoblastoma collaborative laboratory Kenya (RBCOLAB).

The RBCOLAB was started with the aim of establishing a coordinated national RB pathology service. This was an initiative of the wider Kenya National Retinoblastoma strategy that was set up in the year 2008 with the aim of developing a sustainable, locally managed diagnosis and treatment program for RB through various stake holders in the field of RB³⁷.

2.6.1 RB -/- Microscopic: Histiogenesis and Degree of tumour differentiation

RB-/- associated RB is characterized by sheets and nests of small round to polygonal blue cells that have a scanty cytoplasm and large basophilic hyper chromatic nuclei. In addition there is scanty stroma with frequent mitotic figures, calcification and areas of necrosis. The presence of Flexner-Wintersteiner is pathognomonic for RB and confers the degree of differentiation.

Some studies have attempted to grade the histology of RB-/- associated RB based on the presence and proportion of Flexner-Wintersteiner rosettes. These rosettes are characterized by tumour cells which are joined by connections analogous to the retina's external limiting membrane surrounding an empty central lumen. The three-tier system of well, moderate and poorly differentiated is used in majority of the studies^(12, 38) classifying the degree of differentiation according to the estimated percentage of Flexner-Wintersteiner rosettes in the available sections as follows; well differentiated; rosettes in >80% of tumour areas, moderate differentiated; any rosettes to <80% and poorly differentiated tumours no rosettes. Homer-Wright rosettes are not a sign of significant differentiation since they are not specific to RB being also seen in neuroblastoma or medulloblastoma.

Poorly differentiated tumours are more often noted in developing countries compared to the developed countries and this may be attributed to late disease presentation. A study done in France by Khelifaoul found, 42% of cases were well differentiated, 42% moderately differentiated and 16% poorly differentiated¹². In India Seema found poorly differentiated RB presented in 80.3% of eyes and well differentiated in 19.7%³⁹. In Nigeria Ajaiyeoba found 45% of cases were moderate differentiated and 55% cases were poorly differentiated, but no well differentiated cases¹⁰. Maingi et al in Kenya found 9.7% well differentiated, 25.8% moderately differentiated, 48.4% poorly and undetermined 16.1%²³.

A few studies have shown close prognostic association of RB tumour differentiation although not statistically significant^(9, 10) while other studies have shown no association^(11, 12). Due to this inconsistent, it is not a feature used to direct adjuvant chemotherapy and therefore not routinely reported. The current RBCOLAB reporting proforma does not include RB tumour grade (Appendix I).

2.6.2 Microscopic: Extent of tumour spread

Determining the extent of tumour spread helps identify those in need for adjuvant chemotherapy. The sites assessed are: the optic nerve, choroid, and sclera invasion. The following criteria are applied to determine the extent of optic nerve invasion; prelaminar, laminar, retrolaminar and tumour at optic nerve surgical margin⁴⁰. In Tanzania Bowman found 45% with retro-laminar optic nerve involvement and 29% with optic nerve resection margin involvement⁴. In Kenya Maingi found 33.3% optic nerve involvement with 3.2% prelaminar, 12.9%, at laminar, 32.2% post laminar and 51.6% involving the surgical margin²³. Optic nerve invasion especially past the lamina cribrosa has been identified as a significant poor prognostic factor⁴¹. Once the tumour crosses the lamina cribrosa, there is a higher chance of tumour cells having easy access to the pia-arachnoid, with spread to the central nervous system via the cerebrospinal fluid. The risk for extraocular relapse also increases significantly especially if the resection margin is invaded by tumor. It's therefore recommended to reset at least 10 mm of the optic nerve during enucleation⁴².

The extent of choroid invasion (focal or massive) by the tumour should be stated⁴⁰. Focal choroidal invasion; is defined as a solid nest of tumour that measures less than 3 mm in maximum diameter (width or thickness), while massive choroidal invasion; is defined as a solid tumour nest 3 mm or more in maximum diameter (width or thickness) in contact with the underlying sclera. The degree of choroidal invasion varies among RB studies. In the U.S.A Shield found 23% cases⁴¹. In Tanzania Bowman found 62% cases⁴ with choroidal invasion. Massive choroidal invasion has been associated poor prognosis^(12, 43). In Kenya 5 out of 6 patients who had choroidal invasion died within a 3 year period⁵.

Scleral invasion by RB occurs when the tumour extends beyond the choroid into the sclera. True sclera invasion should be differentiated on histopathologic grounds from “floaters,” which are free neoplastic cells that are dragged passively to the sclera during tissue processing thereby simulating scleral invasion. The extent is categorised as intrascleral or extrascleral invasion. Intrascleral, when tumour cells invade the sclera without surpassing the episclera while extra-scleral, when tumour cells invade the whole width of the sclera to the periorbital tissue⁴⁰. At KNH Maingi found 1.1% of cases with intrascleral invasion and 21.5% with extra-scleral invasion²³ while Gichigo found 24% with extra-scleral spread⁵. Any degree of scleral invasion is associated with poor prognosis^(12, 44).

2.6.3. Pathological staging of RB

Following histological evaluation of RB, staging is done in reference to the current Pathologic TNM system 7th edition (Appendix II). Where T is the tumour size; N nodal involvement and M metastasis are demonstrated on histology.

Tumours confined to the retina are staged pT1 while those with minimal optic nerve invasion not beyond the lamina and or focal choroid invasion are staged pT2. Tumours invading the optic nerve past lamina cribrosa but not to surgical resection line and or exhibiting massive choroidal invasion are staged pT3, while tumours invading the optic nerve to resection line and or extra-ocular extension are staged pT4.

2.6.4 Histology of RB1+/+ MYCN^A RB

Has distinct histomorphology features from RB-/- comprising of; undifferentiated large cells with prominent multiple nucleoli, frequent apoptotic bodies, little calcification, necrosis, absent Flexner-Wintersteiner and Homer Wright rosettes. These histopathology features are almost similar to those observed in MYCN-amplified neuroblastoma^(45,46) probably due to their shared genetic mechanism. Due to its aggressiveness, it's mostly associated with involvement of extra ocular structures.

2.7. Management of RB

To optimize RB treatment, a multidisciplinary team that includes; an ophthalmologist, pathologist, paediatric oncologist, and radiation oncologist is involved. The goals of management are: to save the patient's life, preserve as much vision as possible, and decrease risk of late sequela from treatment particularly subsequent neoplasm.

2.7.1 Enucleation

Patients considered for enucleation include those with advanced RB in one or both eyes, active tumour in a blind eye, and painful glaucoma from tumour invasion. More than 99% of patients with unilateral RB without microscopic or macroscopic extraocular disease (pT1) are cured with this procedure.

Critical elements of the surgery include obtaining a long stump of optic nerve usually more than 10 mm and avoiding any perforation of the globe. Enucleated globes are evaluated for high-risk histopathology features.

2.7.2 Systemic chemotherapy

There are two forms systemic therapy pre-enucleation chemo-reduction and post enucleation adjuvant therapy. Side effects when present include myelosuppression with increased susceptibility to bacterial infections and bleeding tendencies. The goal of chemo-reduction is to reduce tumour size, facilitating more focused and safer therapy in advanced cases. However its use has come into scrutiny after a study by Zhao et al found that chemo-reduction leads to down staging and underestimating the histopathology features, hence, increasing the risk of dissemination⁴⁷.

Adjuvant chemotherapy is provided for patients with high risk RB histology features post-enucleation helping in preventing metastasis and improving survival⁶. In a study by Kaliki analysing 52 eyes with high-risk RB features managed with post-enucleation adjuvant chemotherapy using vincristine, etoposide, and carboplatin showed no evidence of systemic metastasis in any case over a mean follow-up of 66 months⁴⁸. Khelfaoul found a higher 3 year disease free interval in patients with high risk histopathology features treated with adjuvant chemotherapy compared to patients with no chemotherapy treatment which was statistically significant¹².

2.7.3 External-beam radiation therapy (EBRT)

RB is very radiosensitive with EBRT doses ranging from 35 Gy to 46 Gy usually result in long-term remissions however EBRT has been associated with the risk of subsequent neoplasms in children with hereditary RB⁴⁹. Newer methods of delivering EBRT are being used at many centers in an attempt to reduce adverse long-term effects. This includes intensity-modulated radiation therapy, stereotactic radiation therapy, and proton-beam radiation therapy⁵⁰.

2.8. Patient outcome

The possible outcomes in patient with RB include: cure, tumour recurrence, tumour metastasis or mortality. In developed countries cure rates of more than 90% have being achieved². This has been attributed to early clinical diagnosis, improved diagnostic criteria and treatment. In developing countries however, the prognosis remains poor with cure rates of less than 50%^{3 and 4}.

Recurrence may occur after aggressive local and systemic therapy or following enucleation. Following chemo-reduction and focal consolidation, tumor recurrence was found in 18% of tumors at 7 years by Shield⁵¹. At KNH Gichigo found 30% of patient with recurrent masses⁵. Orbital RB recurrence occurs within 12 months after enucleation, in a study by Kim et al 69 of the 71 patients (97%) who had tumour recurrence were diagnosed within the first 12 months⁵².

Metastasis generally develops within 1 year of intraocular tumour diagnosis. Those at greatest risk for metastasis are patient with delayed clinical diagnosis and high risk histology features⁵³. Kopelman reported a 2.5 times increased chance of metastasis and death in patient with delayed clinical diagnosis⁵⁴. Patients with evidence of these poor prognostic histology features should therefore be treated with adjuvant chemotherapy to prevent metastases.

The sites of RB metastasis include local extension to the orbit and CNS as well as distant metastasis involving the lungs, bones, and bone marrow. There are different routes of metastasis to these sites: Orbital RB occurs as a result of progression of the tumour through the emissary vessels and sclera, intracranial dissemination occurs by direct extension through the optic nerve while distant metastasis occurs through haematogenous spread following choroidal invasion.

2.8.1 Mortality

Mortality from RB is increased in patient with; extraocular disease, metastasis, trilateral RB and second malignant neoplasms. The prognosis for metastatic RB is dismal and the presence of CNS involvement has been shown to have a worse outcome. In Turkey a study by Gündüz et al assessed 18 patients with RB metastasis and found 9 had CNS metastasis, 4 patients had distant metastasis and 5 had both CNS and other distant site metastasis. At a mean follow-up of 24 months all patients who had any form of CNS metastasis were deceased while the 4 patients who had distant metastasis without CNS involvement were alive⁵⁵. In Mexico Leal et al assessed 81 patients with metastasis, 68 of whom had CNS involvement. 46 of those patients with CNS involvement died despite treatment⁵⁶. In Kenya Gichigo found 21 patients with CNS metastasis, all 21 dying within 2 to 23 months of admission⁵.

Studies done by Paulino and Kivelä showed that patients diagnosed with trilateral RB have median survival of 6 to 9 months^(27, 57). While in Netherlands; Marees et al reported an almost 13 fold increase of second malignancy death while comparing hereditary RB survivors to the general population⁵⁸.

2.9. Retinoma

Retinoma has distinct clinical and histological features from RB^(59, 60). It is frequently found adjacent to RB suggesting that it is a common precursor of RB²⁵. Its incidence in the general population is unknown however its frequency has been described among the population with RB following either clinical or histological evaluation. With those observed clinically ranging from 1.8% (Gallie)⁵⁹ 3.2% (Abouzeid)⁶¹ among RB cases, while those observed histologically range from 6% (Ts'o)¹³ to 20.4% (Eagle)¹⁴.

2.9.1. Retinoma genetics

Several theoretical mechanisms have been proposed to explain the development of retinoma. Recently, it has been demonstrated that the two mutational events inactivating RB1 gene are already present in retinomas. A study by Dimaras²⁵ on retinoma showed that the two hits in RB1 (M1-M2) do not inevitably cause a malignant phenotype, but lead to genomic instability and up regulation of the senescence-associated proteins p16INK4a and p130. These senescence-associated proteins are thought to prevent tumor progression.

2.9.2. Retinoma diagnostic clinical features

They were described by Gallie et al in 1982; characterized by a homogenous translucent grey elevated mass, opaque white calcified flecks having appearance of cottage-cheese and retinal pigment epithelium migration⁵⁹. Singh noted another feature; presence of a zone of chorioretinal atrophy⁶².

2.9.3. Retinoma microscopic features and Immunostains

Retinoma histopathology features described by Ts'o et al and Margo et al are characterized by: smaller and less hyperchromatic nuclei than in RB, abundant eosinophilic cytoplasm and intercellular matrix, absent or very rare mitotic figures, typically absent necrosis, calcification in non-necrotic tumour and differentiation into fleurettes and lack of Homer Wright and Flexner-Wintersteiner rosettes^(13, 60).

The term 'fleurette' denotes a bouquet-like arrangement of cytologically benign cells joined by a series of zonulae adherentes that may form a short segment of neoplastic external limiting membrane. Bulbous eosinophilic processes that represent abortive photoreceptor inner segments form the 'flowers' of the bouquet.

Immunostains Ki67, PCNA, p53 and p130 are used to distinguish between retinoma and RB. Dimaras et al showed, proliferation markers Ki67 and PCNA stained strongly positive in RB, but were undetectable in retinomas. Occasional cell in retinomas stained faintly with p53, but strong staining was observed only in a subset of cells in RB, while p130 was strong in retinoma but not detected in RB²⁵.

2.9.4. Retinoma prognosis and follow-up

The vast majority of adult patients with clinically diagnosed retinoma are asymptomatic and is usually non-progressive therefore does not require treatment. However a few may transform to malignancy with a range of 4 % (Singh et al)⁶¹ to 12% (Abouzeid et al)⁶². Eagle et al in 1989 reported a case of retinoma in a young girl; the tumor was dormant for two years following diagnosis but later underwent malignant transformation and was enucleated at 34 months after presentation⁶³. Hence ocular examination should be performed on an annual basis for possible risk of malignant transformation.

3.0. JUSTIFICATION

Current treatment protocols for RB patients post-enucleation recommend adjuvant therapy for those exhibiting high risk histopathology features such as, post-laminar optic nerve invasion, massive choroid invasion and sclera invasion⁶ that have been associated with significant poor prognosis. The degree of tumour differentiation in most cancers such as colorectal cancer has a bearing in patient management due to its significant prognostic association⁸; however in RB it shows no statistical significant association and therefore not currently used to direct on adjuvant treatment post enucleation. There is no local study assessing whether the degree of tumor differentiation and the high risk histology features have any significant prognostic impact among primarily enucleated RB patients.

The frequency of retinoma in various studies ranges from 6%¹³ to 20.4%¹⁴ while the frequency of the histomorphologically consistent RB1+/+ MYCN^A RB among enucleated RB patients is 1.4%¹⁵. There is no local data for both retinoma and histomorphologically consistent RB1+/+ MYCN^A RB frequencies. This may be attributed to the fact that histopathology features of retinoma are not routinely reported and RB1+/+ MYCN^A RB being recently demonstrated none or few cases have been locally reported.

Determining the prognostic impact of the degree RB tumour differentiation and high risk histology features locally will form a good basis for subsequent patient management. While the frequencies of retinoma and histomorphology consistent RB1+/+ MYCN^A RB among enucleated RB patients will generate local data and also may be of importance in future patients management.

4.0. RESEARCH QUESTIONS

1. Does the degree of tumour differentiation have any prognostic association among primarily enucleated patients with RB at KNH and PCEA-KH?
2. Do the high risk histopathology features have any prognostic association among primarily enucleated patients with RB at KNH and PCEA-KH?

5.0. HYPOTHESIS

The degree of tumour differentiation and the high risk histopathology features will have no prognostic association among RB patients at KNH and PCEA-KH.

6.0. BROAD OBJECTIVE

To determine the prognostic association of the tumour differentiation and the high risk histopathology features among primarily enucleated patient with RB at KNH and PCEA-KH.

7.0. SPECIFIC OBJECTIVES

7.1. Primary Objectives

1. To determine the degree of tumour differentiation and the high risk histopathology features among primarily enucleated RB patients at KNH and PCEA-KH.
2. To determine patient outcome among primarily enucleated RB patients at KNH and PCEA-KH.
3. To correlate the degree of tumour differentiation and the high risk histopathology features with the patient outcome among primarily enucleated RB patients at KNH and PCEA-KH.

7.2. Secondary Objectives

1. Determine the frequency of retinoma among primarily enucleated RB patients at KNH and PCEA-KH.
2. Determine the frequency of histomorphologically consistent RB1^{+/+} MYCN^A RB among primarily enucleated RB patients at KNH and PCEA-KH.

8.0 METHODOLOGY

8.1 Study Design

This was a retrospective cohort clinical-pathological review of primary enucleated RB patient from January 2005 to June 2012 at KNH and PCEA-KH.

Study design illustration

The study population was the primarily enucleated RB patients who were retrospectively followed up from the first day of enucleation. The groups/cohorts were categorized based on presence of the independent variables; poorly differentiated tumour or presence of established high risk histopathology features for exposed group and well and moderately differentiated tumour with absence of established high risk histopathology features for the unexposed group. The dependent variable (prognostic indicator) being the patients survival either dead for poor outcome or alive for good outcome, as illustrated in figure 1 below.

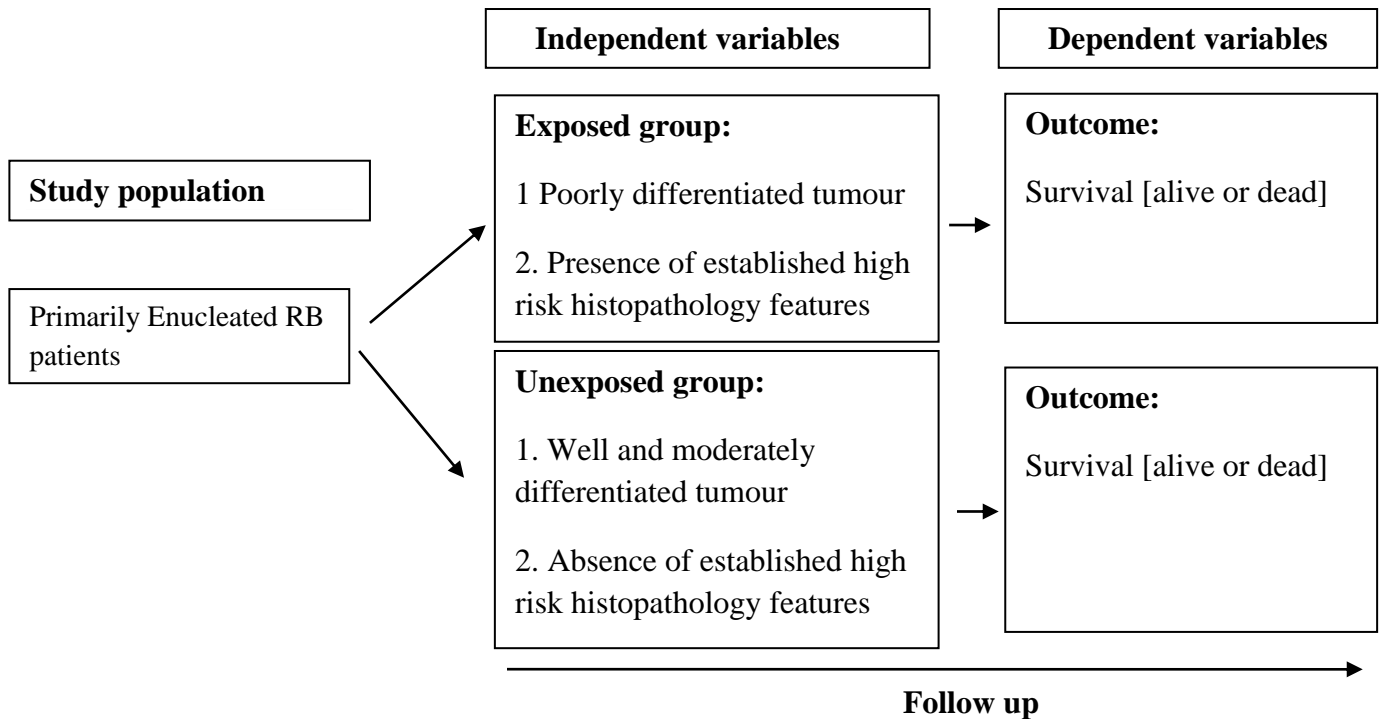


Figure 1: Study design illustration.

8.2 Study Area

The study was conducted at KNH and PCEA-KH Ophthalmology Operating Theatres, Medical Records Registries and KNH, M.P. Shah and RBCOLAB histology laboratories.

The RBCOLAB was established in October 2011 as a centralized laboratory for histopathology evaluation of enucleated RB specimens in Kenya serving most of the hospitals including KNH and PCEA-KH. Prior to its set-up enucleated RB eyes from KNH and PCEA-KH were processed and reported at KNH and M.P. Shah histopathology laboratories respectively

8.3. Study Population

One hundred and forty (140) patients who underwent primary enucleation at KNH and PCEA-KH in the period between January 2005 to June 2012 and their eye specimens histopathologically confirmed to have RB were recruited.

8.3.1 Inclusion Criteria

1. Primarily enucleated patients at KNH and PCEA-KH whose specimen were histopathologically confirmed to have RB from January 2005 to June 2012.
2. Patients with in-complete clinical data and their parents or guardian gave verbal consent to be interviewed.

8.3.2 Exclusion Criteria

1. Secondary enucleation.
2. Missing specimen blocks
3. Missing medical records.
4. Those with fatal outcomes attributable to causes other than ocular RB such as road traffic accident.

8.4 Sample size determination

The sample size was determined using a two proportions formula⁶⁴ illustrated below.

$$n = \left(\frac{z_{\alpha} \sqrt{2\pi_1(1-\pi_1)} - z_{\beta} \sqrt{\pi_1(1-\pi_1) + \pi_2(1-\pi_2)}}{\pi_1 - \pi_2} \right)^2$$

Substitution into the formula
n=108

Where;

- π_1 Estimated proportion of RB survivors at 1 year with poor histopathology feature (optic nerve resection margins involvement) 60 %.(Khelfaoulet al¹²)
- π_2 Estimated proportion of RB survivors at 1 year with no poor histopathology feature (no optic nerve invasion) 95%. (Khelfaoul et al¹²)
- z_{α} Is the two-tailed value of z related to null hypothesis (5%) -1.96
- z_{β} Is the lower one-tailed value of z related alternative hypothesis (80 % power) -0.84

8.5. Data Collection: Medical records retrieval, review and phone interview

Upon ethical approval (Appendix III) permission was sought from relevant authorities at KNH and PCEA-KH to retrieve and review medical records. Once permission was granted (Appendix IV), a list of patients who underwent enucleation from January 2005 to June 2012 for suspected RB was made manually from KNH and PCEA-KH ophthalmology operating theatres records. The medical files of the identified patients were retrieved using the International Classification of Diseases (ICD-10) coding system which codes 69.2 for RB by the help of research assistants well versed in medical records keeping from medical registries both in KNH and PCEA-KH.

Patients who underwent primary enucleation and diagnosed to have RB were identified from the retrieved medical records and accessed for:

1. Demographic data: age at presentation, sex and county of birth.
2. Presenting complaints,
3. RB Laterality: Unilateral or Bilateral,
4. Date and age at enucleation
5. Mode of enucleation: Primary or secondary,
6. Adjuvant therapy if provided: regimen and cycles,
7. Disease state post enucleation (metastasis or recurrence),
8. Patient survival status (alive or dead) where applicable
9. Last follow-up date.

In the case of patients who were lost to follow-up, telephone interviews (Appendix V) were used to collect data from their next of kin after seeking a verbal consent⁶⁵. Information regarding reason for loss of follow-up, patient survival status (alive or dead), date of death and cause of death were obtained and entered in a structured questionnaire (Appendix VI).

8.7. Specimen retrieval and processing

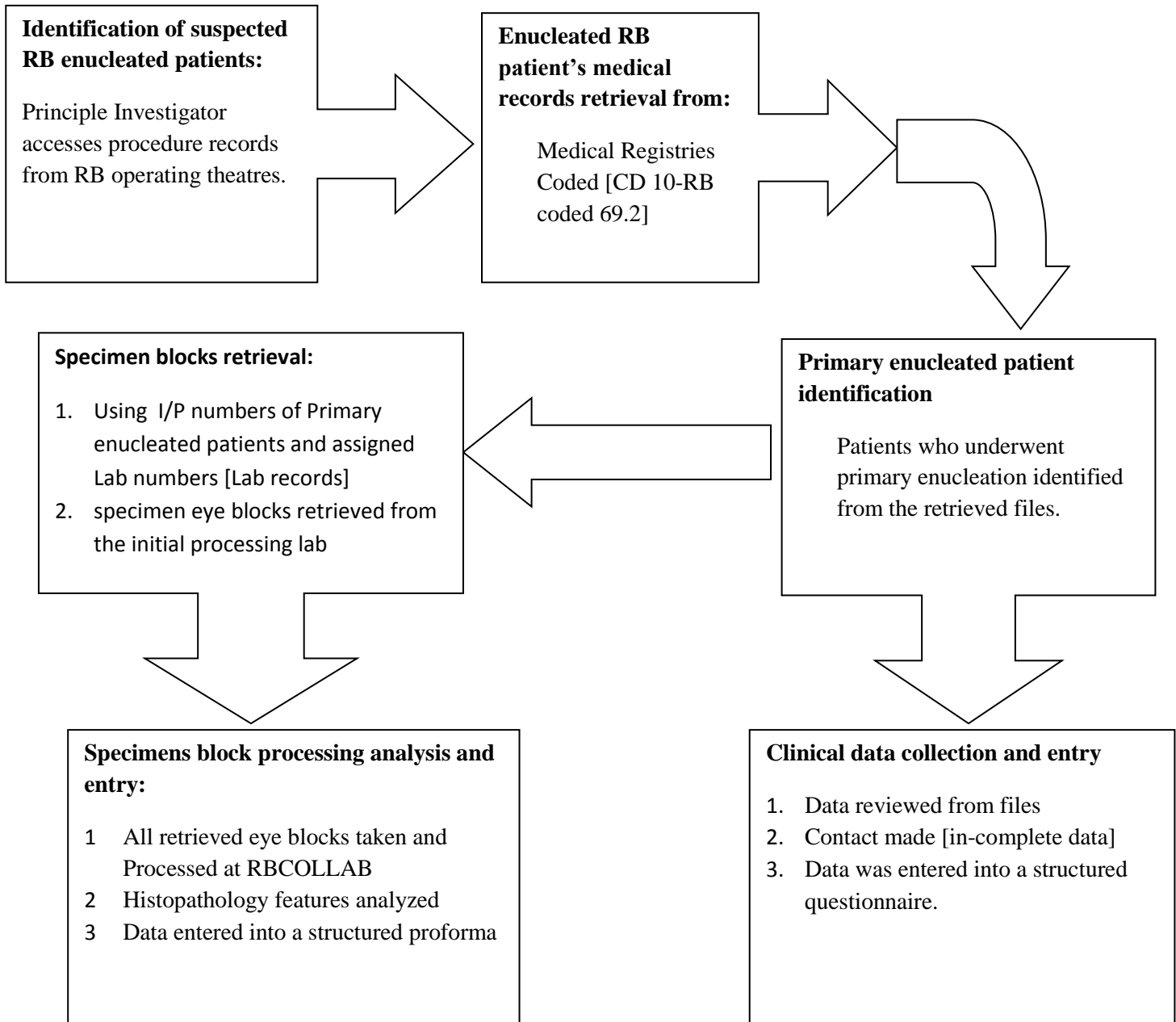
After obtaining permission from chief administrators at KNH, M.P.Shah (Appendix VII) and RBCOLLAB laboratories, eyes specimen blocks of patient who underwent primary enucleation and reported as RB from Jan 2005 to June 2012 at KNH and PCEA-KH were retrieved using the patient's in-patients (I/P) and specimen laboratory numbers. Upon retrieval they were assigned a study number and all processed at RBCOLLAB for standardization adhering to SOP (Appendix VIII).

8.8. Examination and reporting

All processed slides were assessed by the principal investigator and two pathologists (supervisors) and recorded in a proforma (Appendix IX). Where there was lack of consensus the slides were reviewed by a third blinded pathologist as the tie-breaker. The features assessed were:

1. Re-Confirmation of RB.
2. Presence of retinoma features,
3. Histomorphology features consistent with RB1+/+ MYCN^A RB (neuroblastoma like).
4. Degree of tumor differentiation (three tier system) based on the percentage of Flexner-Wintersteiner rosettes on the Pupil-Orbit section:
 - A. Well differentiated: more than 80% of the tumor area
 - B. Moderately differentiated: any to 80% of the tumor area
 - C. Poorly differentiated: complete absence of rosettes
5. High risk histopathology feature:
 - A. Extent of Optic nerve invasion; none, pre-laminar, laminar, post-laminar and surgical margins involvement.
 - B. Extent of Choroidal invasion; none, focal or massive invasion
 - C. Extent of Sclera involvement; none, intra scleral or extra sclera invasion.
6. Involvement of other ocular structures: Iris, ciliary body, lens and anterior chamber,

FIGURE 2: flow chart illustrating patients identification, medical records retrieval, specimen block retrieval, data collection and entry



8.9. Quality Assurance

- A trained technologist on histology eye processing was hired.
- The retrieved eye blocks were clearly labeled.
- The retrieved blocks were processed adhering to standard operating procedure (S.O.P.).
- The principle investigator reviewed the histopathology features and diagnosis, two blinded supervising pathologist independently confirmed these findings. In case of lack of consensus that case was reviewed by a third blinded pathologist as the tie-breaker.
- Every tenth case, slides were also reviewed by the third blinded pathologist.
- Data was carefully entered into respective data collections forms to avoid mix-ups.

8.10. Ethical considerations

- Permission to conduct this study was sought and obtained from KNH/UON-ERC (Appendix III).
- Written authorization to access patient's medical records and retrieve eye specimen block was obtained from PCEA-KH and M.P. Shah (Appendix IV and VII respectively).
- Information regarding the outcome of the children's who were lost to follow-up was obtained from their guardian or parent after seeking a verbal consent.
- Confidentiality was maintained, with only the principal investigator, supervisors and statistician allowed to view the data with identifiers.
- This study had no adverse effects on subjects' health and no extra cost was accorded to the patient.
- The retrieved blocks were returned to their corresponding archives after processing.

8.11. Data collecting instruments

- Clinical and histology data was collected using predesigned questionnaire (Appendix VI) and reporting proforma (Appendix IX) respectively

8.12. Variables

1. The Independent variables were the degree of tumour differentiation, established poor prognostic histological features, gender, laterality, age at tumor presentation, age at enucleation, treatment and presence of tumour recurrence and metastasis.
2. The dependent variables (prognostic indicator): Time to event from enucleation to present, determined by patient survival either dead or alive.

8.13. Data management and statistical analysis plan

- All participants were assigned a unique study number and data collected using a structured questionnaire and proforma. Once collected data was stored safely in a locked drawer. The data was then entered into access program and cleaned using Epi-info 7.
- All statistical tests were performed at 5% level of significance (95% confidence interval) using SSPS 20.0 software
- Data frequencies were generated using bar charts, pie charts and graphs. Continuous variables were analyzed using measure of central tendency, measure of variation and Student t-test, while categorical variables were analyzed using Chi square test.
- Univariate and Multivariate regression methods analysis were used to determine prognostic factors associated with patient outcome. The overall disease free and survival interval were analyzed with Kaplan Meier method.
- A P-value of < 0.05 was considered to be statistically significant.

9.0 RESULTS

In the period under review a total of 280 patients from KNH and PCEA-KH underwent enucleation for suspected RB. 140 of these patients were excluded from the study: 70 cases had missing clinical files, 13 cases had RB ruled out on histology, 31 cases had undergone secondary enucleation, 3 patients died due to unrelated ocular RB (two had trilateral RB and one had pulmonary tuberculosis) and 23 cases had missing laboratory blocks. 140 cases were eligible for the study, with 106 known and 34 Unknown outcomes respectively. Figure 3 below demonstrates the cohort overview.

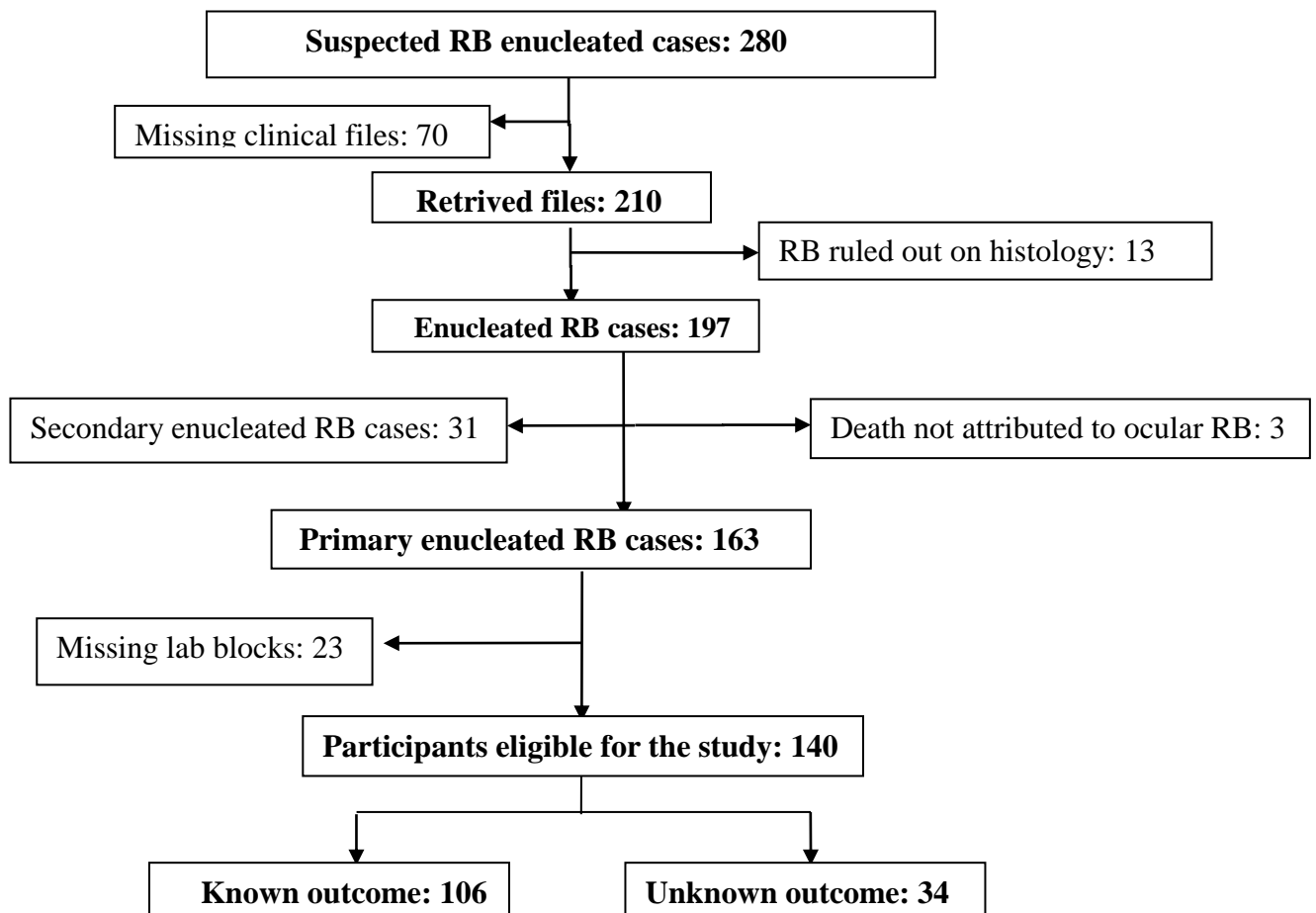


Figure 3: Overview of the cohort participants.

Key RB = Retinoblastoma.

Hospital where primary enucleation was done

Of the 106 participants with known outcome majority 69 (65.09%) were enucleated at KNH compared to 37 (34.91%) at PCEA-KH, as illustrated in figure 4 below.

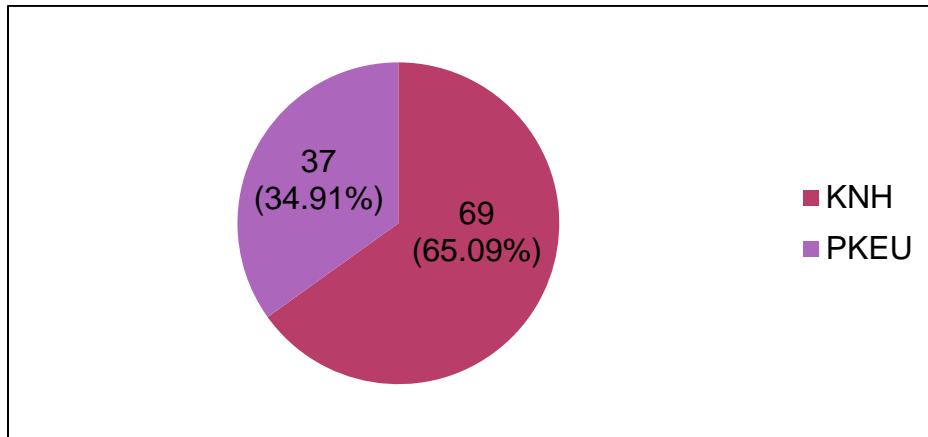


Figure 4: Distribution of hospitals where enucleation was done (n=106)

Obtained clinical data

Of the 106 participants whose outcome was known, 88 (83.02%) had complete clinical data and were on follow-up, while 18 (16.98%) had incomplete clinical data due to loss of follow-up with their outcome being established after contacting their guardian or parents on phone.

Mean follow-up period in months for patients with complete clinical data (n=88) was 44.93, median 41.50, Range 2.00 - 118.00.

Mean follow-up period in months for patients with in-complete clinical data (n=18) was 10.3, median 7.0, Range 0.10 – 37.0.

Distribution for the reasons of loss of follow-up for those contacted

The major reason for loss of follow-up among the 18 cases whose parents or guardians were contacted, was financial constraints in 12 (66.67%) of cases, as illustrated in figure 5 below

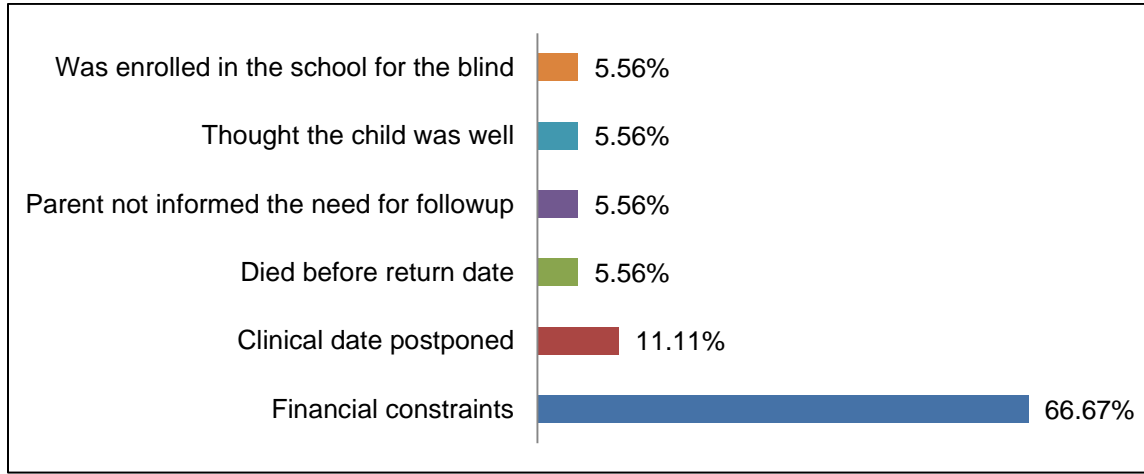


Figure 5: Distribution for the reasons for loss of follow-up for those contacted (n=18).

Distribution of participants by sex

There was no significant sex difference observed between, male 56(52.8%) and female 50(47.2%), with a Ratio of 1.30:1 and $p = 0.627$. Figure 6 below illustrates the distribution of participants by sex.

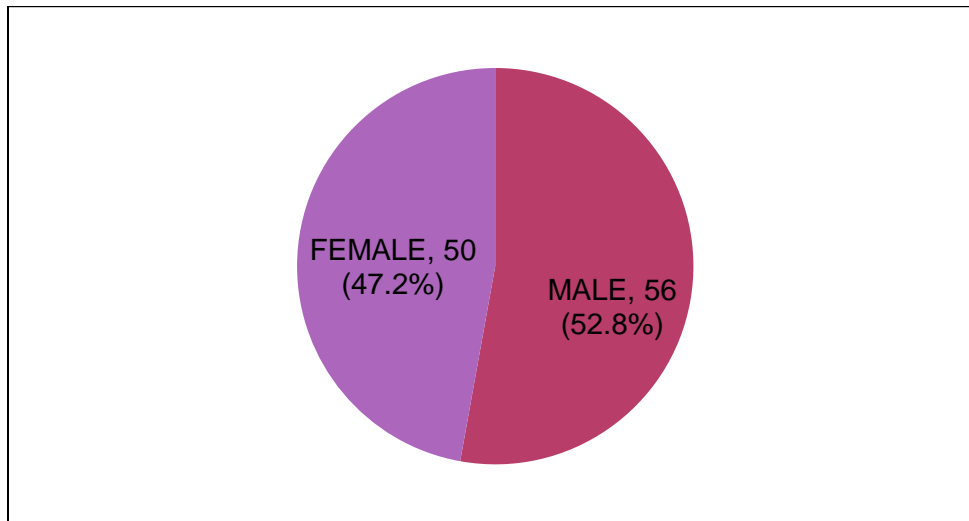


Figure 6 Distribution of participants by sex (n=106).

Distribution of participants by laterality

Majority of patients 84 (79%) had unilateral RB with 22 (21%) having bilateral disease, as illustrated in figure 7 below.

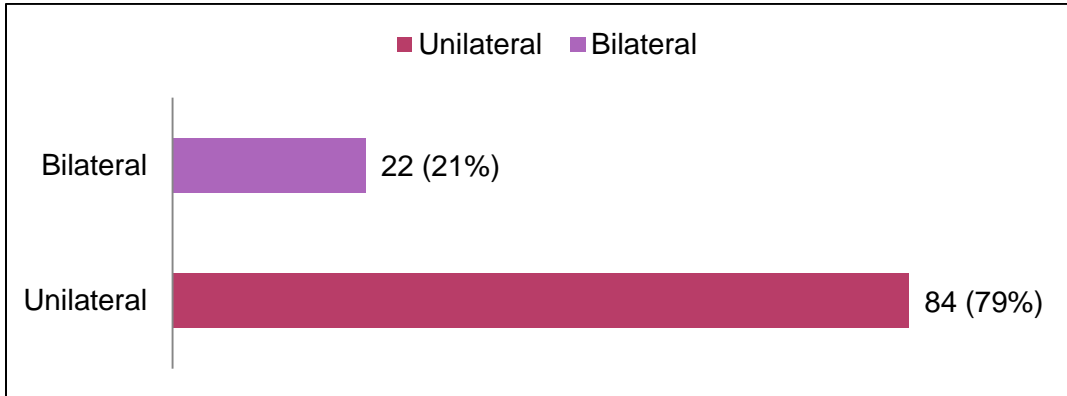


Figure 7: Distribution of participants by laterality (n=106).

Distribution of participants by age at presentation

The mean age at presentation was 26.8 months (SD 16.82), median 24.00 months, mode 36.00 months and Range 2.00 - 81.00 months. Majority of patients were diagnosed by 5 years of age or less (98%), as shown in figure 8 below.

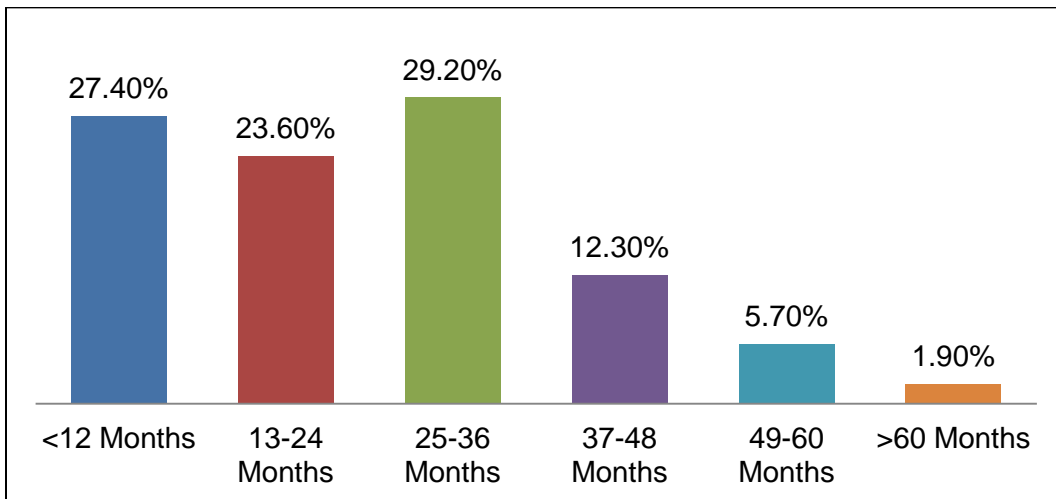


Figure 8: Distribution of participants by age at presentation (n=106).

Age at presentation of participants in months vs. laterality

Patients with bilateral disease presented at an earlier age with all cases presenting below 48 months, in comparison to unilateral disease where some patients presented above 60 months of age.

The mean age of the patients with unilateral disease was 28.99 months, compared to 18.45 months among the patients with bilateral disease; the mean difference was 8.75 which was statistically significant ($p=0.008$).

Figure 9 below illustrates the age at presentation of participants in months vs. laterality.

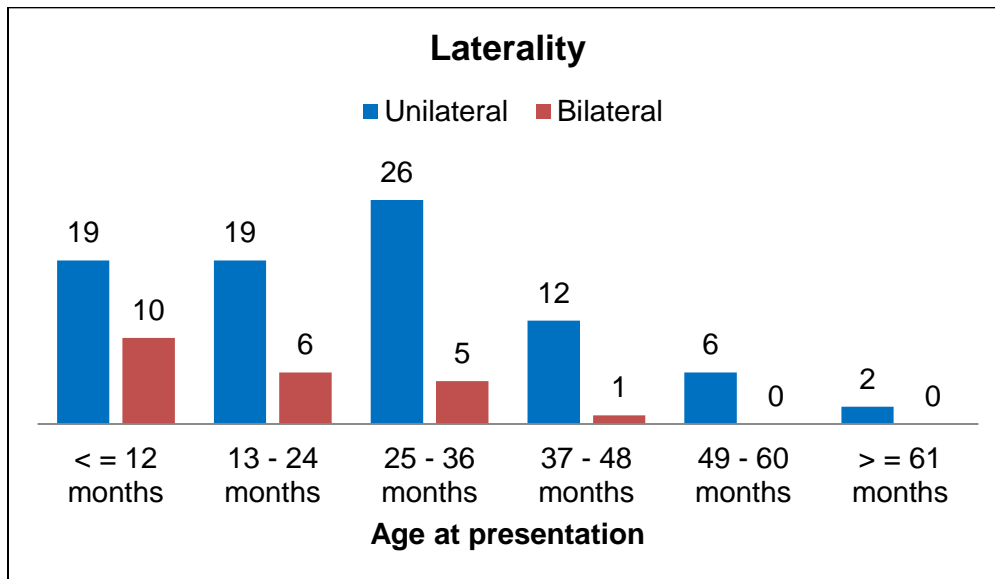


Figure 9: Age at presentation of participants in months vs. laterality (n=106).

Overview of outcome of participants

A total of 140 participants were eligible for the study with 34 unknown outcomes. Of the 106 participants whose outcome was known, 76 were alive and 30 were dead. Figure 10 below indicate the overview of the outcome of participants.

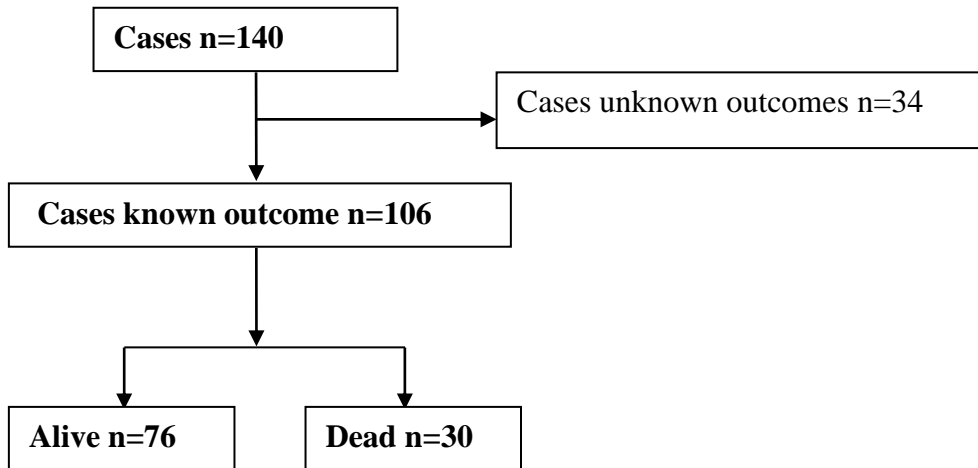


Figure 10: Overview of the outcome of participants.

The mean survival time of those with fatal outcome (n=30) following enucleation was 17.7 months (SD 14.1), Range 2 - 54, for the surviving group (n=76) was 59.5 months (SD 25.4) Range 13 – 118.

The difference in the mean age between the two groups (41.8 months) was statistically significant ($p = <0.001$).

Association among laterality, family history, leukocoria, proptosis and outcome of participants

n =106

Patient with Bilateral disease were 3 times more likely to die than those with Unilateral disease which was statistically significant (**p=0.016**)

Positive family history was not associated with a significant poorer outcome (**p=0.415**)

Patients who presented with leukocoria were not associated with poor outcome (**p=0.324**)

The patient who presented with proptosis had a 4 times risk of fatal outcome which was statistically significant (**p=0.039**).

Table 1: Association among laterality, family history, leukocoria, proptosis and outcome of participants

(n=106)	OUTCOME		OR(95%CI)	P Values
	DEAD n (%)	ALIVE n (%)		
LATERALITY				
Bilateral	11 (50.0%)	11(50.0%)	3.2 (1.07 - 9.46)	0.016
Unilateral	20(23.0%)	64 (77.0%)		
FAMILY HISTORY				
Positive	3(42.86%)	4 (57.14%)	1.9(0.26- 11.96)	0.415
Negative	28(28.28%)	71(71.72%)		
LEUKOCORIA ONLY				
Yes	21 (25.9%)	60 (74.1%)	.622 (.239 - 1.619)	0.324
No	9 (36.0%)	16 (64.0%)		
PROPTOSIS				
Yes	5 (62.5%)	3 (37.5%)	4.867(1.084 -21.848)	0.039
No	25 (25.5%)	73 (74.5%)		

Presenting Complaints of participants

Majority of the patients presented with white reflex only 86 (81.3%), while 8 (7.5%) had an initial white reflex but presented with orbital swelling. Figure 11 below shows the distribution of presenting Complaints.

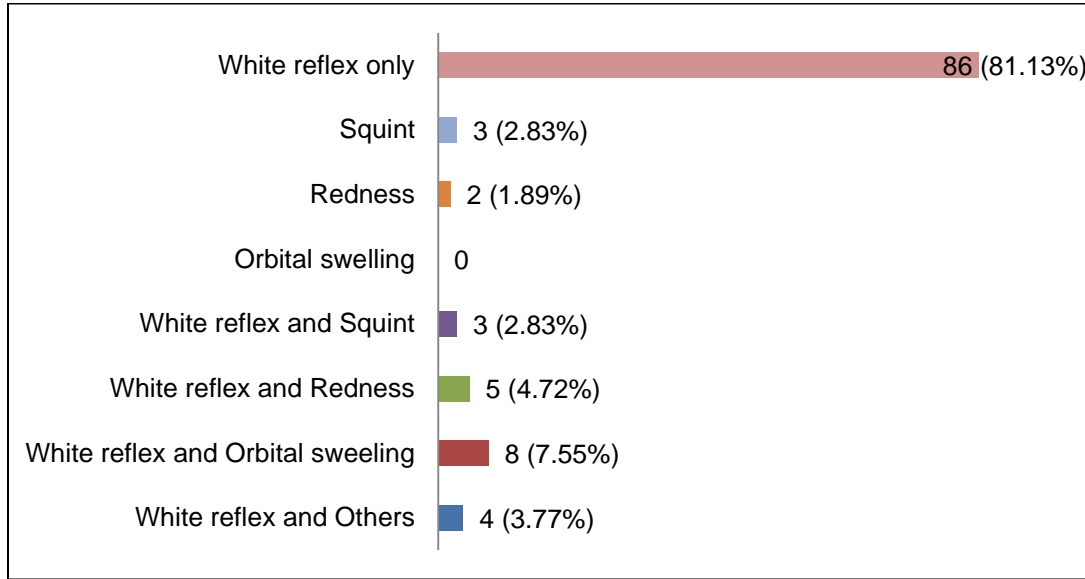


Figure 11: Distribution of presenting Complaints (n=106)

Types of adjuvant chemotherapy regimens administered to participants

Fifty five patients were initiated on adjuvant chemotherapy 36 (65.45%) of whom received Vincristine, Ectoposide and Carboplatin (VEC) regimen and 19 (34.55%) received Vincristine, Adriamycin, Carboplatin and Cisplatin (VACIS) regimen, as indicated in figure 12 below.

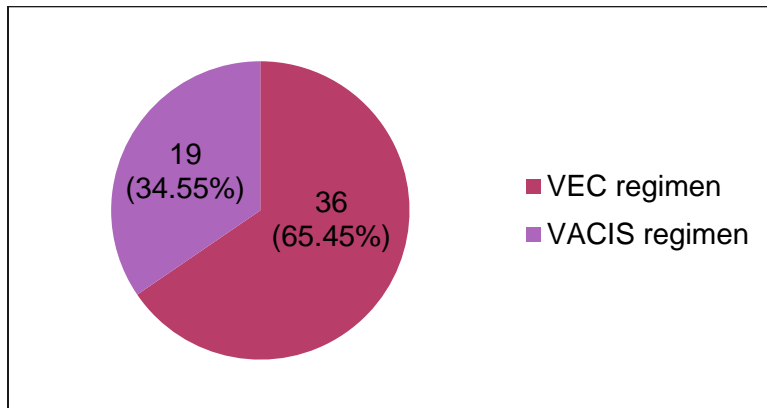


Figure 12: Types adjuvant chemotherapy regimens administered to participants (n=55).

Completion rate of adjuvant chemotherapy by participants

Of the 55 patient initiated on adjuvant chemotherapy, 44 (80%) completed the cycles whereas 11 (20%) did not complete. The majority of patients in the latter group were lost to follow up. Figure 13 below shows completion rate of adjuvant chemotherapy by participants.

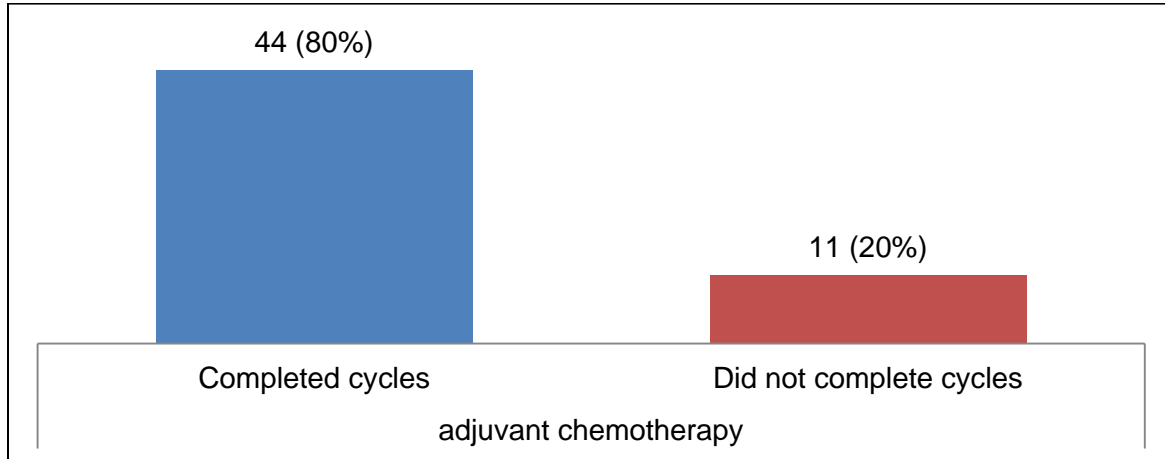


Figure 13: Completion rate of adjuvant chemotherapy by participants (n=55)

Association between completion adjuvant chemotherapy and outcome of participants

The patient who did not complete adjuvant chemotherapy had a poorer outcome in comparison to those who completed adjuvant chemotherapy which was statistically significant (**p=0.042**).

Table 2: Association between completion adjuvant chemotherapy and outcome of participants

n=55 Adjuvant chemotherapy	OUTCOME		OR(95%CI)	P Values
	DEAD n (%)	ALIVE n (%)		
Not completed	8(72.7%)	3 (27.3%)	4.24 (0.84 - 27.49)	0.042
Completed	17(38.6%)	27(61.4%)		

Association among metastasis, recurrence and outcome of participants

Patients who had metastasis or recurrence had a poor outcome (100% and 90% mortality respectively), both being statistically significant ($p < 0.001$).

Table 3: Association among metastasis, recurrence and outcome of participants

n=106 Metastasis	Outcome		OR(95%CI)	P Values
	Dead n (%)	Alive n (%)		
Yes	7 (100.0%)	0 (0.0%)	4.263(2.88 - 6.32)	<.001
No	19 (23.5%)	62 (76.5%)		
Recurrence				
Yes	9 (90.0%)	1 (10.0%)	32.294 (3.819 -273.049)	<.001
No	17 (21.8%)	61 (78.2%)		

Characteristic histopathology findings

All the 106 were confirmed to have RB -/- features. No case histomorphologically consistent with RB +/- MYCN^A RB was noted and 2.8% had retinoma features.

The majority of participants had moderately differentiated tumour 57 (53.8%) with 40 (37.7 %) cases having massive choroidal invasion and only 8 (7.6%) having scleral invasion. 34 (32.1%) had post laminar optic nerve invasion. Histopathologic features are summarised in table 4 below.

Table 4: Summary of characteristic histopathologic features of participants enucleated eyes

n=106

Parameter	Frequency	Percentage (%)
Histomorphology features:		
RB -/-	106	100%
RB +/- [MYCN ^A]	0	0%
Retinoma features present;		
Yes	3	2.8%
No	103	97.2%
Degree of differentiation:		
Well Differentiated,	9	8.4 %
Moderately Differentiated,	57	53.8%
Poorly Differentiated,	40	37.8%
Choroidal Invasion:		
Not Involved,	55	51.9 %
Focal Invasion,	11	10.4%
Massive Invasion,	40	37.7%
Scleral Invasion:		
Not Involved,	98	92.5%
Intrasclera,	2	1.9 %
Extrasclera,	6	5.7%
Optic Nerve Involvement:		
Not Involved,	48	45.3 %
Pre Laminar,	15	14.2 %
At Laminar,	9	8.5 %
Post Laminar but margins free,	18	17.0 %
Post Laminar and margins.	16	15.1%
TNM Staging:		
pT1	34	32.1%
pT2	11	19.8%
pT3	35	33.0%
pT4	16	15.1%

Association among degree of differentiation, choroidal invasion, scleral invasion, optic nerve invasion and outcome of participant

Patient's with poorly differentiated tumour had a poorer outcome in comparison to those who had well and moderately differentiated tumours which was statistically significant (**p= 0.037**).

Massive choroidal Invasion was also associated with a poorer outcome which was statistically significant (**p=0.002**). Patient's with sclera involvement had a poorer outcome in comparison to those with no involvement which was statistically significant (**p= 0.006**)

Patient's with post laminar or surgical margin optic nerve invasion were 8 times more likely to die which was statistically significant (**p=<0.001**).

Table 5: Association among degree of differentiation, choroidal invasion, scleral invasion, optic nerve invasion and outcome of participant

n=106. Degree of differentiation	OUTCOME		OR(95%CI)	P Values
	DEAD n (%)	ALIVE n (%)		
Poorly	16(40.0%)	24 (60.0%)	2.48 (1.95 - 6.43)	0.037
Well & Moderately	14 (21.2%)	52(78.8%)		
Degree of Choroidal invasion				
Massive	25 (62.5%)	15(37.5%)	16.67 (1.92 -748.27)	0.002
Focal	1 (9.1%)	10 (90.9%)		
Sclera invasion				
Involved	6 (75%)	2(25%)	9.25 (1.75 - 48.9)	0.006
Not involved	24(24.5%)	74(75.5%)		
Degree of Optic nerve invasion				
Post Laminar and at Surgical Margin	20 (58.8%)	14 (41.2%)	8.86 (3.09 - 25.82)	<0.001
Pre laminar and at Laminar	10 (13.9%)	62 (86.1%)		

Association between TNM Staging and outcome of participants

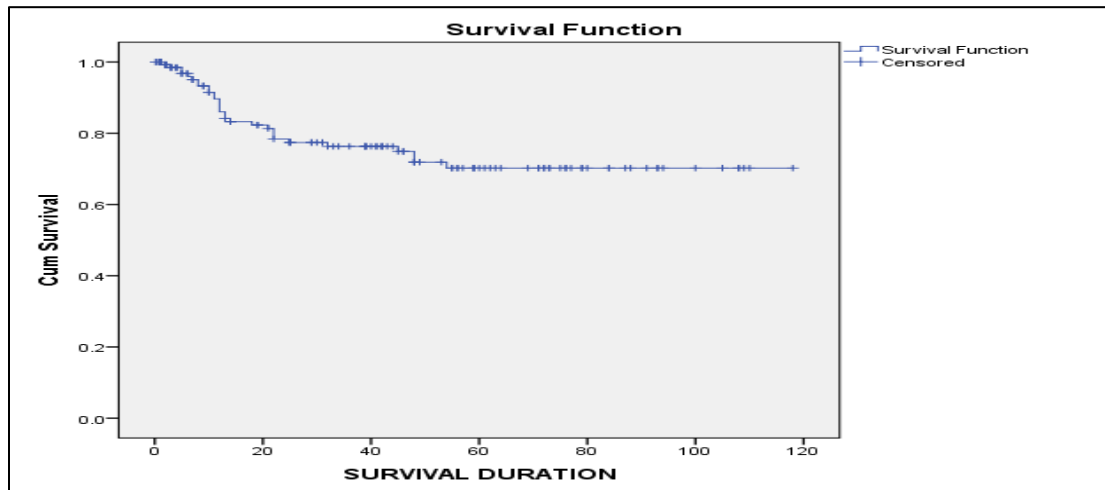
Patient who had a late stage (\geq pT3a) tumour had a poorer outcome which was statistically significant ($p < 0.001$)

Table 6: Association between TNM Staging and outcome of participants:

n=106 TNM Staging	Outcome		OR(95%CI)	P Values
	Dead n (%)	Alive n (%)		
\geq pT3a	28 (56.0%)	22 (44.0%)	34.36 (7.35 - 310.26)	<0.001
\leq pT2b	2 (3.6%)	54 (96.4%)		

Kaplan Meier overall survival probability curve of participants

A total of 140 participants eligible for the study were included in the generation of the survival curves i.e. with known (106) and unknown (34) outcome. The latter 34 patients whose outcome was unknown were lost to follow-up but their parents or guardians were not contacted due to inaccurate, change or lack of mobile phone numbers. Each of these 34 patients was censored as at the last day known alive on the Kaplan Meier curve. Start point was day of enucleation, while the event was death. Figure 14 below indicates the overall survival probability curve of participants.



Probability of survival at 12 months is **0.85**, while at 36 months is **0.78** and at 60 months is **0.70**.

Figure 14: Kaplan Meier overall survival probability curve of participants (n=140)

Comparison of the participant's survival according to the degree of differentiation

The survival distributions for the three tumour grade groups were statistically significantly different [$p=0.032$], as shown in figure 15 below.

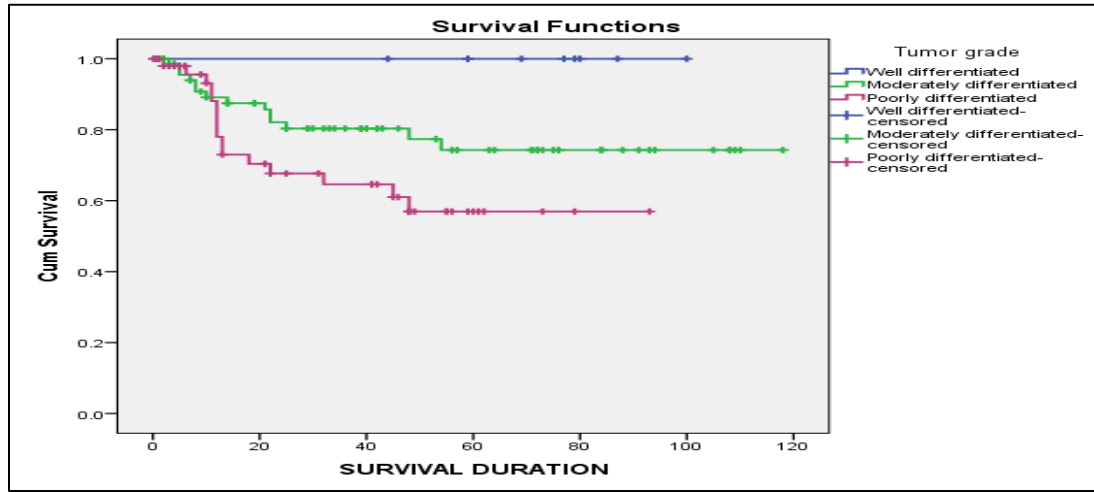


Figure 15: Comparison of the participant's survival according to the degree of differentiation (n=140)

Comparison of the participant's survival according to the degree of choroidal Invasion

The survival distributions for the three choroidal invasion groups were statistically significantly different ($p=0.001$), as indicated in figure 16 below.

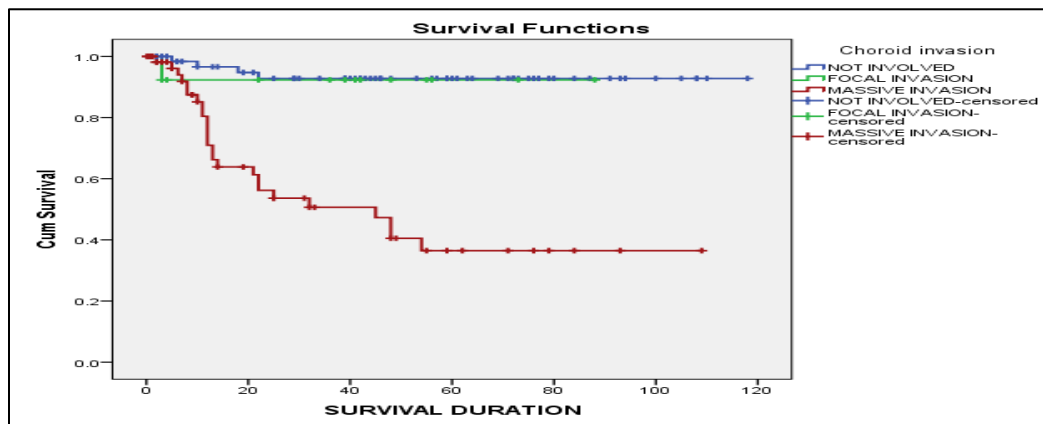


Figure 16: Comparison of the participant's survival according to the degree of choroidal invasion (n=140)

Comparison of the participant's survival according to the degree of sclera invasion

The survival distributions for the three sclera invasion groups were statistically significantly different ($p = 0.02$), as illustrated in figure 17 below.

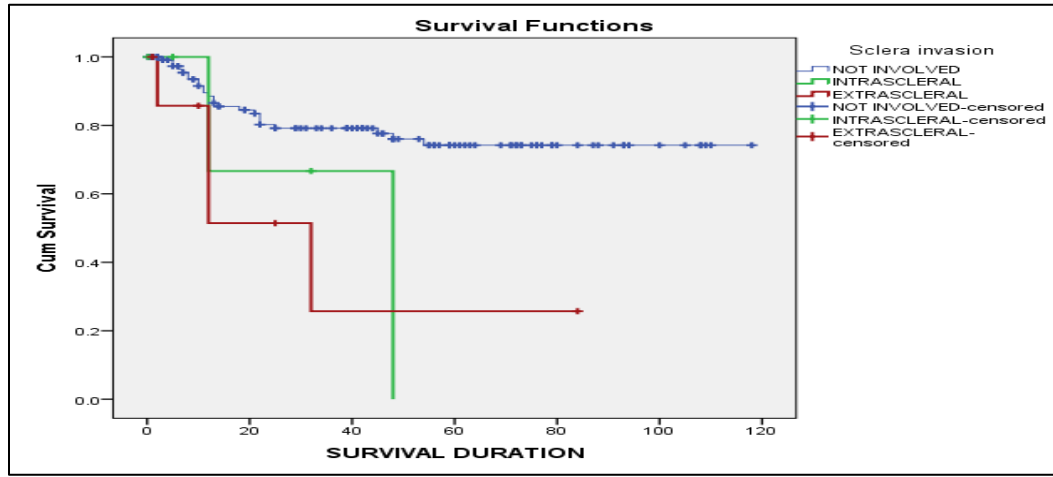


Figure 17: Comparison of the participant's survival according to the degree of sclera invasion (n=140)

Comparison of the participant's survival according to the degree of optic nerve invasion

The survival distributions for the five groups were statistically significantly different, ($p < 0.001$), as indicated in figure 18 below.

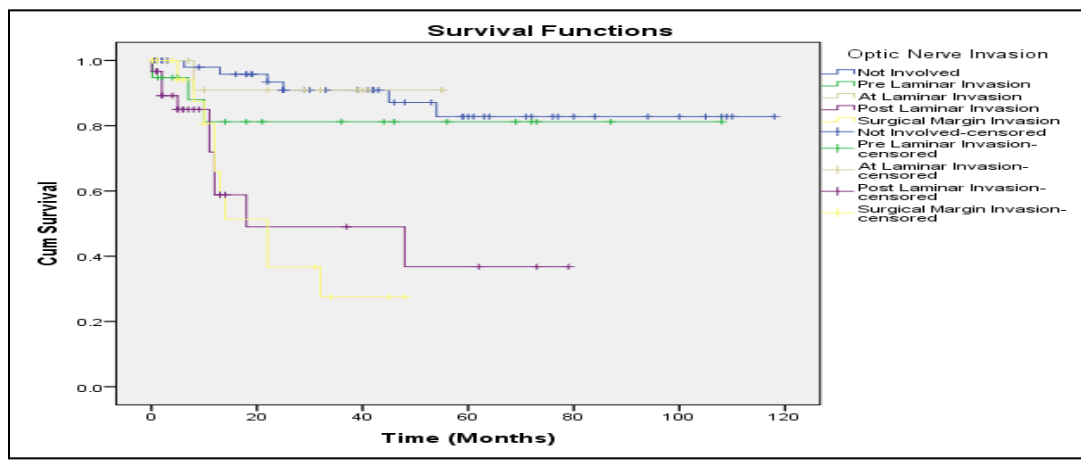


Figure 18: Comparison of the participant's survival according to the degree of optic nerve invasion (n=140)

Comparison of the participant's survival according to TNM staging

The survival distributions for the six groups were statistically significantly different, ($p < 0.001$), as illustrated in figure 19 below.

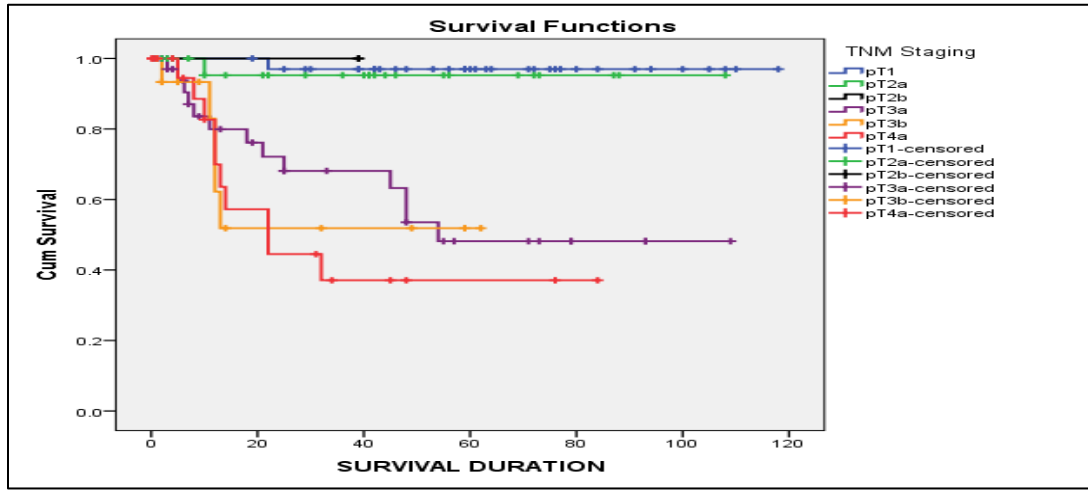


Figure 19: Comparison of the participant's survival according to TNM staging (n=140)

Multivariate Analysis

n=140.

The variables which had a significant statistical association with outcome of the participants i.e. bilateral RB, proptosis, non-completion of adjuvant chemotherapy, metastasis, recurrence, poorly differentiated tumour, massive choroidal invasion, sclera invasion and post laminar optic nerve invasion were included in the multivariate analysis model.

The variables with significant impact on outcome after analyses were: bilateral RB, orbital swelling, metastasis, recurrence, poorly differentiated tumour, sclera invasion, massive choroidal invasion and post laminar optic nerve invasion. However non completion of adjuvant chemotherapy was not found to have a statistically significant impact (**p= 0.073**). As illustrated in table 7 below.

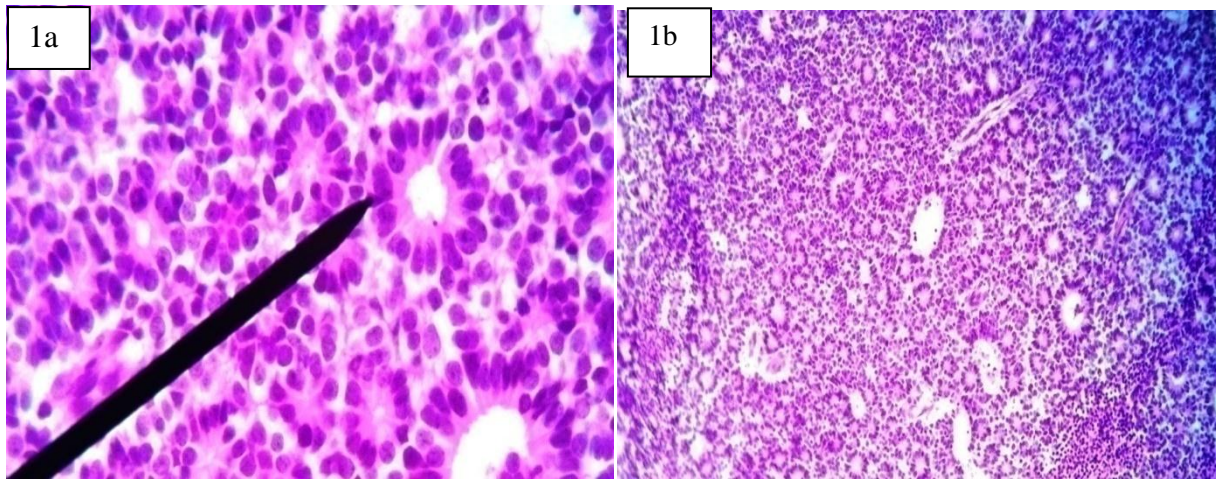
Table 7: Multivariate Analysis

Variables	Hazard Ratio	95% confidence interval Hazard Ratio	P=Value
Bilateral RB	0.376	0.179 – 0.791	0.010
Proptosis	3.436	1.301 – 9.079	0.013
Non completion of adjuvant chemotherapy	0.458	0.195 – 1.077	0.073
Metastasis	7.390	3.111 – 17.552	0.001
Recurrence	6.421	2.906 – 14.188	0.001
Poorly differentiated tumour	0.477	0.231 – 0.984	0.045
Massive choroid invasion	0.088	0.030 – 0.253	0.001
Sclera invasion	0.256	0.088 – 0.742	0.012
Post laminar optic nerve invasion	0.133	0.048 – 0.369	0.001

10.0 HISTOPATHOLOGY APPERANCES (PHOTOMICROGRAPHS)

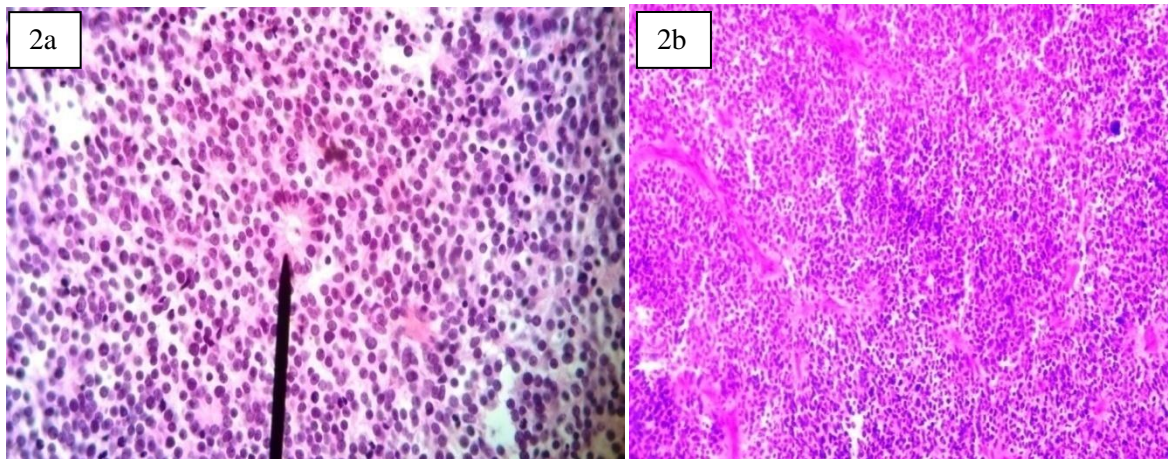
Tumour differentiation and high risk histology features as prognostic factors among patients with retinoblastoma at Kenyatta National Hospital and Presbyterian Church of East Africa Kikuyu Hospital.

Plate 1:



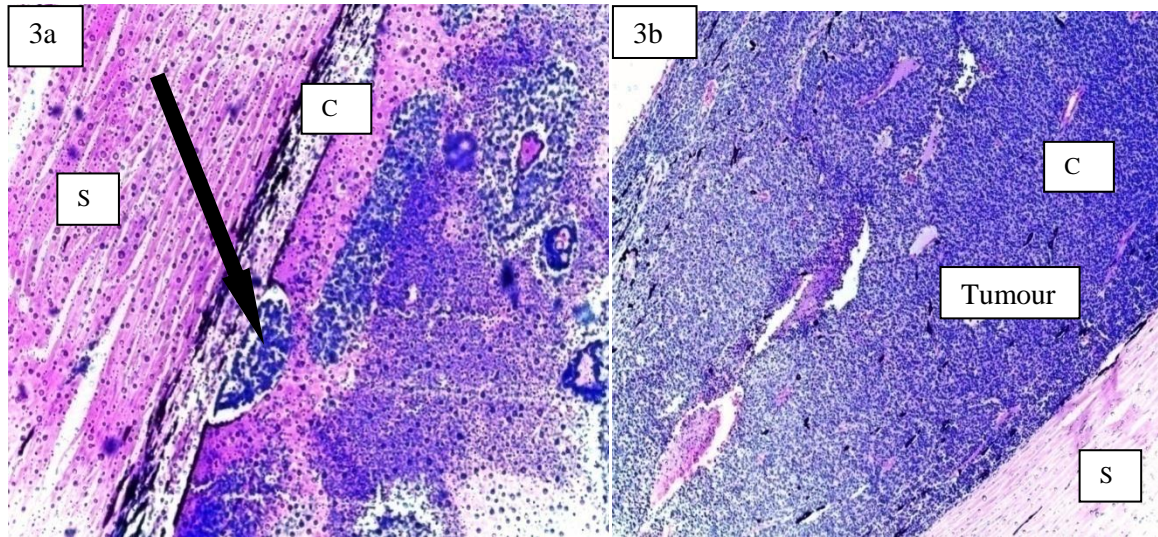
(1a) characteristic Flexner Wintersteiner rosette ($\times 400$) illustrated by the black pointer, (1b) Well differentiated RB ($\times 40$) exhibiting numerous Flexner Wintersteiner rosettes' appearing in $> 80\%$ of the tumour.

Plate 2:



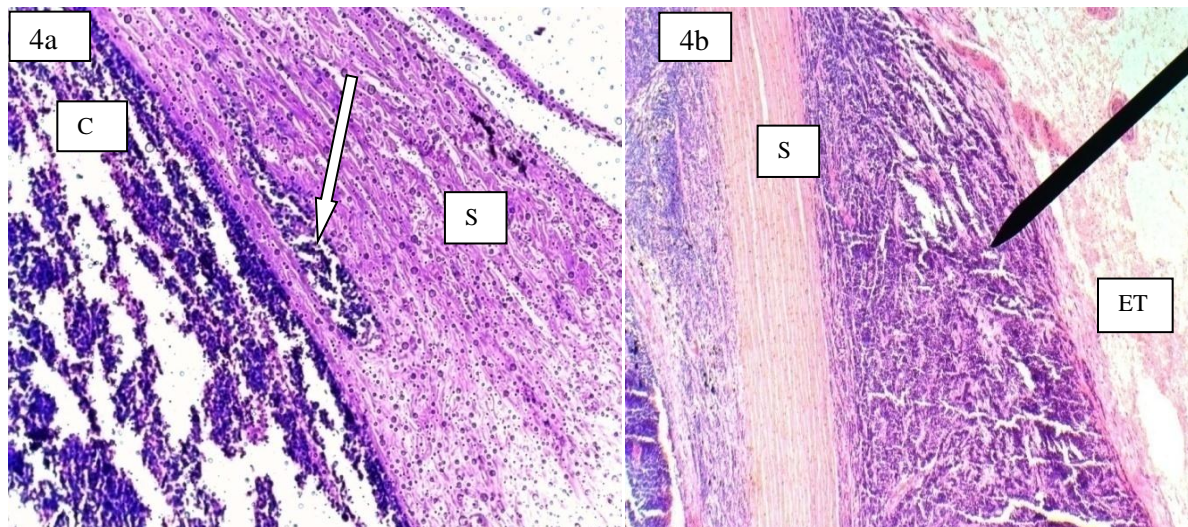
(2a) Moderately Differentiated RB exhibiting few Flexner Wintersteiner rossets ($\times 400$) illustrated by the black pointer (2b) Poorly differentiated RB comprising of sheets of small round blue cells with no Flexner Wintersteiner rossets ($\times 100$).

Plate 3



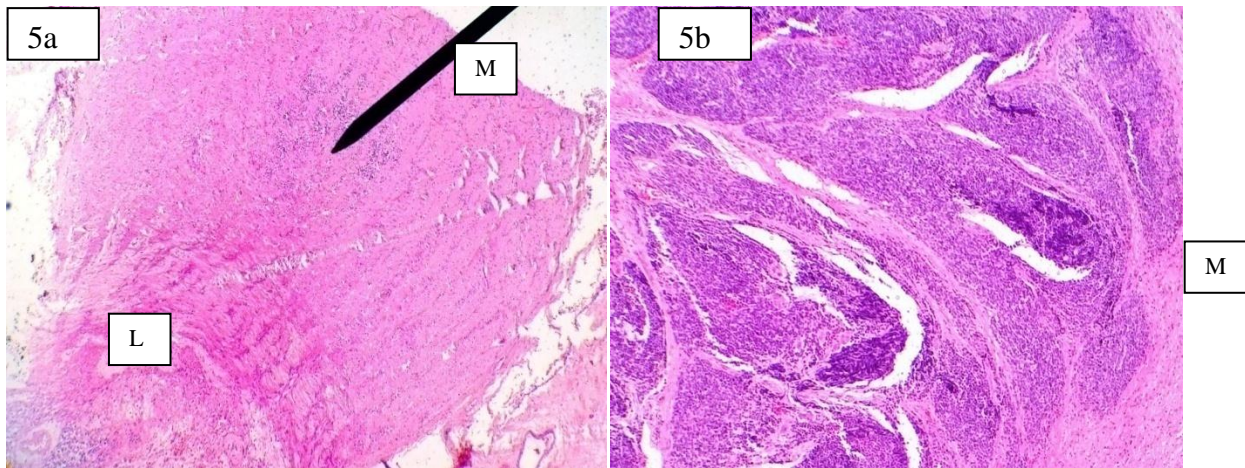
(3a) Focal choroidal invasion (C) < 3mm illustrated by the black arrow, with no sclera involvement (S) ($\times 400$). (3b) Massive choroidal by tumour (C) >3mm with no sclera invasion (S) noted ($\times 400$).

Plate 4



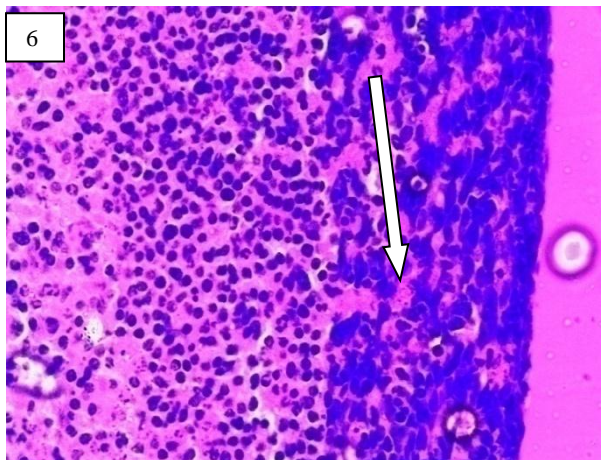
(4a) Massive choroidal invasion(C) > 3mm with intrasclera invasion(S) illustrated by the white arrow. ($\times 400$). (4b) Massive extrasclera soft tissue involvement (ET) illustrated by black pointer. ($\times 400$).

Plate 5



(5a) Post laminar optic nerve invasion (L) illustrated by the black pointer surgical margins are tumour free (M) ($\times 100$). (5b) Optic nerve invasion to the surgical margins (M) ($\times 400$).

Plate 6



(6) Features of retinoma, characterized by small and less hyperchromatic nuclei than in retinoblastoma and differentiation into fleurettes pointed by the white arrow ($\times 400$)

11.0 DISCUSSION

DEMOGRAPHICS OF THE PARTICIPANTS

There was a slight male preponderance with a male: female ratio of 1.12:1. This compares with other local studies where Kimani et al²² and Gichigo et al⁵ found ratios of 1.26:1 and 1.16:1 respectively. The majority of patients (80.9%) presented by the age of 3 years, the mean age at presentation was 26.8 months, with a median of 24 months, and a range of 2–81 months. The patients in our study presenting much earlier in comparison to other local studies undertaken at KNH; in Gichigo et al⁵ study 63% presented by age 3 years with mean age at presentation 35 months, Kimani et al²² the mean age at presentation was 32.4 months, while Maingi et al²³ was 32.8 months. This may be explained by the fact that our study only considered patients who underwent primary enucleation, while the other studies included all RB groups. It could also mean that the Kenya National Retinoblastoma strategy³⁷ has had an impact on reducing the number of patients presenting with late disease.

The proportion of those with unilateral RB was 79% and bilateral 21% which was similar to Nyamori et al²¹ study where 74.2% of cases with unilateral RB and 25.8% bilateral RB cases were found. The mean age at presentation for unilateral RB was 28.99 months compared to 18.45 months for bilateral RB which was statistically significant ($p= 0.008$). Both groups in our study presented earlier compared to other local studies Nyamori²¹ found unilateral cases presenting at 35.9 months and bilateral cases at 26 months, while Gichigo⁵ found unilateral cases presenting at 39.8 months and bilateral at 24.3 months. This may still be attributed to earlier presentation of the cohort in our study in comparison to the other studies and positive impact from the Kenya National Retinoblastoma strategy has described above. However in comparison to studies in the developed countries our cohort still presented much later. In Britain patients with Unilateral RB mean age at presentation was 18 months and 5 months for bilateral disease².

In this study positive family history was identified in 7 cases (6.6%) which compares well to Nyamori et al. who found 4.3 % of cases²¹. In developed countries however, higher frequencies are noted, with Britain recording 12%². The difference is more likely explained by the fact that children with RB in developed world have better survival and therefore more likely to attain adulthood and have offspring.

CLINICAL PRESENTATION OF THE PARTICIPANTS

The most common presenting symptom was white reflex which was the only presenting feature in 81% of patients. Other symptoms which presented together with white reflex were; proptosis (7.6%), redness of the eye (6.5%), squint (5.7%) and poor vision (3.8%). Gichigo et al found 72% had white reflex, with fewer (38%) presenting with white reflex only, while the remainder had associated proptosis⁵. In Nigeria Owoeye et al found majority 84.6% to have proptosis and chemosis¹⁶. In the USA leukocoria (60%), strabismus (20%) and ocular inflammation (5%) were noted to be the common presenting signs¹.

Local orbital recurrence was noted in 10 (9%) patients while metastasis was reported in 7 (6.6%) of cases where all being to the central nervous system. Gichigo et al⁵ however noted higher occurrence of both recurrence and metastasis with 30 % of patient having recurrence and 17 (16.1%) having metastasis mainly to the central nervous system.

HISTOPATHOLOGY FINDINGS

In our study, all the specimens were found to have the RB-/- histomorphology features. The frequency of retinoma was 2.8% which was lower in comparison to other studies where it ranges from 6%¹³ to 20.4%¹⁴. No case with histomorphologic features consistent with RB +/+ MYCN^A RB was noted. Rushlow et al¹⁵ having analyzed 1068 patients with non-familial unilateral RB found 29 (2.7%) of patients with RB+/, 15 (1.4%) of whom had MYCN^A and neuroblastoma like histomorphology features. This may probably be explained by the small numbers in our study of 106 patients in comparison to 1068 patients in the study by Rushlow et al. Patients with RB+/- MYCN^A RB may also have presented with advanced disease where primary enucleation was not feasible in view of its aggressive behaviour.

Majority of tumours in this study were moderately differentiated 53.8%, 37.8% were poorly differentiated and 8.5% were well differentiated. These findings are different from other studies in developing countries; in Kenya Maingi et al found 9.7% cases to be well differentiated, 25.8% were moderately differentiated and 48.4% poorly differentiated and 16.1% undetermined²³. In Nigeria, Ajaiyeoba et al found no case of well differentiated tumor with 45% being moderately differentiated and 55% poorly differentiated¹⁰. However in developed countries majority are usually well differentiated with Khelfaoul et al¹² in France finding 42% well differentiated, 42%

moderately differentiated and 16% poorly differentiated. It may be that patients who present early are more likely to have well differentiated tumours. In our study the distribution of the high risk histology features was as follows: Post laminar and surgical margins involvement in 34 (58.6%) of those with optic nerve involvement, choroidal invasion in 48% of cases, of which 37.7% of these had massive invasion and Sclera involvement was in 7.5% of cases. This differs from the study of Maingi et al. where 83.8% of those with optic nerve invasion had Post laminar cribrosa and surgical margin involvement, 62.6% had massive choroidal invasion and 22% had sclera invasion²³. Fewer cases with late RB stage > pT3a (48.1%) were found in this study in comparison to other studies where Gichigo et al found late disease in 71.7%⁵ while Nyamori et al found 74%²¹. There were fewer cases of the high risk histologic features noted in our study compared to other previous local studies^(5 and 23). This may be attributed to the early presentation in our cohort.

SURVIVAL OUTCOME OF THE PARTICIPANTS

Of the 106 patients with known outcome, 70 (71.6%) were alive, and 30 (28.4%) dead, this differs from a study done locally at KNH by Gichigo et al⁵ where 26.7% were alive and 73.4 % dead. There was a higher overall survival rate in our study of 0.85 at 12 months, 0.78 at 3 years and 0.70 at 5 years in comparison to studies by Gichigo et al⁵ KNH and Bowman et al⁴ in Tanzania where the probability of survival at 3 years was lower at 0.2 in both studies. This may be attributed to the early presentation of our cohort in comparison to the two studies, where only patient amenable to primary enucleation were considered in our study while the latter studies considered all RB groups. This shows that early RB presentation and diagnosis improves survival.

ASSOCIATION BETWEEN CLINICAL PRESENTATIONS WITH OUTCOME OF THE PARTICIPANTS

Patients with bilateral RB were 3 times more likely to die than those with unilateral RB which was statistically significant ($p=0.016$). This differs from Gichigo et al⁵ where no statistical difference ($p=0.532$) between the two groups was found the later study incorporating all RB groups.

Advanced disease was found to have a poorer outcome, with patients having orbital swelling associated with a 65% mortality which was statically significance ($p=0.039$) and compares to Gichigo et al⁵ found 100% mortality in those presenting with orbital swelling which was statically significant ($p=0.001$)

Patients whom adjuvant chemotherapy was administered but failed to complete the cycles were 4 times likely to die compared to those who completed treatment which was statistically significant ($p=0.042$). Adjuvant chemotherapy for RB patients with high-risk histological features has been shown to improve patient's survival; Kaliki et al⁴⁸ observed that 57 patients on follow-up and completed treatment were disease free at 66 months.

Recurrence or Metastasis were associated with poor outcome with a 90% and 100% mortality respectively both being statistically significant ($p=< 0.001$). This compares with Gichigo et al⁵ where there was 100% mortality at 12 months for those with metastasis and Gündüz et al⁵⁵ in Turkey where 100% mortality at 24 months for those with metastasis was noted.

HISTOPATHOLOGY ASSOCIATION AND OUTCOME OF PARTICIPANTS

The degree of tumour differentiation was found to have an impact on patients survival with those having poorly differentiated RB being 2.8 times likely to die than those with well or moderately differentiated tumour which was statistically significant ($p=0.037$). Ajaiyeoba et al¹⁰ found a close association between the poorly differentiated RB with poor outcome though not statistically significant ($p=0.057$) while other studies however have reported no association between the tumour grade and outcome¹². The survival distribution between the three groups was also statistically significant ($p = 0.034$) in this study, however this differed with Khelfaoul et al¹² where no statistical difference ($p=0.11$) was found.

The high-risk histological features were confirmed to impact significantly on patient's poor outcome; massive choroidal invasion was $p=0.002$, sclera invasion was $p=0.006$ and optic nerve Post laminar and surgical margins was $p<0.001$. This compares well with other studies, where Andrea et al in Argentina found massive choroidal invasion to be associated with poor outcome ($p=0.04$)⁴³ while Cuenca et al still in Argentina found both sclera involvement and Optic nerve invasion post laminar invasion being associated with significant poor outcome with $p=0.05$ and $p=0.02$ respectively⁴⁴. The survival distributions among the five groups of optic nerve invasion was significant $p<0.001$, which is consistent with findings by Khelifaoul et al at $p=0.000$ ¹².

MULTIVARIATE STUDIES

The variables which had a significant association with outcome i.e. bilateral RB, proptosis, non-completion of adjuvant chemotherapy, metastasis, recurrence, poorly differentiated tumour, massive choroidal, invasion sclera invasion and post laminar optic nerve invasion were included in the model. Non-completion of adjuvant chemotherapy was the only variable that showed no statistically significant association ($p=0.073$) with the other variables showing significant association with poor outcome. Gichogo et al⁵ found leucokoria only and tumour confined to the globe being associated with better outcome while Khelifaoul et al¹² found Massive choroidal invasion and retro-laminar optic nerve invasion being associated with poor outcome following multivariate analysis.

12.0 STUDY LIMITATIONS

The challenges encountered included:

- Missing and incomplete patient clinical records.
- Inability to contact the guardian or parent of those patients lost to follow-up due to missing or wrong telephone contacts.
- Missing specimen blocks.
- Different trimming techniques at KNH and M.P Shah Laboratories before the establishment of RBCOLAB. To overcome this, the specimens were re-blocked for standardized processing.

13.0 CONCLUSIONS

1. The distribution of degree of tumour differentiation was; 9 (8.5 %) for well differentiated, 57 (53.8%) for moderately differentiated and 40 (37.8%) for poorly differentiated tumours. For the high risk histopathology features; massive choroidal invasion was found in 40 (37.7%) of cases, with few cases 8 (7.6%) having sclera invasion (intrascleral and extrascleral) and 34 (32.1%) with optic nerve post lamina cribrosa involvement.
2. There was a higher survival probability which was 85% at 12 months, 78% at 36 months and 70% at 60 months in comparison to previous studies done locally. This may be attributed to the earlier presentation of our cohort since only those who had undergone primary enucleation were considered, while the other previous studies included all the RB patients.
3. The degree of tumour differentiation was found to have a prognostic impact, with patients having poorly differentiated tumour being associated with a poorer survival. The high risk histologic features i.e. massive choroidal invasion, sclera invasion (intrascleral and extrascleral) and optic nerve post lamina cribrosa involvement were associated with poor outcome which compares to other studies.
4. The retinoma frequency was 2.8% which was low in comparison to other studies. While no single case was histomorphologically consistent with RB +/+ MYCN subtype was defined in this study.

14.0 RECOMMENDATIONS

1. The degree of tumour differentiation was found to have a prognostic impact among patients with RB, we therefore advocate for a consensus in the grading criteria and its inclusion in the reporting of RB.
2. Standardized synoptic reporting should be maintained with continued careful evaluation of the high risk RB histopathology features to guide on management.
3. A larger study is recommended to determine the frequency of histomorphologically consistent RB +/- MYCN subtype.

15.0 REFERENCES

1. Shields J. and Shields C. "Retinoblastoma," in *Intraocular Tumors- A Text and Atlas*, Eds.pp. 294–318, Lippincott Williams and Wilkins, Philadelphia, PA; 2008 293–365.
2. MacCarthy A, Birch J.M, Draper G. J et al. Retinoblastoma: treatment and survival in Great Britain 1963 to 2002. *British Journal of Ophthalmology* 2009; vol. 93, no. 1, pp. 38–39.
3. Wessels G and Hesselting P. Outcome of children treated for cancer in the Republic of Namibia. *Med Pediatr Oncol.*1996; 27:160-4
4. Bowman J, Mafwiri M, Luthert P, et al. Outcome of Retinoblastoma in East Africa. *Pediatric Blood Cancer* 2008; 50: 160- 162
5. Gichigo N, Kimani K, and Kariuki M. survival among retinoblastoma patients treated at Kenyatta National Hospital: A retrospective audit. *Journal of Ophthalmology of Eastern Central and Southern Africa.* 2013; 1:16-19.
6. Honavar G, Singh A, Shields L, et al. Postenucleation adjuvant therapy in high-risk retinoblastoma. *Arch Ophthalmol.* 2002; 120:923–31.
7. Sohn A, Charles J, John L, et al. Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. *Journal of Gastrointestinal Surgery.* Dec 2000; Volume 4, pp 567-579.
8. Carolyn C, Fielding P, Lawrence J et al. Prognostic Factors in Colorectal Cancer, College of American Pathologists Consensus Statement 1999.*Arch Pathol Lab Med.* 2000 July; Vol 124,
9. Brown D. The clinicopathology of retinoblastoma. *American Journal of Ophthalmology,* 1966; 61, 508.
10. Ajaiyeoba A, Komolafe O, Olusanya A, et al. Histological determinant of mortality in retinoblastoma in Ibadan. *Br J Ophthalmol* September 2008; Vol 92 No 9
11. Roshmi G, Geeta K, Vijay A, et al. Histopathologic Risk Factors in Retinoblastoma in India. *Arch Pathol Lab Med.* 2009; 133: 1210–1214.
12. Khelfaoui F, Validire P, Auperin A, et al. Histopathologic risk factors in retinoblastoma: a retrospective study of 172 patients treated in a single institution. *Cancer.* 1996; 77(6):1206–1213

13. Ts'o O, Zimmerman E and Fine S. The nature of retinoblastoma, I: photoreceptor differentiation—a clinical and histopathologic study. *Am J Ophthalmol.*1970; 69(3):339–349.
14. Eagle C Jr. High-risk features and tumor differentiation in retinoblastoma: a retrospective histopathologic study. *Arch Pathol Lab Med* 2009; 133 (8): 1203-9,.
15. Diane E., Berber M, Gallie B et al. Characterization of retinoblastomas without RB1 mutations: genomic, gene expression, and clinical studies. *Lancet Oncol* 2013; 14: 327–34
16. Owoeye F, Afolayan P, Ademola-Popoola S, et al. Retinoblastoma – a clinicopathologic study in Ilorin Nigeria. *Afr J Health Sci* 2005; 12(3-4): 94-100
17. Broaddus E, Topham A, and Singh A. Incidence of retinoblastoma in the USA: 1975-2004. *Br J Ophthalmol.* Jan 2009; Vol.93, No.1, pp. 21-23, ISSN 0007-1161
18. Ochicha O, Gwarzo A and Gwarzo D. Pediatric malignancies in Kano, Northern Nigeria, *Nigeria World J Pediatr* 2012;8(3):235-239
19. Bunin R, Orjuela M. Geographic and environmental factors. In Singh D, Damato E Pe'er j, et al, eds. *Clinical ophthalmic oncology*. Philadelphia: Saunders-Elsevier, 2007: 410-16
20. Parkin M, Kramarova E, Drapper J et al. International incidence of childhood cancer, IARC *SciPubl* 1998; vol II: 1-391
21. Nyamori J, Kimani K, Njuguna M et al. The incidence and distribution of retinoblastoma in Kenya. *Br J Ophthalmol* 2011; doi:10.1136/bjophthalmol-2011-300739
22. Kimani K, Illako D and Kollmann M. A review of retinoblastoma; presentation, diagnosis and management at Kenyatta National Hospital. M.Med Thesis, University of Nairobi, 2000.
23. Maingi M, Waweru W, Kimani K et al. An audit and review of the histologic reporting of retinoblastoma at Kenyatta National Hospital. M.Med Thesis, University of Nairobi, 2009.
24. Knudson G Jr: Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 1971; 68:820-823.
25. Dimaras H, Khetan V, Gallie B et al. Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. *Hum Mol Genet.* 2008; 17(10):1363–1372.
26. Fletcher O, Easton D, Anderson K, et al. Lifetime risks of common cancers among retinoblastoma survivors. *J Natl Cancer Inst.*2004; 96:357–363.

27. Paulino, C. Trilateral retinoblastoma: is the location of the intracranial tumor important? *Cancer*. Jul 1999; Vol.86, No.1, pp. 135-141, ISSN 0008-543X
28. Roarty J, McLean I, Zimmerman L, et al. Incidence of second neoplasms in patients with bilateral retinoblastoma. *Ophthalmology*. 1988; 95:1583–1587.
29. Sadia B, Aziz-ur-R, Israr A, et al. Presentation Pattern of Retinoblastoma *Pak J Ophthalmol* 2011; Vol. 27 No. 3
30. Kayembe-Lubeji D. Retinoblastoma in Zaire. *Tropical Doctor* 1990; 20: 38
31. Chuah C, Lim M, Seah L et al. Pseudo retinoblastoma in enucleated eyes of Asian patients. *Singapore Med J*. 2006;47:617–620
32. Karcioğlu A, Gordon A, and Karcioğlu L. Tumor seeding in ocular fine needle aspiration biopsy. *Ophthalmology*. 1985; 92:1763-67.
33. Pearce S, Salotti A, Little P et al. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: *lancet* 2012; 380(9840):499-505.
34. Moscinski C, Pendergrass W, Weiss A, et al. Recommendations for the use of routine bone marrow aspiration and lumbar punctures in the follow-up of patients with retinoblastoma. *J Pediatr Hematol Oncol* 1996; 18 (2): 130-4,
35. College of American Pathologists. Protocol for the examination of specimens from patients with retinoblastoma web:www.cap.org.2011
36. The Royal College of Pathologists. Standards and datasets for ocular retinoblastoma histopathology reports web: www.rcpath.org.2010
37. Helen D, Abby W, and Brenda G. The Kenyan National Retinoblastoma Strategy: Building local capacity in the diagnosis and management of pediatric eye cancer in Kenya: *Ophthalmology rounds*. July/August 2008; Vol 6, Issue 4
38. Joaño P, Zelia S. Odashiro, et al. Histopathological Features and P-glycoprotein Expression in Retinoblastoma I OVS. October 2005; Vol. 46, No. 10
39. Seema K, Sumita S, Rachna M, et al. A Histopathologic Analysis of Eyes Primarily Enucleated for Advanced Intraocular Retinoblastoma From a Developing Country: *Arch Pathol Lab Med*—Vol 136,

40. Sastre X, Chantada L, Doz F, et al. Proceedings of the consensus meetings from the International Retinoblastoma Staging Working Group on the Pathology Guidelines for the examination of enucleated eyes and evaluation of prognostic risk factors in retinoblastoma. *Arch Pathol Lab Med* 2009; 133: 1199–1202.
41. Shields L, Shields A, Baez K. et al. Optic nerve invasion of retinoblastoma. Metastatic potential and clinical risk factors. *Cancer*. 1994; 73:692–8.
42. Abramson H and Ellsworth M. The surgical management of retinoblastoma. *Ophthalmic Surg*. 1980; 11:596–8.
43. Andrea B, Claudia S, Verónica S, et al. Outcome of Children with Retinoblastoma and Isolated Choroidal Invasion. *Arch Ophthalmol*. 2012; 130(6):724-729.
44. Adriana C, Flor G, Daisy C, et al. Microscopic Scleral Invasion in Retinoblastoma Clinicopathological Features and Outcome. *Arch Ophthalmol*. 2009;127(8):1006-1010
45. Tornóczy T, Semjén D, Shimada H, et al. "Pathology of peripheral neuroblastic tumors: significance of prominent nucleoli in undifferentiated/poorly differentiated neuroblastoma." *Pathol Oncol Res* 2007; 13(4): 269-75.
46. Hogarty D. "The requirement for evasion of programmed cell death in neuroblastomas with MYCN amplification." *Cancer Lett* 2003; 197(1-2): 173-9.
47. Zhao J, Dimaras H, Gallie B, et al. Pre-Enucleation Chemotherapy for Eyes Severely Affected by Retinoblastoma Masks Risk of Tumor Extension and Increases Death From Metastasis. *J Clin Oncol*. 2011 Mar 1;29(7):845-51
48. Kaliki S, Shields C, Shah S, et al. Postenucleation adjuvant chemotherapy with vincristine, etoposide, and carboplatin for the treatment of high-risk retinoblastoma. *Arch* 2011 Nov; 129 (11): 1422-7.
49. Kleinerman R, Tucker M, Tarone R, et al. Risk of new cancers after radiotherapy in long-term survivors of retinoblastoma: An extended follow-up. *J Clin Oncol* 2005; 23:2272-2279.
50. Reisner M, Viégas C, Graziotin R, et al. Retinoblastoma--comparative analysis of external radiotherapy techniques, including an IMRT technique. *Int J Radiat Oncol Biol Phys* 2007; 67 (3): 933-41.
51. Shields C, Mashayekhi A, Demirci H, et al. Practical Approach to management of Retinoblastoma. *Arch Ophthalmol* 2004; 122: 729- 735.

52. Kim J, Kathpalia V, Wong R, et al. Orbital recurrence of retinoblastoma following enucleation. *Br J Ophthalmol* 2009; 93:463-467.
53. Singh D. and Shields L. Post-enucleation prophylactic chemotherapy in high-risk retinoblastoma. *Arch Ophthalmol* 2002;120:923-931
54. Kopelman E, McLean W, Rosenberg H et al. Multivariate analysis of risk factors for metastasis in retinoblastoma treated by enucleation. *Ophthalmology*. 1987;94(4):371-7
55. Gündüz K, Müftüoğlu O, Günalp I, et al. Metastatic retinoblastoma clinical features, treatment, and prognosis. *Br J Ophthalmol*. 2006 Sep; 113(9):1558-66.
56. Leal A, Rivera R, Flores M, et al. Survival in extra-orbital metastatic retinoblastoma: treatment results. *ClinTranslOncol*. 2006 Jan;8(1):39-44
57. Kivelä, T. Trilateral retinoblastoma: a meta-analysis of hereditary retinoblastoma associated with primary ectopic intracranial retinoblastoma. *J ClinOncol* Jun 1999; Vol.17, No.6, pp. 1829-1837, ISSN 0732-183X.
58. Marees T, van Leeuwen E, de Boer R, et al. Cancer mortality in long-term survivors of retinoblastoma. *Eur J Cancer*2009; 45 (18): 3245-53.
59. Gallie L, Ellsworth M, Abramson H, et al. Retinoma: spontaneous regression of retinoblastoma or benign manifestation of the mutation? *Br Cancer*. 1982; 45(4):513–521.
60. Margo C, Hidayat A, Kopelman J, et al. Retinocytoma: a benign variant of retinoblastoma. *Arch Ophthalmol* 1983; 101(10):1519–1531.
61. Singh D, Santos M, Shields L. et al. Observations on 17 patients with retinocytoma. *Arch. Ophthalmol* 2000; 118, 199–205.
62. Abouzeid H, Balmer A, and Moulin A. Phenotypic variability of retinocytomas: preregression and postregression growth patterns *Br J Ophthalmol* 2012; doi: 10.1136.
63. Eagle R, Shields A, Donoso L, et al. Malignant transformation of spontaneously regressed retinoblastoma, retinoma/retinocytoma variant. *Ophthalmology*.1989;96(9):1389–1395
64. Fleiss J, Tytun A, and Ury H. A Simple Approximation for Calculating Sample Sizes for Comparing Independent Proportions. *Biometrics* Jun 1980;Vol. 36, No. 2 pp. 343-346
65. Burke, Lisa A, Miller, et al. (Phone Interviewing as a Means of Data Collection: Lessons Learned and Practical Recommendations (30 paragraphs). *Forum Qualitative Sozial forschung / Forum: Qualitative Social Research*2001; 2 (2), Art. 7.

MICROSCOPIC EXAMINATION

Percentage of retinal involvement: %

Microscopic involvement of ocular structures.

- None
- Vitreous
- Ciliary body
- Angle/Schlemm’s canal
- Other (specify):
- Sclera
- Extrascleral extension
- Iris
- Cornea
- Optic disc
- Vortex veins
- Anterior chamber
- Lens

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 tumour cannot be assessed
- pT0 No evidence of primary tumour
- pT1 Tumour confined to eye with no optic nerve or choroidal invasion
- PT2a Tumour superficially invades optic nerve head but does not extend past lamina cribrosa or tumour exhibits focal choroidal invasion.
- PT2b Tumour superficially invades optic nerve head but does not extend past lamina cribrosa and exhibits focal choroidal invasion.
- PT3a Tumour invades optic nerve past lamina cribrosa but not to surgical resection line or tumour exhibits massive choroidal invasion.
- PT3b Tumour invades optic nerve past lamina cribrosa but not to surgical resection line and exhibits massive choroidal invasion.
- PT4a Tumour invades optic nerve to resection line but no extra-ocular extension identified.
- PT4b Tumour invades optic nerve to resection line and extra-ocular extension identified.

FINAL REPORT

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

Signature: _____

APPENDIX II: TNM PATHOLOGICAL CLASSIFICATION OF OCCULAR RB

(ICD-O C69.2) (TNM 7th edition)

T Primary tumour

Pox: Primary tumour cannot be assessed

pT0: No evidence of primary tumour

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

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

- pT2a: Tumour superficially invades optic nerve head but does not extend past lamina cribrosa or tumour exhibits focal choroidal invasion
- pT2b: Tumour superficially invades optic nerve head but does not extend past lamina cribrosa and exhibits focal choroidal invasion

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

- pT3a: Tumour invades optic nerve past lamina cribrosa but not to surgical resection line or tumour exhibits massive choroidal invasion
- pT3b: Tumour invades optic nerve past lamina cribrosa but not to surgical resection line and exhibits massive choroidal invasion

pT4: Tumour invades optic nerve to resection line or exhibits extraocular extension elsewhere

- pT4a Tumour invades optic nerve to resection line but no extraocular extension identified
- pT4b Tumour invades optic nerve to resection line and extraocular extension identified

pN: Regional lymph nodes

- pNX Regional lymph nodes cannot be assessed
- pN0 No regional lymph node involvement
- pN1 Regional lymph node involvement (pre-auricular, cervical)
- pN2 Distant lymph node involvement

pM: Distant metastasis

- M0 No distant metastasis.
- pM1 Distant metastasis.
- pM1a Single metastasis to sites other than CNS.
- pM1b Multiple metastases to sites other than CNS.
- pM1c CNS metastasis.
- pM1d Discrete mass(es) without leptomeningeal and/or cerebral spinal fluid (CSF) involvement.
- pM1e Leptomeningeal and/or CSF involvement.

APPENDIX III: KNH/UON ETHICAL APPROVAL LETTER



UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke



KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/19

Link: www.uonbi.ac.ke/activities/KNH/UoN

30th January 2014

Dr. John Muthuri
Dept. of Human Pathology
School of Medicine
University of Nairobi



Dear Dr. Muthuri

RESEARCH PROPOSAL: TUMOUR DIFFERENTIATION AS A PROGNOSTIC MODIFIER AMONG PATIENTS WITH RETINOBLASTOMA AT KENYATTA NATIONAL HOSPITAL AND P.C.E.A KIKUYU EYE UNIT (P496/09/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 30th January 2014 to 29th January 2015.

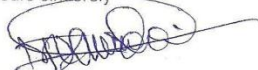
This approval is subject to compliance with the following requirements:

- a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
- b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
- c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
- d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
- e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
- f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
- g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website www.uonbi.ac.ke/activities/KNH/UoN.

Protect to Discover

Yours sincerely



PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N.Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Dean, School of Medicine, UoN
The Chairman, Dept.of Human Pathology, UoN
Assistant Director/Health Information, KNH
Supervisors: Dr. E. Dimba, Dr.W. Waweru, Dr. J. Githanga, Dr. K. Kimani, Dr. H. Dimaras

Protect to Discover

APPENDIX IV: PCEA-KH APPROVAL LETTER



P.C.E.A Kikuyu Hospital

P.O. Box 45-00902 Kikuyu, Tel: (020) 2044766-68, (020) 2044769-71
Fax: (020)2044765/772 Mobile:0722-207636 / 0733-606133 / 0736-270192

9th April 2014

Dr. John Muthuri
Department of Pathology
UON
P.O. Box 19676-00202
NAIROBI

Dear Dr. Muthuri

RE: REQUEST TO ACCESS MEDICAL RECORDS

Your request to access medical records for your research study has been accepted.

You are welcome to the Eye Unit for data collection and will work with Dr. Kabiru who has been attending to retinoplastoma patients.

Dr. Kabiru is available on Wednesdays (morning hours) and Thursdays. You can get in touch with her on 0722-743807 for an appointment to discuss on logistics.

Yours faithfully,

Dr. Daniel Mundia
Ag. Director of Clinical Services – Eye Unit

Cc Dr. Alain M'bongo
Dr. Joy Kabiru



General Hospital



Eye Unit



Rehabilitation Centre



Dental Unit

Email: kikuyu@pceakikuyuhospital.org / Website: www.pceakikuyuhospital.org

"The love of Christ through healing"

APPENDIX V: TELEPHONE INTERVIEWS MANUSCRIPT

Tumour differentiation and high risk histology features as prognostic factors among patients with retinoblastoma at Kenyatta National Hospital and Presbyterian Church of East Africa Kikuyu Hospital.

Step 1: Introduction and Confirmation of the legal parent/guardian:

Hello, I am Dr John Muthuri from the department of pathology at the University of Nairobi. Am I talking to Mr./Mrs..... the parent or guardian to (child's name) (If yes go to step 2. If no; thank the receiver and verify the number dialed)

Step 2: How the parent or guardian address was obtained:

I obtained your telephone number from the records at KNH or PKEU after approval from both the Kenyatta National Hospital/University of Nairobi Ethical research committee and KNH/PCEA-KH institution. Can you spare a few minutes (if yes go to step 3, if no confirm a better time to get back to them)

Step 3: Explaining the purpose of the call to the legal parent/guardian and obtaining a verbal consent.

This is in regard to a study am undertaking of the children who underwent eye surgery and were on follow up at KNH and PCEA-KH.

First, I'd like to explain to you more about the study and please feel free to stop me at any time you have a question.

The purpose of carrying out this study is to try to determine if some of the laboratory findings seen on the eye specimen after surgery have any significance on children wellbeing. This will enable us improve the care of children with RB. The information you will provide will be confidential and in no way will it be used to victimize you or your child.

Now that I've given you a basic idea concerning the study what questions do you have?
(Answer appropriately)

Am kindly seeking your consent to inquire some details regarding your child. (If yes go to 4 if no thank them for their time)

Step 4: Inquiring of the knowledge on the child's illness and clarification,

Before I continue, do you know what affected your child eye/eyes? (If yes let them expound if No take time and explain that the child had RB an eye cancer). Then let them know that eye specimen where taken to the laboratory for evaluation after surgery to confirm RB and its extent. (Then proceed to 5)

Step 5: How the child is doing.

Thank you, some of the questions may be a bit sensitive Please feel free to stop me at any time you get uncomfortable or you have any issues you would like me clarify.

How (child's name) is doing? (Will get an idea if alive or dead).

(If alive go to step 6.If deceased go to step 7)

Step 6: Questions if child Alive.

- A. How old is your child (child's name)?
- B. Which hospital was (Child's name) being followed up?
- C. The last follow-up month?
- D. The reason for stopping the follow-up?
- E. If the child is healthy? If no inquire more
- F. If any other sibling or family member are affected by RB?

Thank you very much for your time but please (child's name)'s need to resume follow-up (if not on follow-up).

Step 7: How parent/guardian is coping after the child's Death.

Am so sorry for your loss how are you coping? (Follow as below)

- If coping well proceed to step 8.
- If not coping well: ask whether they require counseling and link them to KNH adult counseling team at clinic 24: by informing them that fare is to be refunded once they attend the clinic and that at-least five sessions of counseling will be paid for. The in-charge clinic 24 will be informed to facilitate in booking the appointment.

Step 8: Questions if the child is dead.

Kindly need to ask you few questions and in case you're uncomfortable answering them or you have any issues you would like me clarify, feel free to stop me at any stage.

- A. At what age did (child's name)'s pass on?
- B. Did it occur in hospital (which) or at home?
- C. Please describe the circumstance surrounding the child's death? (Aim to determining if RB related or due to other courses).
- D. The last follow-up month?
- E. The reason for loss follow-up?
- F. If any other sibling or family member is affected.

Step 9: If other siblings or family members are affected link them to relevant RB care institutions.

Please for the other siblings or family members they need to be reviewed by eye specialists (refer them accordingly to either primary or secondary RB care center)

Thank you very much for your time.

APPENDIX VI: QUESTIONNAIRE

Tumour differentiation and high risk histology factors as prognostic factors among patients with retinoblastoma at Kenyatta National Hospital and Presbyterian Church of East Africa Kikuyu Hospital .

1.0. Demographic, Pre-surgical, surgical and post-surgical clinical information.

1.1. Study number _____

1.2. Hospital managed _____

1.3. Sex: Male female

1.4. Age at presentation (months) _____

1.5. Presenting complaints:		Duration in months
1. White reflex	<input type="checkbox"/>	<input type="checkbox"/>
2. Squint	<input type="checkbox"/>	<input type="checkbox"/>
3. Redness	<input type="checkbox"/>	<input type="checkbox"/>
4. Orbital swelling	<input type="checkbox"/>	<input type="checkbox"/>
5. Others. _____		<input type="checkbox"/>

PRESENTING COMPLAINS CODE: 1. YES, 2. NO

1.6 County of Birth _____

1.7 Guardian/parent contacts Provided YES NO

1.8 Guardian/parent occupation _____

1.9 Guardian/parent level of education _____

CODE: 1. No Formal education, 2. Primary School, 3. secondary school, 4. Tertiary Institution, 5. Not Indicated.

1.10 Family history of RB: YES NO NOT INDICATED

1.11. Laterality: Unilateral Bilateral Trilateral

1.12. Affected Eye: RE LE

1.13. Date of enucleation _____

1.14 Eye enucleated: RE: LE:

1.15 Type of enucleation: Primary Secondary

1.16. Age at enucleation (months): _____

2.0 Mode of management post primary enucleation:

2.10. Chemotherapy YES NO

2.11. Date Initiated _____

2.12. Duration from date of enucleation in days: _____

2.13 Regimens: VACI'S Cycles Number: VACI'S

VEC Cycles Number: VEC

CODE REGIMENS: 1 YES, 2 NO.

Completed Not completed

2.2 Radiotherapy: YES NO

2.21 Number of Sessions _____

Completed Not completed

3.0. Outcome post primary enucleation and duration in months: Outcome. Duration.

A] Alive disease free	<input type="checkbox"/>	<input type="checkbox"/>
B] Recurrence	<input type="checkbox"/>	<input type="checkbox"/>
C] Metastasis	<input type="checkbox"/>	<input type="checkbox"/>
D] Dead	<input type="checkbox"/>	<input type="checkbox"/>
E] Unknown.	<input type="checkbox"/>	

OUTCOME CODE: 1. YES, 2. NO

4.0. Cause of death: Retinoblastoma related **not Retinoblastoma related**

CODE: 1. Retinoblastoma related (Metastasis, recurrence, Chemotherapy related)

2. Not Retinoblastoma related (others)

4.1. Cause of death not retinoblastoma related where applicable _____

5.0. Date of last follow-up _____

6.0. Follow up period in months _____

7.0 Clinical data Information from records: complete In-complete

8. Telephone interview for those with in-complete clinical data.

8.0. Guardian/parent contacts provided: YES NO

8.1. Contact made: YES NO

8.2. Verbal consent granted: YES NO

8.3 Patient survival status: Alive Dead

8.4 Date alive/dead _____

8.5 Duration from enucleation in months _____

8.4 Cause of death: Retinoblastoma related **Not Retinoblastoma**

8.5. Cause of death not retinoblastoma related where applicable _____

8.6 Reason why lost to follow up _____

8.7 Parent/guardian needing and linked to counseling: YES NO

APPENDIX VII: M.P. SHAH APPROVAL LETTER



SOCIAL SERVICES LEAGUE
M.P. SHAH HOSPITAL
P.O. Box 14497-00800,
WESTLANDS, NAIROBI, KENYA.
TEL: 374 2763 /64 /65 FAX: 375 4686
Email: Laboratory@mpshahhosp.org
Website: www.mpshahhosp.org

DEPARTMENT OF LABORATORY MEDICINE

John Muthuri
P. O. Box 19676-00200
Nairobi
Mobile: 0721 920 648

16th July 2014

Dear Sir,

Ref: Tissue Blocks Issued

Kindly find the attached cases of retinoblastoma list that was issued. We would like to have the blocks back for our audit purposes.


Thank you.

Yours sincerely,

Dr. Timothy Onyuma
Pathologist

Collected by:

Name: Dr. John Muthuri

Signature: 

Date: 16/7/2014

APPENDIX VIII: SOP FOR RETRIEVED SPECIMEN BLOCKS HANDLING AND PROCESSING

1. The retrieved blocks were sectioned using microtome [3-5 microns] and the sections floated in warm water to remove wrinkles.
2. The sections were then be picked on a slide and placed in a warm oven for 15 minutes so to adhere to the slide.
3. The sections were then de-paraffined by dipping them in xylene to alcohol and then water.
4. Staining was done using standard Haematoxylin and Eosin techniques which entailed:
 - A. Staining in Harris Haematoxylin for 5minutes. Then,
 - B. Washing in running tap water for 1minute. Then,
 - C. Dipping 3 to 5 times in 1% Acid Alcohol. Then,
 - D. Wash in running tap water for 1minute. Then,
 - E. Rinse in 95% alcohol 10 dips
 - F. Stain in working eosin Y, making sure stain covers slides completely.
 - G. Wash in running tap water for 30 seconds
 - H. Dehydrate in ascending alcohols levels and clear in three changes of xylene
5. The quality of staining was confirmed first before mounting.
6. A cover slip was applied to the slide and after drying microscopically examine

APPENDIX IX: STUDY LABORATORY PROFORMA

Tumour differentiation and high risk histology features as prognostic factors among patients with retinoblastoma at Kenyatta National Hospital and Presbyterian Church of East Africa Kikuyu Hospital.

1.1 Study number _____

1.2 Blocks Retrieved: YES NO

1.3 Laboratory initially processed _____

2. MICROSCOPIC EXAMINATION:

2.1 Histomorphologically consistent with:

- A].RB -/- associated RB
B].RB +/- MYCN ^A RB
C]. Retinoma features

CODE: 1 YES, 2. NO

2.2 Histologic tumour grade P-O section:

- A]. Well differentiated
B]. Moderately differentiated
C]. Poorly differentiated

2.3. Extent of tumour spread:

2.3.1. Tumour limited to retina and or vitreous cavity only

CODE: 1 YES, 2. NO

2.3.2. Extent of choroid invasion:

- A].Not involved
B].Focal choroidal invasion [<3 mm].
C].Massive choroidal invasion [>3 mm]

2.3.3. Extent of Sclera invasion:

- A] Not involved
- B] Intrascleral
- C] extra-sclera.

2.3.4. Extent of optic nerve invasion:

- A] Not involved
- B] Pre laminae optic nerve invasion
- C] At Laminae involvement
- D] Post laminae optic nerve invasion
- E] Optic nerve surgical margin involvement
- F] Cannot be determined.

2.4. Involvement of other ocular structures:

- A].Not Involved
- B].iris
- C].Optic disc
- D].Ciliary body
- E] Lens
- F] Anterior Chamber.

CODE: 1. YES, 2. NO.

- 3.0. Staging: Pathologic TNM staging system:**
- pTx. Primary tumour cannot be assessed
- pT0. No evidence of primary tumour
- pT1. Tumour confined to retina with no optic nerve or choroidal invasion
- pT2a. Tumour superficially invades optic nerve head but does not extend past lamina cribrosa or tumour exhibits focal choroidal invasion.
- pT2b. Tumour superficially invades optic nerve head but does not extend Past lamina cribrosa and exhibits focal choroidal invasion
- pT3a. Tumour invades optic nerve past lamina cribrosa but not to surgical resection line or tumour exhibits massive choroidal invasion
- pT3b. Tumour invades optic nerve past lamina cribrosa but not to surgical resection line and exhibits massive choroidal invasion
- pT4a. Tumour invades optic nerve to resection line but no extra-ocular extension identified
- pT4b. Tumour invades optic nerve to resection line and extra-ocular extension.