CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

To Study the Incidence of Retinoblastoma in Islamabad & Rawalpindi & KAP Assessment of Parents Towards Screening and Genetic Testing

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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I dedicate my thesis to Allah Almighty, my mother, Khushnood Bibi and my father Noor Ahmed.

CERTIFICATE OF APPROVAL

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Abstract

Retinoblastoma, a rare childhood cancer, remains a significant public health challenge despite advancements in diagnosis and treatment. Understanding its incidence patterns is crucial for targeted prevention and early intervention strategies. While the global incidence rate ranges from 1 case per 15,000 to 20,000 live births, studies suggest higher rates in Pakistan, with estimates reaching 4.0 cases per 100,000 children under 5. This study aims to comprehensively explore the incidence of retinoblastoma across Islamabad and Rawalpindi population. Islamabad is the capital city of Pakistan and Islamabad and Rawalpindi, twin cities, are located in north of Pakistan. There is no data available for this region so, information regarding incidence of retinoblastoma in the general public of this region is desirable. My investigation of retinoblastoma in Islamabad and Rawalpindi was thorough. I also studied retinoblastoma patients' demographics and clinical features. I also examined parental knowledge about retinoblastoma screening and genetic testing.

The main objective of this study was to estimate the incidence of retinoblastoma and the level of awareness among parents of retinoblastoma patients. In this study, the departmental records of all retinoblastoma patients from 1st January, 2021 to 31st December, 2023 at Al Shifa Trust Eye Hospital, Rawalpindi, which is the biggest eye hospital in this region, were reviewed and retinoblastoma patients of Islamabad and Rawalpindi region were identified. From each patient's record following information was extracted: patient's name, date of diagnosis, laterality, age, sex, region and socioeconomic status of patient's family. A total of 51 patients of retinoblastoma were identified for this region. Out of these 29 (56.86%) were male and 22 (43.14%) were female. 33 (64.7%) had unilateral while 18 (35.29%) had bilateral retinoblastoma. The overall incidence of retinoblastoma in the population was 3.5 patients per 100,000 individuals. 24 (47.06%) patients were under the age of 5 while 27 (52.94%) above the age of 5. 20 patients were reported in 2021, 12 in 2022 and 19 in 2023. In addition, a simple but well-constructed questionnaire with seven closed-ended questions assessed parental comprehension

of retinoblastoma screening and genetic testing. Parental knowledge of retinoblastoma screening and genetic testing was low. This study concluded that the overall incidence rate of retinoblastoma is very high in this region.

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Abbreviations

CSF	Cerebrospinal Fluid
GD2	Ganglioside
IMRT	Intensity-modulated radiation therapy
IRSS	International Retinoblastoma Staging System
Indel	Insertion and deletion
LOH	Loss of Heterozygosity
LP	Lumbar puncture
M1, M2, Mn	Mutation 1, Mutation 2, Mutation n
MRI	Magnetic Resonance Imaging
NMRI	Nuclear Magnetic Resonance Imaging
RB1	Retinoblastoma gene
Rb	Retinoblastoma
bp	Base Pair
pRb	Retinoblastoma Protein

Chapter 1

Introduction

1.1 Retinoblastoma Cancer

Retinoblastoma, also known as OMIM#180200, is a kind of intraocular malignant tumour that causes immature cells in the retina to develop into the disease. This type of tumour is often seen in children. The nerve tissue that lines the internal wall of the back of the eye is called the retina. The retina is responsible for taking in light, converting it into signals, and then transmitting those impulses to the brain via the optic nerve. By decoding the impulses, the brain is able to provide you with the ability to perceive the picture. Although the tumour first causes the patient to have impaired vision, it eventually leads to the development of additional malignancies and even blindness. Should the cancer not be treated, there is a possibility that the patient may perish [1].

This condition, commonly referred to as "cancer of the infant retina," generally presents itself either before or soon after birth. According to the available data, the age at which bilateral and unilateral Rb first appeared is 15 and 24 months, respectively, whereas the average age at which Rb first appeared is 19.5 months [2]. In spite of the fact that certain studies have shown an extremely rare late onset that occurs between the ages of 20 and 60 and has an incidence rate of four per million, only ninety-five percent of patients really experience this [3]. According to the studies, there are around 9000 cases that occur annually, which is equivalent

to one case for every 20,000 live births. It has been noted that there are no biases for racial or gender-based predispositions that are associated with the occurrence of Rb [4]. The only exception to this is in regions that have huge populations and high birth rates, such as those in Africa and Asia [5].

1.2 Introduction of Genetic and Non-Sporadic Diseases

The development of Rb may occur in two distinct ways: (60%) it can be non-hereditary or sporadic, and (40%) it can be hereditary or familial [6]. Patients who have a hereditary form of the illness are individuals who have a history of the ailment in their family and who have a new germline mutation either at the time of conception or while the embryo is developing. Genetic mutations of this kind occur naturally and are very long-lasting in the germline. It is quite probable that the patient would have a constitutional heterozygous mutation and an early development of bilateral and multifocal tumours in both of these inherited conditions. Because of the limited penetrance and expressivity of some germline mutations, ten to fifteen percent of kids from families with Rb may develop unilaterally when they are born [7].

Germline mutations are more likely to be present in persons who are unilateral and who acquire sickness at an early stage, while non-hereditary mutations are more likely to be present in patients who are unilateral and who develop illness at a later stage. On the other hand, those who have inherited Rb are more likely to acquire osteosarcomas than they are to develop other secondary non-ocular cancers. Individuals who have radiation are at an increased risk of developing cancers of the lungs, bladder, soft tissues, skin, and brain, in addition to other organs, throughout their whole lives. This risk is especially high for those who have a history of cancer. The sporadic or non-heritable type of Rb is often unifocal, unilateral, and non-heritable in sixty percent of all instances, with a frequency of

eighty-five percent, according to the experts. The incidence of multifocal tumours, on the other hand, is much lower [8].

Two further mutational events, referred to as M1 and M2, cause the biallelic inactivation of the Rb causal gene. More than 95% of people with hereditary Rb have it, and it is passed down in a way called autosomal dominant inheritance. This means that the first mutation in hereditary Rb usually only affects one allele. It is possible for it to take many different forms, ranging from a little nucleotide mutation to a significant insertion and deletion (indel) [9]. The neighboring retinoblast allele needs the second mutation in order to develop into malignancy. This is due to the fact that the second mutation is responsible for the development of LOH, which is a condition that typically occurs in bilateral Rb. In cases with sporadic Rb, somatic retinoblast cells go through both mutational processes. However, constitutional cells often do not have either mutation present in their DNA. Approximately ten to fifteen percent of people who are sporadic have been found to have a germline mutation that has a 45 percent chance of being passed down to their offspring [10]. Instead of directly creating Rb, M1 and M2 in humans are responsible for initiating the precursor lesion for Rb, which is retinomas. These retinomas are also benign and euploid. Other genetic abnormalities, such as M3-Mn in Rb patients, are also responsible for the development of later malignancies that are not of the eye [11].

1.3 Signs and Symptoms

- The most prevalent clinical sign of Rb, affecting 60–80% of individuals, is the development of an aberrant white pupillary reflex, commonly referred to as cat's eye or leukocoria [12]. Figure 1.1

- .In contrast, the second-most common clinical symptom in children with Rb is the development of a squint, also known as strabismus or being "cross-eyed" [12].

- Retinoblastoma or other medical problems may cause these signs and symptoms. The pupil of the eye exhibits a white colour instead of red when illuminated by light.

- Redness or pain in the eye (Figure 1.2)

- Larger eyeball than normal

- Pupil and coloured part of the eye look cloudy

- Infection around the eye [12, 13]

FIGURE 1.1: Crossed Eyes

FIGURE 1.2: Redness in the eye

1.4 Risk Factors of Rb

A risk factor is any element that increases an individual's likelihood of developing an ailment, such as cancer. The risk factors for different types of cancer differ. Several lifestyle-related variables, such as body weight, physical activity, diet, and tobacco use, have a substantial role in the development of various types of cancer in adults. Nevertheless, the influence of these variables on cancer risk is often gradual, and it is widely accepted that they have little effect on paediatric cancers such as retinoblastomas. Retinoblastoma is associated with a limited number of known risk factors [14].

1.5 Age

Children under the age of 3 are most commonly diagnosed with retinoblastoma. Most congenital (heritable) retinoblastomas are discovered in the first year of life, while nonheritable retinoblastomas are often diagnosed between the ages of one and two. After age 6, retinal tumours are uncommon [15].

1.6 Heredity

Offspring of individuals with the hereditary variant of retinoblastoma have a much higher probability of acquiring the condition. This particular variant often leads to the development of bilateral retinoblastomas, which are tumours affecting both eyes. Nevertheless, most youngsters with retinoblastoma do not possess a familial background of the ailment. This remains true irrespective of whether they have the heritable or non-heritable type of retinoblastoma [14].

Nevertheless, kids of children who acquire the heritable variant of retinoblastoma face a 50% probability of inheriting the RB1 gene mutation, which leads to the development of the tumour. Offspring of individuals who inherit the non-heritable kind are not at an increased risk [14].

1.7 Unclear Risk Factors

Some studies have suggested some parental factors that might be linked to an increased risk of retinoblastoma, such as:

- Nutrient deficient diets among mothers during pregnancy [16]
- Exposure to certain chemicals during pregnancy [17]
- Exposure of fathers to radiation [18]
- Older age among fathers [19]

The possible link between these factors and retinoblastoma is still being studied.

1.8 Problem Statement

Assessing the incidence rate of retinoblastoma in Islamabad and Rawalpindi is very crucial in order to estimate the burden this disease puts on the population. But there is no data available on the incidence rate of retinoblastoma in Islamabad and Rawalpindi as well as other cities of Pakistan except Karachi, previously. Before this study, there was only one study conducted in Karachi, Pakistan regarding the prevalence of the disease, which is above 20 years old. Therefore, it is important to present an updated data on incidence and awareness of retinoblastoma in Islamabad and Rawalpindi.

1.9 Scope

Calculating the total number of infected people in Rawalpindi and Islamabad would aid in planning resource allocation and understanding the burden of the disease. The awareness of parents of patients about retinoblastoma is also estimated by this study.

1.10 Aims and Objectives

Aim

The aim of this study was to find out the incidence of retinoblastoma disease in Islamabad and Rawalpindi population along with the demographic and clinical features of the patients and parent knowledge about screening and genetic testing of retinoblastoma.

Objectives

1. To find out incidence of retinoblastoma in Islamabad and Rawalpindi population
2. To find out demographic and clinical features of retinoblastoma children
3. To find out parental knowledge of screening and genetic testing of retinoblastoma

Limitations

There are certain limitations of this study such as:

- The study only captured cases referred to Al Shifa Trust Eye Hospital, Rawalpindi, potentially missing those diagnosed elsewhere or not seeking care at all. This may skew the results towards more severe cases or certain demographics with better access to specialized healthcare.
- The study relies on past records, so changes in diagnostic practices or record-keeping systems over time could impact the comparability of the data.
- The study has not collected data on relevant environmental factors that could influence retinoblastoma risk, hindering understanding of potential causes.
- These results may not be generalizable to a broader population, as the hospital serves a specific demographic and geographic area.

- The survey was conducted at a single hospital, which might have increased the margin of error and reduced the study's ability to detect real differences in incidence rates. Broader surveys with a larger number of study sites should be conducted to generate accurate data.
- This study did not consider the risk factors linked to the incidence of the disease.

It's important to consider these limitations and to look for studies that address these limitations through broader sampling, comprehensive data collection, and multi-center designs.

Chapter 2

Literature Review

2.1 Definition of Retinoblastoma

Retinoblastoma is a rare malignant intraocular tumour affecting the developing retina, primarily in children under five years old. It arises from the uncontrolled proliferation of immature retinal cells, known as retinoblast [1].

2.2 Types of Rb

Retinoblastoma, a rare eye cancer primarily diagnosed in children, exhibits diverse characteristics and progression patterns. Understanding these variations, classified as types, is crucial for accurate diagnosis, effective treatment, and optimal patient outcomes [20]. Following are the key types of retinoblastoma, their defining features, potential causes, and clinical implications.

2.2.1 Based on Laterality

Unilateral Retinoblastoma

This form, affecting approximately 60% of cases, involves tumour growth in only one eye. It's often non-hereditary and arises from a spontaneous mutation in the

RB1 gene within a single retinal cell. Unilateral retinoblastoma typically presents at a later age than the bilateral type [21].

Bilateral Retinoblastoma

Comprising roughly 40% of cases, this type features tumours developing in both eyes. The main cause of this type is an inherited RB1 gene mutation passed from a parent or arising spontaneously during early embryonic development. Bilateral retinoblastoma often manifests at a younger age and has more aggressive tumour characteristics [22].

2.2.2 Based on Genetics

Germline Mutation

This category encompasses retinoblastoma cases where the RB1 gene mutation is present in all body cells due to inheritance or early embryonic development. It's associated with a higher risk of developing bilateral retinoblastoma and other cancers later in life [23].

Somatic Mutation

In this type, the RB1 gene mutation occurs only in retinal cells, not affecting other tissues. It typically results in unilateral retinoblastoma and carries a lower risk of future cancer development [24].

Indicators of the heritable type of retinoblastoma in a kid include the following factors:

- There is a hereditary predisposition to retinoblastoma within the family
- Multiple tumours are present in the eye
- Bilateral ocular neoplasms are present [25]

2.2.3 Based on Tumour Spread

Intraocular Retinoblastoma

This type further categorizes tumours within the eye structures, such as the retina, choroid, or optic nerve. Early detection and treatment can often achieve successful outcomes with vision preservation [26].

Extraocular Retinoblastoma

The spread of cancer cells behind the eye(s) is classified as extraocular retinoblastoma. This may include neighboring tissues like the orbit or far organs such as the brain, bone marrow, or lymph nodes. The complexity of treatment increases as the prognosis is contingent upon the degree of dissemination [27].

2.2.4 Other Classifications

Trilateral Retinoblastoma

This rare form (less than 5% of cases) involves additional tumours in the brain, most commonly a pinealoblastoma. It's exclusively associated with germline RB1 mutations and requires aggressive multimodal therapy [28]. The condition known as trilateral retinoblastoma, in which the ocular tumours are accompanied by a midline intracranial primitive neuroectodermal tumour (PNET), affects around 5–7% of bilateral people (and a small number of unilateral patients). Because they originate in the pineal gland area, these tumours were once known as pinealomas. PNETs are seen as a primary intracranial malignancy that occurs concurrently or consecutively, rather than as extensions (metastases) of intraocular tumours to the central nervous system. A thorough examination for extraocular illness should include radiographic screening for PNET [14].

Macular Retinoblastoma

When the tumour involves the central vision area (macula), it presents unique challenges for preserving vision while controlling cancer spread. Understanding

the various types of retinoblastoma empowers healthcare professionals to tailor diagnostic approaches, predict disease progression, and implement optimal treatment strategies. Early detection and prompt intervention remain key to achieving successful outcomes for children battling this challenging eye cancer [28].

2.3 Fundamental Composition of Rb

The RB transcript is encoded by 27 exons within a genomic DNA sequence of about 200 kilobases (kb). Exons have a length ranging from 31 to 1889 base pairs (bp). The smallest intron measures a mere 80 base pairs, whereas the largest extends over 60 kilobases [29].

2.4 Rb's Historical Background

In 1809, Dr. James Wardrop first identified Rb as a type of ocular cancer and recommended enucleation as the only therapeutic option (Wardrop, 1809). Subsequently, researchers used terms such as retinoma, neuroepithelioma, and retinal glioma to refer to this medical illness. Verhoeff and Jackson termed retinoblastoma in 1926 to indicate its derivation from retinal progenitor cells, which have the capacity to differentiate into many cell lineages. Retinoblastoma (Rb) is a cancer that develops from the unregulated proliferation of immature cells in the retina. Berkeley and Kalita first documented this in 1977. This finding is substantiated by histological exams, studies of structural characteristics, cell culture investigations, immune cytochemistry, and genetic analyses of the cancer [30]. Retinoblastoma cure rates have significantly improved due to a number of reasons, with 5-year survival rates up to 98% in developed nations. First of all, the discovery of chemotherapy was coincidental; it resulted from findings made during World War II that those who were exposed to nitrogen mustard had lower levels of white blood cells. This led to studies looking at mustard agents' ability to stop cell division, especially in cancer cells. Many medications were then quickly created

and used to treat cancer; in the 1990s, first-line chemotherapy was launched, allowing for the treatment of micrometastases and intraocular tumors, which decreased mortality and improved the chances of eye preservation. The most often utilized combination was found to be vincristine, carboplatin, and etoposide, with changes according to the severity of the illness. Disseminated metastatic illness also requires autologous bone marrow transplantation in addition to rigorous treatment. Second, there has been a notable improvement in survival and eye preservation rates as a result of the use of local treatment. Specialized treatment teams for retinoblastoma agree that chemotherapy is not sufficient to manage intraocular illness; other local therapeutic modalities, such as diode laser transpupillary thermotherapy, laser, cryoapplication, and brachytherapy, are necessary. Targeted information campaigns help in early diagnosis, which allows for local treatment or a combination of carboplatin and trans pupillary thermotherapy to ablate tiny tumors. To further increase the effectiveness of treatment, local therapies are also used as second-line treatments for local recurrence. Finally, a major factor in increasing patient survival and eye preservation rates has been the development of specialized centers across Western nations. These centers house multidisciplinary teams consisting of ophthalmologists, oncologists, pediatric oncologists, radiation physicians, radiologists, geneticists, and anesthetists. Due to the high degree of interdisciplinary knowledge, retinoblastoma management patient results can be optimized by customized therapies based on thorough first ophthalmological tests performed under general anesthesia [31].

2.5 Diagnosis

The following tests and procedures may be used:

2.5.1 Physical Exam and Health History

During a comprehensive physical examination, healthcare professionals assess the overall health status by thoroughly evaluating for any indications of pathological

conditions, such as palpable masses or other atypical findings. In addition, the physician will extensively review the patient's medical history to determine past illnesses, treatments, and lifestyle practices. The physician will inquire about familial occurrences of retinoblastoma [32].

2.5.2 Eye Exam with Dilated Pupil

A comprehensive examination of the eye involves the administration of medicated eye drops to dilate the pupil, enabling a clear view of the retina and optic nerve through both the lens and pupil. A specialized light source carefully inspects the internal structures. In cases involving young children, this process may require anesthesia [33].

Various kinds of eye examinations are conducted with the dilation of the pupil:

2.5.3 Ophthalmoscopy

During ophthalmoscopy, a miniature magnifying lens and an illumination source are used to inspect the posterior segment of the eye, including the retina and optic nerve [34]. The process entails using a miniature magnifying lens and an illumination source to see these formations [34].

2.5.4 Fluorescein Angiography

Fluorescein angiography is a medical procedure that includes the study of blood vessels and their circulation in the eye. In order to carry out this process, a highly visible orange fluorescent dye called fluorescein is introduced into a blood vessel in the arm, where it subsequently enters the circulation. Specialized imaging equipment takes pictures of the retina and choroid as it flows through the blood vessels of the eye in order to detect any blockages or leaks in the vessels The biggest diameter retinal arteries should be seen "feeding" the tumor in a dilated and convoluted manner in a fundus-based retinoblastoma. Furthermore visible

are arteriovenous shunts, micro aneurysms, and retinal hemorrhages. In the same research, the aberrant vascularization was completely confined to the tumors itself and became inconsistent with normal retinal structure as the tumors grew. The intrinsic vessels within the tumor exhibited complicated and disordered branching patterns, uneven diameter, and early termination within the tumor's body. When comparing RB to Coat's disease, which is characterized by massive dilated arteries that remain within one level of the retina and exhibit significant peripheral non-perfusion, the physician may more easily recognize RB due to its multi-level involvement of vascular abnormalities. Finally, the physician should attempt to gather all important information during the first three minutes of the investigation since after that time, diffuse leaking from retinal arteries may cause an inability to distinguish minute aspects of the fundus [35].

2.5.5 Electroretinography

Electroretinography is an ocular examination that utilizes diminutive electrodes and light to scrutinize the retina, the photosensitive layer of the eye. This procedure measures and documents the faint electrical impulses emitted by retinal cells when exposed to varying degrees and types of light. It can be employed for assessing retinal function during and after therapy [36].

2.5.6 RB1 Gene Test

A laboratory test that examines a sample of blood or tissue for any alterations in the RB1 gene is referred to as an RB1 gene test [37].

2.5.7 Ultrasound Exam of the Eye

Throughout the whole of this procedure, we direct ultrasonic waves of high intensity against the tissues that are located inside the eye in order to generate reverberations. Before beginning the therapy, the eye is put under anaesthesia

by means of ophthalmic drops. A knowledgeable and skilled professional carefully places an accurate device onto the surface of the eye, which is capable of both sending and receiving sound waves. The echoes provide a visual representation of the internal anatomy of the eye, making it easier to determine the distance between the cornea and the retina. During an ultrasound process, sonograms show visual depictions on a monitor screen. Additionally, sonograms can be printed for further analysis [38]. Sonograms are a visual representation of an image.

2.5.8 MRI (Magnetic Resonance Imaging)

Magnetic resonance imaging (MRI) is a diagnostic technique that uses a magnet, radio waves, and advanced computer technology to provide precise images of internal body structures, including the eye region. This technology may alternatively be known as nuclear magnetic resonance imaging (NMRI) [39, 40].

2.5.9 Biopsy

Retinoblastoma may usually be diagnosed using methods other than biopsy. If the illness exists in one eye, it might show up in the other eye that is not afflicted. Hence, assessments of the latter are carried out to ascertain the hereditary status of retinoblastoma [41].

Multiple variables contribute to the determination of both prognosis and therapy choices.

2.5.10 Variables Influencing Forecast & Available Courses of Therapy

The prognosis and treatment choices are contingent upon the following factors:

- The determination of whether the cancer is affecting one side (unilateral) or both sides (bilateral).

- The assessment of the size and quantity of the tumour.
- The evaluation of whether the tumour has spread to nearby tissue, the brain, or other parts of the body (metastasis).
- Age of the patient
- Likelihood of preserving visual acuity.
- Whether there is a presence of secondary malignancy
- Whether the cancer is in its first diagnosis or has returned [42].

2.6 Retinoblastoma Stages

After diagnosing retinoblastoma, medical practitioners conduct examinations to determine the presence of malignant cells inside the eye or in other areas of the body. Medical practitioners use the International Retinoblastoma Staging System (IRSS) to ascertain the stage of retinoblastoma [43].

i. Stage 0

The tumour is only situated in the eye and was managed without the need for surgical intervention.

ii. Stage I

The tumour is only located in the eye yet, it has been surgically removed and no cancerous cells are left.

iii. Stage II

The surgical procedure successfully eliminated the eye tumour, nevertheless, microscopic analysis indicates the presence of remaining malignant cells.

iv. Stage III

Stage III of the malignancy is further separated into two subcategories: IIIa and IIIb. In stage IIIa, the cancer has extended beyond the eye and has progressed to the nearby tissues surrounding the orbit.

In stage IIIb, the cancer has spread beyond these sites and has affected lymph nodes located near the ear or around the neck.

v. Stage IV

Designated as stage IVa or IVb:

In stage IVa, the cancer has spread to one or more bodily components, such as the bones or liver [43].

On the other hand, in Stage IVb, the spread of the disease includes the invasion of brain tissue and the involvement of the spinal cord, with the potential for affecting other areas of the body as well [43].

The following examinations and methodologies may be used throughout the staging procedure:

2.6.1 MRI (Magnetic Resonance Imaging)

Magnetic resonance imaging (MRI) is an advanced diagnostic technique that utilises a magnetic field, radio waves, and computer technology to generate detailed pictures of interior structures inside the body. This imaging technique is often known as nuclear magnetic resonance imaging (NMRI) [40].

2.6.2 Bone Scan

A method for identifying actively proliferating cells, such as those present in malignant bone tissue. A small dose of a radioactive substance is injected into a vein and then travels throughout the circulation. A scanner that records a visual representation of the body can detect the concentration of the radioactive chemical in regions of the bones impacted by cancer. These regions appear more vividly on

screen due to their greater absorption of radioactive matter compared to normal bone cells [44].

The child receives a small dose of radiopharmaceuticals via intravenous injection, which then travels throughout the skeletal system. Lying still on a motorized bed that moves through the scanning device, images are captured and transferred onto a computer monitor by means of radiation detection technology employed within this medical apparatus [44].

2.6.3 Bone Marrow Aspiration and Biopsy

This procedure involves the extraction of bone marrow and a tiny section of bone by inserting a hollow needle into the hipbone or breastbone. The pathologist uses microscopic examination to meticulously assess the bone marrow specimen in order to detect any signs of malignant cells.

If there is a suspicion that the cancer has extended beyond the eye, the pathologist conducts a bone marrow aspiration and biopsy [41, 45].

2.6.4 Lumbar Puncture

Lumbar puncture: Physicians get cerebrospinal fluid (CSF) from the spinal canal via the procedure of lumbar puncture. During this procedure, physicians inject a needle into the cerebrospinal fluid (CSF) surrounding the spinal cord, between two vertebrae, to extract a fluid sample. The cerebrospinal fluid (CSF) sample is scrutinised using a microscope to detect indications of cancer metastases in the brain and spinal cord.

Moreover, it is feasible to assess the presence of ganglioside GD2, a biomarker for tumours. Medical practitioners may also use the terms ”spinal tap” or ”lumbar puncture” to describe this technique [46].

2.7 Metastasis

Cancer, a malignant illness, has the ability to metastasize and spread from its original location to other parts of the body. This movement is called metastasis. This phenomenon arises when the deviant cells disengage from their initial site (the main tumour) and traverse either the lymphatic system or circulation [47].

Cancer may spread via several mechanisms, including tissue invasion, infiltration of the lymphatic system, and dissemination through the bloodstream.

2.7.1 Tissue

In terms of tissue dissemination, cancerous cells proliferate into adjacent areas.

2.7.2 Lymph System

As for lymphatic system involvement, cancer cells invade this network and travel via lymph vessels to distant sites within the body.

2.7.3 Blood

When cancer enters the bloodstream it can use blood vessels as a conduit to migrate to other areas throughout the body [47, 48].

2.8 Treatment for Rb

In the last ten years, there have been notable progressions in the management of retinoblastoma, the prevailing cancer that occurs inside the eye in youngsters. The use of safer techniques for intravitreal chemotherapy injection and ophthalmic artery chemosurgery, along with other important improvements in local drug administration, has made it possible to save eye globes that were not possible with

systemic chemotherapy or external beam irradiation before. Possible strategies for treating intraocular retinoblastoma include oncolytic viruses, immunotherapy, and novel medicines.

Current studies on the spatial distribution of tumours and the localised delivery of drugs may provide preliminary advancements in the development of novel therapies for metastatic diseases [49].

The care of retinoblastoma depends on its location inside the eye, either within or outside the eye.

2.8.1 Intraocular Retinoblastoma

Intraocular retinoblastoma pertains to the existence of the retinoblastoma malignancy within the ocular region. In instances of intraocular retinoblastoma, neoplastic cells inhabit either one or both eyes.

The cancerous growth may be localised solely in the retina or may have progressed to other regions such as the choroid, ciliary body, or a segment of the optic nerve. Notably, at this stage, there is no indication that metastasis has occurred in periorbital tissues or any remote sites throughout the body [50].

2.8.2 Extraocular Retinoblastoma (Metastatic)

Cancerous cells spreading beyond the eye is referred to as extraocular retinoblastoma. Orbital retinoblastoma is the formation of tumours in the tissues around the eye.

Extraorbital retinoblastoma is the term used to describe the spread of cancer cells to regions inside the central nervous system, such as the brain and spinal cord, or to distant parts of the body, such as the liver, bone marrow, or lymph nodes [48].

2.9 Overview of Treatment Options

The treatment options for retinoblastoma typically involve six categories of standard therapies:

2.9.1 Cryotherapy

Cryotherapy, or cryosurgery, uses very cold temperatures to eliminate aberrant tissue. A very cold metal probe is used to freeze and kill malignant cells near the tumour on the surface of the eye. This procedure is often used to treat tiny retinoblastoma tumours situated in the anterior region of the eye. Furthermore, thermotherapy can serve as an alternative form of therapy. Small peripheral and equatorial retinal tumors up to 2 mm thick and 4 mm in basal diameter are treated with cryotherapy.Cryotherapy (triple freeze-thaw) is administered at intervals of 4-6 weeks until total tumor remission. An even bigger scar than the tumor results during cryotherapy. Retinal tears, rhegmatogenous retinal detachment, and transitory serous retinal detachment are among the side effects of cryotherapy. There is a synergistic effect when cryotherapy is given 2-4 hours before chemotherapy because it can improve the distribution of chemotherapeutic drugs across the blood retinal barrier [51].

2.9.2 Thermotherapy

Thermotherapy, a technique that utilizes heat to eradicate cancer cells, can be implemented through the targeted emission of laser beams either directly onto the external surface of the eyeball or via a dilated pupil. This treatment modality may be utilized as an independent therapy for smaller tumours or in tandem with chemotherapy for larger ones. It is classified as a form of laser therapy. In thermotherapy, tumor necrosis is induced by applying sub photocoagulation amounts of concentrated heat produced by infrared radiation to tissues.21 The objective is to preserve the retinal vessels by slowly and steadily raising the tumor's temperature to between 40 and 60 ^{o}C. Transpupillary thermotherapy is often treated

with infrared radiation from a semiconductor diode laser given using an indirect ophthalmoscope delivery method with a big spot of 1300 microns. As an alternative, transpupillary administration can be carried out via a trans scleral pathway using a diopexy probe or under an operating microscope. Small tumors with a basal diameter of 4 mm and a thickness of 2 mm can be well controlled with thermotherapy. With three to four sessions of thermotherapy, approximately 85% of tumors can have complete tumor shrinkage. Retinal traction, serous retinal detachment, focal iris atrophy, and focal paraxial lens opacity are the most frequent side effects [52].

2.9.3 Chemotherapy

Chemotherapy is a therapeutic approach for cancer that employs medications to halt the proliferation of cancerous cells, either by inducing cell death or by preventing cell division. The administration of chemotherapy varies based on the cancer's stage and its location within the body. Chemotherapy encompasses various modalities: intravenous, oral, topical, and intra-arterial drug delivery [31].

Chemotherapy encompasses various modalities:

2.9.3.1 Systemic Chemotherapy

Administering chemotherapy orally or via intravenous or intramuscular injection enables the medications to enter the bloodstream and effectively target cancer cells in various parts of the body. Systemic chemotherapy is utilized for chemoreduction, reducing tumour size, and preventing surgical removal of the eye. Post-chemoreduction therapies may include radiation therapy, cryotherapy, laser therapy, or localised chemotherapy. However, the treatment for extraorbital illness requires intensive chemotherapy, which may include high-dose chemotherapy and autologous stem cell transplantation, with or without radiation therapy. Systemic chemotherapy can eliminate residual cancer cells following the initial treatment or eradicate cancer cells located beyond the eye [53].

2.9.3.2 Adjuvant Therapy

Adjuvant therapy refers to post-treatment administered to reduce cancer recurrence likelihood. Initiated in the 1970s, studies on the effectiveness of adjuvant treatment to reduce the risk of metastasis were characterized by inconsistent outcomes and offered no definitive advice. Information from a recent long-term follow-up research is helpful. It comprised a subgroup of individuals who had primary enucleation for unilateral sporadic retinoblastoma. Patients were chosen for the trial based on specified histopathologic features.

Metastatic occurrences, which typically happen a mean of 9 months after enucleation, were permitted to be included in a minimum follow-up of 1 year.13. Those who got adjuvant treatment had a 4% incidence of metastasis, whereas those who did not had a 24% incidence. The results of the study showed that in patients with high-risk histopathological features, adjuvant treatment administration dramatically decreased the probability of metastasis. Orbital external beam radiation treatment and systemic chemotherapy are examples of adjuvant therapy [54].

Considering adjuvant chemotherapy after enucleation to prevent metastasis in high-risk patients makes sense given our growing understanding of the risk variables that predict metastasis and the availability of efficient chemotherapy regimens for intraocular retinoblastoma. Adjuvant chemotherapy's usefulness in these situations is still up for debate, and its function is yet unclear. Patients with tumor invasion of optic nerve transection, scleral and extrascleral extension, spontaneous or inadvertent ocular perforation, and intraocular surgery for undiagnosed retinoblastoma are advised to have adjuvant orbital external beam radiation after enucleation. But the effectiveness of this kind of therapy is still up for debate.

There is debate about the histopathologic prognostic markers that determine who is "high-risk" for developing metastasis, which exacerbates the dispute around adjuvant treatment. Adjuvant therapeutic experience has been limited because to the general rarity of retinoblastoma, which includes the unique occurrence of extraretinal involvement [54].

2.9.4 Regional Chemotherapy

Regional chemotherapy involves the direct administration of chemotherapeutic agents into specific areas of the body, including but not limited to the cerebrospinal fluid, organs such as the eye, and various cavities. This method enables a targeted approach to eradicating cancer cells in those particular regions.Regional chemotherapy, including regional intra-arterial chemotherapy (RIAC), has been developed as a strategy to enhance cancer survival rates.

This approach has been under investigation since the 1950s and has shown promising results for certain localized and metastatic malignancies. By delivering high concentrations of chemotherapy drugs directly to targeted areas, such as the liver in hepatic artery infusion (HAI) for unresectable colorectal cancer with liver metastases, significant response rates have been achieved. In some cases, tumors have become operable following this treatment. Clinical studies have also indicated that localized intra-arterial infusion of gemcitabine (GEM) has been well-tolerated by patients with advanced pancreatic cancer (APC), leading to increased response and resectability rates. Furthermore, regional chemotherapy, particularly intra-arteri

al approaches like hepatic artery infusion (HAI) and gemcitabine (GEM) infusion, has demonstrated efficacy in managing advanced pancreatic cancer (APC) and unresectable colorectal cancer with liver metastases.

By directly administering chemotherapy drugs to the affected regions, these techniques achieve high drug concentrations while minimizing systemic exposure, thus reducing systemic side effects. This targeted approach has led to improved response rates and increased opportunities for surgical intervention, ultimately contributing to better outcomes for patients with these challenging malignancies.

Ongoing research continues to refine and expand the use of regional chemotherapy, offering hope for further advancements in cancer treatment and improved survival rates. Treating retinoblastoma [55] involves utilizing multiple forms of localized chemotherapy.

2.9.4.1 Ophthalmic Artery Infusion Chemotherapy

Ophthalmic artery infusion chemotherapy is a targeted method for administering chemotherapeutic drugs directly to the eye. This procedure involves inserting an artery catheter to precisely deliver anticancer treatment to the affected area. Healthcare providers may sometimes use a small balloon inserted into the artery to restrict blood circulation and concentrate the medicine near the tumor site. Ophthalmic artery infusion chemotherapy can serve as a primary treatment option for localized intraocular malignancies or those resistant to other therapies. Specialized retinoblastoma treatment institutes with expertise in this technique deliver this specific type of chemotherapy. These specialized retinoblastoma treatment institutes with expertise in ophthalmic artery infusion chemotherapy play a crucial role in providing comprehensive care for patients with intraocular malignancies. By utilizing this targeted approach, they can effectively deliver chemotherapy directly to the tumor site, maximizing treatment efficacy while minimizing systemic side effects. This advanced technique requires specialized training and equipment, ensuring safe and precise administration of chemotherapy drugs. By offering ophthalmic artery infusion chemotherapy as part of their treatment arsenal, these institutes provide patients with access to cutting-edge therapies tailored to their specific needs, ultimately improving outcomes and quality of life for individuals battling intraocular malignancies. Furthermore, the expertise and proficiency of these specialized retinoblastoma treatment institutes in ophthalmic artery infusion chemotherapy allow for a multidisciplinary approach to patient care. Collaborating closely with ophthalmologists, oncologists, radiologists, and other healthcare professionals, they develop personalized treatment plans that integrate this advanced technique with other modalities such as surgery, radiation therapy, and systemic chemotherapy when necessary. This comprehensive approach ensures that patients receive optimal care tailored to their individual condition, maximizing the chances of tumor control and preserving vision whenever possible. By offering state-of-the-art treatments like ophthalmic artery infusion chemotherapy in conjunction with expert medical care, these institutes play a vital role in improving outcomes and quality of life for patients with intraocular malignancies [56].

2.9.4.2 Intravitreal Chemotherapy

Intravitreal chemotherapy delivers anticancer drugs directly into the vitreous humor, a gel-like fluid within the eye, to target and treat cancer cells. Physicians recommend this approach for cases of metastatic disease in the vitreous humor that have not responded to previous treatments or have recurred after prior therapy. This method allows for precise delivery of chemotherapy drugs to the affected area, enhancing treatment efficacy while minimizing systemic side effects. Intravitreal chemotherapy offers a targeted approach to managing intraocular malignancies, providing patients with a potentially effective treatment option in challenging cases. This localized treatment method plays a crucial role in managing intraocular malignancies by directly targeting cancer cells within the eye. By delivering chemotherapy drugs directly into the vitreous humor, intravitreal chemotherapy maximizes the concentration of anticancer agents at the tumor site while minimizing systemic exposure and associated side effects. This approach is particularly beneficial for cases of metastatic disease in the vitreous humor that have not responded to conventional therapies or have recurred following prior treatment. Through its targeted and precise delivery mechanism, intravitreal chemotherapy offers a promising treatment option for patients facing challenging intraocular malignancies, ultimately contributing to improved outcomes and quality of life [57].

2.9.4.3 Intrathecal Chemotherapy

Intrathecal chemotherapy refers to the direct introduction of anticancer medications into the cerebrospinal fluid (CSF). This treatment addresses metastatic cancer that has metastasized to the brain [58].

2.9.5 Radiotherapy

Radiation therapy is a very successful cancer treatment that uses high-energy x-rays or other forms of radiation to destroy cancerous cells or prevent their growth [59].

There are two separate modes of radiation treatment that may be utilized:

2.9.5.1 External-Beam Radiation Treatment

External-beam radiation treatment use an external apparatus to precisely target and administer radiation to the exact area of the body afflicted by cancer . Due to the significant risk of sarcoma in the irradiated area and the possibility of local consequences, external beam radiation treatment (EBRT) is currently utilised less often. Based on a very wide series, a team in New York estimated the incidence of second cancer to be 51% at 50 years, both within and outside the irradiated region. Treatment-related variations in this incidence are observed: 3.4% in children who are completely enucleated without receiving chemotherapy or radiation therapy; 3% in patients receiving systemic chemotherapy; and up to 20% in patients receiving EBRT (typically for sarcomas in the irradiated region). In this investigation, the incidence of recurrent malignancies was not increased by brachytherapy or systemic chemotherapy in the absence of external beam radiation. When brachytherapy, intravitreal chemotherapy, and other salvage therapies are available, radiotherapy is seldom ever employed; but, in nations where this treatment option is the only one accessible, it is still used. According to a recent research, 35x% of people get cancer again 40 years after being diagnosed with retinoblastoma [60].

Applying precise methods of delivering radiation treatment may successfully reduce the likelihood of radiation-induced damage to nearby healthy tissue.

2.9.5.2 Intensity-modulated Radiation Therapy

Intensity-modulated radiation therapy (IMRT) is an advanced external radiation treatment approach that uses computer-generated imagery to accurately determine the size and form of the tumour in three dimensions (3-D). The tumour is targeted with several beams of different intensities from various angles to provide the most effective administration of therapeutic dosages while minimising damage

to nearby healthy tissue. Usually, inverse planning is used, which enables a computer to recommend a plan based on predefined target dosage parameters that the attending physician deems clinically appropriate. One possible disadvantage of IMRT is that, as dosage to target volumes grow more conformal, misdelineating the target or normal structures may result in partial or total misses. As such, precise target volume and important normal structure delineation is essential for IMRT. Dosimetric studies of IMRT for NPC have shown better tumor coverage and normal tissue sparing; however, clinical research demonstrating doctors' capacity to accomplish this consistently are still in their infancy. Utilizing IMRT, recent clinical investigations—two of which were phase III trials—have shown that salivary function is better protected. For NPC, IMRT has emerged as the preferred method of administering radiation treatment, pending more research. As a result, this article examines current developments in IMRT-based NPC therapy [61].

2.9.5.3 Proton Beam Radiation Therapy

Proton beam radiation therapy is an advanced kind of external radiation treatment that uses high-energy streams of positively charged particles, known as protons, to eliminate cancerous cells. Proton beam radiation therapy can reduce the extent of damage to adjacent healthy tissue near a tumour caused by radiation exposure [62]. Internal radiation treatment is a medical process that involves the insertion of radioactive chemicals near or directly into malignant cells using needles, seeds, wires, or catheters. Utilizing specific approaches in the administration of radiation might greatly decrease the probability of causing damage to nearby healthy tissue [63].

2.9.5.4 Internal Radiation Therapy

Plaque radiation involves the attachment of radioactive seeds to a disc, called a plaque, which is then placed directly on the outer surface of the eye in close proximity to the tumour. Orienting the side of the plaque that holds the seeds

towards the eyeball focuses radiation on the cancer. The plaque functions as a safeguarding barrier that safeguards adjacent tissue from radiation exposure [64].

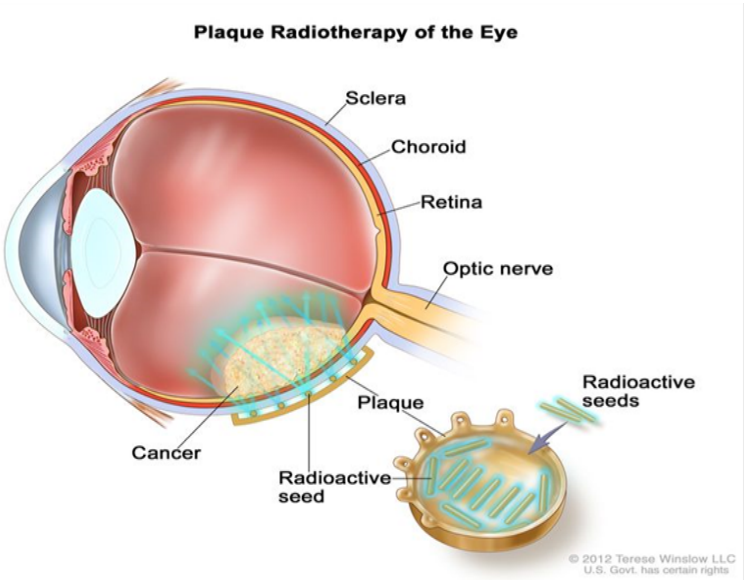

FIGURE 2.1: Plaque Radiotherapy of the eye, a therapy used to treat eye tumours

2.9.5.5 Intensive Chemotherapy Treatment Followed by the Transplantation of Stem Cells

Chemotherapy eliminates cancer cells by delivering high concentrations of medication. However, it also targets normal cells, including hematopoietic cells responsible for blood production. Stem cell rescue aims to replace these cells. Hematopoietic stem cells, found in bone marrow or blood, are extracted from the patient and stored at low temperatures for future use. After completing chemotherapy, healthcare professionals thaw and administer these cryopreserved stem cells intravenously. The reintroduced stem cells multiply and renew, replenishing the body's blood cells. To treat extraorbital disease effectively, patients undergo intensive chemotherapy followed by autologous stem cell transplantation and consolidation with high-dose chemotherapy. Depending on specific conditions, radiation treatment may also be considered. This comprehensive treatment approach involves rigorous chemotherapy to eradicate cancer cells, followed by autologous stem cell transplantation and consolidation with high-dose chemotherapy to ensure thorough treatment. Depending on the individual patient's condition and response to

treatment, radiation therapy may also be incorporated into the treatment plan. By utilizing this multifaceted approach, healthcare professionals aim to maximize the effectiveness of therapy while minimizing the risk of cancer recurrence, ultimately improving the patient's prognosis and quality of life [65].

2.9.5.6 Enucleation Surgery

One of the surgical procedures known as enucleation includes the removal of a piece of the eye as well as the optic nerve. A skilled pathologist who is knowledgeable about retinoblastoma and other eye illnesses will examine a specimen of ocular tissue taken through a microscope to identify any signs of cancer spreading to other parts of the body.

It is recommended that the assignment be carried out by a competent pathologist who is knowledgeable about retinoblastoma as well as other eye illnesses. In situations where the tumour is large, does not respond to therapy, or returns after having been treated in the past, the surgeon will conduct enucleation with the expectation that there will be a limited chance of retaining eyesight. One of the procedures that will take place is the implantation of a prosthetic eye in the patient.

A minimum observation time of two years is required to evaluate the unaffected eye and identify any disease recurrence in the damaged area around the eye [66].

2.10 Evaluation of Novel Therapeutic Modalities in Clinical Studies

An overview of the treatments that are presently being studied in clinical trials can be found in the section that follows. It might not include all of the current research being done on cutting-edge therapies.

2.10.1 Targeted Therapy

Targeted therapy is a kind of cancer treatment that involves the identification and precise targeting of particular cancer cells using drugs or other substances. Targeted treatments often inflict less damage on healthy cells in comparison to radiation or chemotherapy. Due to the successes of multi-omics technologies like Next-Generation Sequencing (NGS) and proteomics, our understanding of the molecular composition of retinoblastoma has recently advanced, offering us a great variety of possible targets for targeted therapy. Many studies are being conducted to investigate different small molecule inhibitors for the goal of targeted treatment against retinoblastoma, since these targets show great therapeutic potential. Nevertheless, the successful integration of these drugs into clinical practice continues to be a persistent problem. Targeted therapies are still undergoing clinical trials, but the results so far have been inadequate, underscoring the need for more study to close the gap between these treatments' preclinical and clinical effectiveness [67].

2.10.2 Oncolytic Virus Therapy

It's a therapy that uses a virus to target cancer cells and destroy them while leaving healthy ones intact. Therapeutic effects against retinoblastoma may result from direct administration of an adenovirus unique to the tumour that targets the RB1 gene. Researchers are actively studying the application of targeted therapy to treat advanced or recurrent retinoblastoma. Over the past ten years, (pre)clinical research has been developing and evaluating vectors based on adenovirus, herpes simplex virus, and vaccinia virus, revealing obstacles to the field's progress. In this article, we present a set of selection criteria or ideal characteristics for an effective oncolytic virus. These criteria include genomic stability, low seroprevalence, lack of pathogenicity, and selectivity in infection and replication. Utilizing these prerequisites, we assess the current state of oncolytic viruses and identify the vesicular stomatitis virus as a potentially ideal species for platform development [68].

2.11 Adverse Effects of Treatment of Retinoblastoma

The long-term adverse effects that follow cancer treatment and last for several months or even years are referred to as late effects. The following are potential long-term effects of treatment for retinoblastoma:

1. Physical disabilities such as limitations in vision or hearing [69]
2. The shape and size of the surrounding bone may change after the eye is removed until a prosthetic eye is inserted. The likelihood of this happening is higher in younger children (under 3) [70].
3. Changes in memory, mood, emotions, thought process, or knowledge gain [71]
4. Secondary cancers can arise from other malignancies such as soft tissue sarcoma, melanoma, lung and bladder tumours, or osteosarcoma [72].

The following risk factors could increase the chance of getting a second cancer:

a Having the retinoblastoma genetic variation that is heritable from generation to generation [73]

b Preceding radiation therapy administration, especially before the patient turned one year old [74]

c Having had a secondary cancer in the past [75]

2.12 Treatment of Progressive

Late effects are the enduring and detrimental consequences that occur after cancer therapy and last for an extended period, ranging from months to years. The following are plausible enduring consequences of retinoblastoma treatment:

1. Physical disabilities, such as visual or auditory impairments [69].

2. The morphology and dimensions of the adjacent bone may undergo alterations from the removal of the eye until the insertion of a prosthetic eye. The probability of this occurrence is greater among children who are under the age of 3 [70].

3. Alterations in memory, mood, emotions, cognitive processes, or acquisition of information [71].

4. Secondary cancers may develop as a result of other malignant conditions, including soft tissue sarcoma, melanoma, lung and bladder tumours, or osteosarcoma [72].

The following risk factors may elevate the likelihood of developing a secondary malignancy:

a Researchers have shown the presence of the heritable retinoblastoma genetic variant over several generations [73].

b Prior to administering radiation treatment, particularly in children under the age of one [74]

c Previously had secondary cancer [75]

2.12.1 High Dose Chemotherapy

For the purpose of destroying cancer cells, chemotherapy is administered in high quantities. In addition, the cancer treatment results in the elimination of healthy cells, including those that are accountable for the formation of blood. It is possible to replenish blood-forming cells via a process known as stem cell rescue. In order to get hematopoietic stem cells, which are blood cells that have not yet matured, the process includes collecting them from the patient's bone marrow or blood and then cryopreserving them for use in the future. Defrosting and reintroducing the cryopreserved stem cells into the patient is the next step that the medical team

takes once the treatment has been completed. As a result of these reintroduced stem cells, the blood cells of the body experience multiplication, which also helps the blood cells recover. In the case of extra-orbital illness, intensified chemotherapy is necessary. Furthermore, the treatment of this condition may require radiation therapy, autologous stem cell transplantation, and consolidation with high-dose chemotherapy [76].

2.12.2 Surgery (Enucleation)

One of the surgical procedures known as enucleation includes the removal of the eye as well as a portion of the optic nerve. A competent pathologist knowledgeable about retinoblastoma and other visual issues will examine the removed eye tissue microscopically to detect any indications of metastasis to other anatomical locations. It is recommended that this treatment be carried out by a competent pathologist who is also knowledgeable about retinoblastoma and other visual issues. Pathologists who are skilled in the art of enucleation do the procedure when the tumour is enormous, does not respond to treatment, or returns after treatment, and there is little to no chance of maintaining eyesight. In order to provide the patient with a prosthetic eye, the surgeon will implant it. It is essential to do routine monitoring of the damaged eye for a period of at least two years in order to identify any indications of a recurrence. Additionally, it is essential to do an examination of the second eye of the patient [66].

2.13 Treatment of Unilateral, Bilateral, and Cavitary Retinoblastoma

If it is likely that the eye can be saved, treatment may include cryotherapy, thermotherapy,plaque radiotherapy, systemic or opthalmic artery infusion chemotherapy [51, 52, 64]. Physicians use external-beam radiation therapy to treat bilateral intraocular retinoblastoma that does not respond to previous therapies [60].

If the tumour is of significant size and the preservation of the eye is improbable, the therapy may include the following measures:

2.13.1 Surgery (Enucleation)

Physicians may administer systemic chemotherapy after the operation to reduce the likelihood of the disease spreading to other areas of the body [67]. When both eyes are affected by retinoblastoma, the treatment administered to each eye may be different depending on the size of the tumour and the possibility that the eye will be preserved. The eye with a larger disease load often determines the dose of systemic chemotherapy [54]. Individuals who have been diagnosed with cavitary retinoblastoma have a favourable response to treatment and have great long-term outcomes, which are equivalent to those who have been diagnosed with noncavitary retinoblastoma. One of the surgical procedures known as enucleation includes the removal of the eye as well as a portion of the optic nerve. A competent pathologist who is knowledgeable about retinoblastoma and other visual issues will examine the removed eye tissue under a microscope to detect any signs of metastasis and its spread to other anatomical sites. A competent pathologist who is knowledgeable about retinoblastoma and other visual issues should carry out this treatment. When the tumour is big, does not respond to treatment, or returns after treatment, and there is little to no chance of maintaining vision, the pathologist should recommend enucleation as the treatment of choice. In order to provide the patient with a prosthetic eye, the surgeon will implant it. During the first two years after the injury, the surgeon should carefully monitor the damaged eye for any indications of a recurrence and also examine the other eye [66].

2.14 Treatment of Extraocular Retinoblastoma

Possible interventions for extraocular retinoblastoma, which refers to the spread of malignancy to the surrounding eye region, may include the following:

- The treatment includes the administration of chemotherapy throughout the body and the use of external-beam radiation therapy [53, 60].
- Enucleation [66]
- Following surgery, systemic chemotherapy, enucleation, external-beam radiation treatment, and further chemotherapy may be administered [53, 60, 66]. Possible interventions for extraocular retinoblastoma, when the disease has metastasized to the brain, may include the following:
- Administration of chemotherapy either systemically or intrathecally, as well as external-beam radiation treatment targeting the brain and spinal cord [53, 58].
- Administration of chemotherapy, followed by the administration of high-dose chemotherapy together with stem cell rescue, with or without the addition of radiation treatment [76].
- The efficacy of chemotherapy, radiation therapy, or high-dose chemotherapy plus stem cell rescue in prolonging the survival of patients with extraocular retinoblastoma remains uncertain [58, 68].

2.15 Some Drugs of Rb

The treatment of retinoblastoma typically involves the use of Vincristine (Oncovin, Vincasar PFS), carboplatin (Paraplatin), and etoposide (Toposar, VePesid). When the size of the tumour is taken into consideration, it may be recommended to combine two or more drugs. Every kind of treatment, including chemotherapy, is associated with a broad variety of adverse effects that might occur over the course of treatment. Additionally, a number of drugs have the potential to cause unpleasant side effects that continue to manifest themselves for a considerable amount of time.

Studies conducted in preclinical settings have shown that cyclosporine has a key role in the treatment of retinoblastoma tumours. Actinomycin-D, on the other

hand, has a lower e-total value, which indicates that it binds to Rb more effectively. This indicates that the relationships between the two groups are more intimate. As a consequence of this, specialists advise their patients to consider the medicine as a possible treatment option for retinoblastoma and other tumours that are associated with it [77].

Chapter 3

Material and Methods

3.1 Study Design

Retrospective cross-sectional analysis of patients with new RB diagnoses at Al Shifa Trust Eye Hospital, Rawalpindi between 1stJanuary, 2021 and 31st December, 2023 was the focus of the study.

3.2 Study Settings

This research was conducted at Al Shifa Trust Eye Hospital, Rawalpindi. Al Shifa Trust Eye hospital is the biggest eye hospital in this region. It offers complete ophthalmology services, including retinoblastoma diagnosis and medicinal and surgical management. In the northern regions of the nation, it is the primary RB treatment facility in Rawalpindi and Islamabad. Majority of RB patients of this region and nearby areas are usually referred to and seek treatment at this hospital.

3.3 Study Population

The study subjects included in first part of the study to find out incidence of retinoblastoma in Islamabad and Rawalpindi are permanent residents of Islamabad and Rawalpindi. The subjects comprised of both sexes. Total 51 subjects were included in this part of the study.

In second part of the study a well prepared questionnaire was used. It had seven closed-ended questions. A total of 36 subjects participated in this part of the study. These participants belonged to different parts of the country.

The research consisted of three steps:

3.3.1 Step 1

Information of some demographic and clinic features of retinoblastoma patients such as age, date of diagnosis, gender, region and socioeconomic status, was obtained from hospital electronic records.

3.3.2 Step 2

A questionnaire about screening and genetic testing in retinoblastoma was used to assess the parent knowledge. Face-to-face interviews of parents of retinoblastoma patients were carried out by me. The survey began with a brief introduction that focused on using the respondents' present information to answer each question rather than speculating. Following every interview, socioeconomic details such as household income, education, and health insurance were gathered, and all clinical data were taken directly from the electronic medical files.

3.3.3 Step 3

Each subject included in this study was given explanation about the objectives and purpose of the study. All subjects participated on voluntary basis. Identity and any personal information obtained or gathered were kept confidential.

3.4 Method of Data Collection

3.4.1 Duration of the Study

The study was carried out for the period of 4 and a half month from September 2023 to the mid of January 2024.

3.4.2 Collection of Data

The departmental records of every patient who visited the paediatric ophthalmology of Al Shifa Trust Eye Hospital, Rawalpindi was reviewed in order to identify any new RB patients who present from 1^{st} January, 2021 to 31^{st} December, 2023. Age, date of diagnosis, sex, region, laterality, and socioeconomic status was all taken from each patient's medical history.

Also, a seven-item simplified English questionnaire on screening children was used to check parental knowledge. The survey consisted of 7 closed-ended questions and covered the key facets of genetic testing and retinoblastoma screening. In order to discourage guessing, each question contained three possible answers: "yes", "no" and an additional "I don't know" choice (in order to avoid guessing). The following were the evaluation standards for each query: 1 for a correct response, 0 for "I don't know," and -1 for an improper response. The interviewee's level of knowledge is reflected by the sum of all of the questions' scores. Higher ratings indicate stronger parent knowledge, with possible sum values ranging from -7 to 7. I performed face-to-face interviews with the participants. Prior to the survey, a succinct introduction was given, with a focus on answering each question based on

the respondents' existing understanding rather than speculating. Following each interview, socioeconomic data such as household income, education level, and health insurance were gathered, and all clinical information was obtained from the electronic medical records.

3.4.3 Inclusion criteria

Patients diagnosed for retinoblastoma during 1^{st} January 2021 and 31^{st} December 2023 and parents of those patients who were diagnosed during study duration were included.

3.4.4 Exclusion criteria

Retinoblastoma patients of all other regions and the parents of those being hospitilized and treated before and after study duration were excluded.

3.5 Study Variables

- Age at diagnosis (crucial for calculating incidence rates)
- Sex
- Race/Ethnicity
- Geographic location (urban vs. rural, developed vs. developing country)
- Socioeconomic status
- Laterality (unilateral vs. bilateral involvement)

3.6 Statistical Analysis

Analysis of data was performed by using SPSS version 23. The results obtained were explained in simple frequencies. The variations were assessed using independent T test for statistical significance. The level of significance was considered as P value less than 0.05.

1. At first I explored frequency distribution of demographic and clinical features of the subjects.

2. Described statistics had been used to summarize and present information in the form of mean, frequencies, percentages and tables.
3. Independent T test was used to calculate statistical significance.

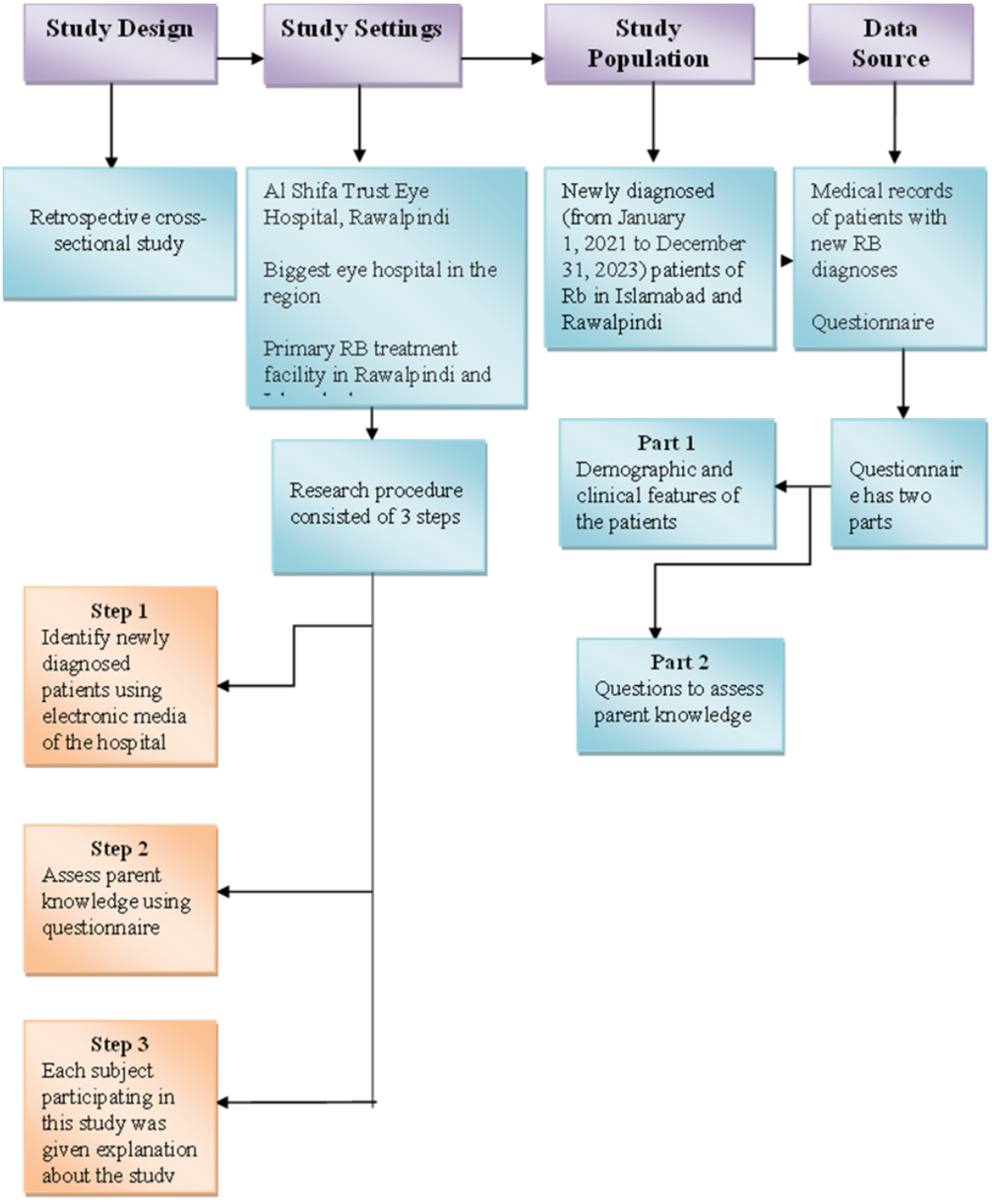

FIGURE 3.1: Flowchart for methodology

Chapter 4

Results

Incidence is a measure of the number of new cases of a characteristic that develop in a population in a specified time period [80]. Incidence rate can be calculated by the following formula:

$$Incidence\ rate = \frac{Number\ of\ new\ cases}{Population\ size\ \times\ Timeframe\ (Years)}$$

The population of Islamabad (2023) is 2.36 million (or 2,363,863) [79]. 5 August 2023 and the population of Rawalpindi is estimated to be 2.43 million (or 2,430,788) [80].

The total population of both $cities = 2,363,863 + 2,430,788 = 4,794,651$

$$Incidence\ rate = \frac{51}{4,794,651\ \times\ 3}$$

$$= \frac{51}{14,383,953}$$

$$= 0.0000035$$

$$Incidence\ rate = 3.5\ per\ 100,000$$

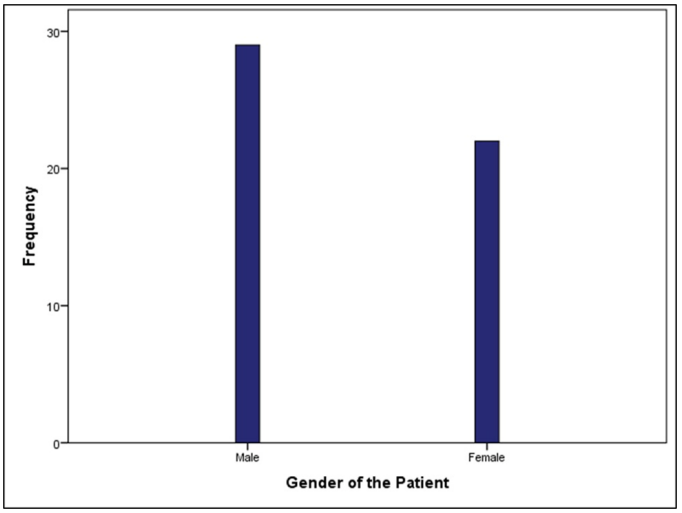

FIGURE 4.1: Gender of Patients

Out of 51 retinoblastoma patients, 29 patients were male and 22 patients were female. P value for gender was calculated to be 0.19 which is insignificant. Retinoblastoma incidence is not affected by the gender.

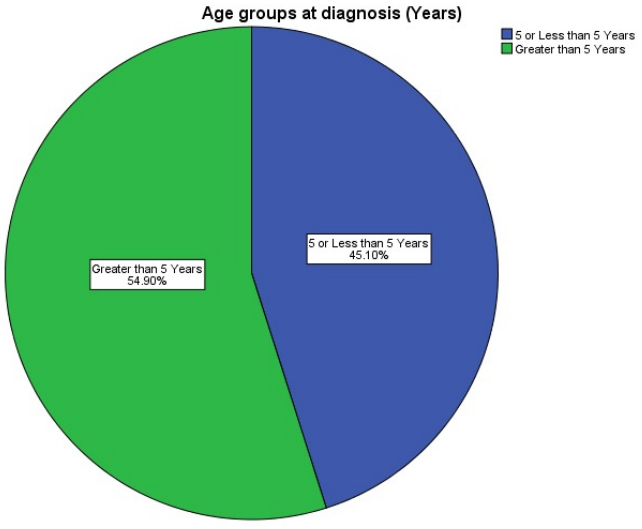

FIGURE 4.2: Age of Patients at diagnosis in months

Patients of retinoblastoma belonged to different age groups. Out of 51 patients 24 (47.06%) patients were under 5 or 5 and 27 (52.94%) patients were above 5. Mean age at diagnosis ranged from 2 months to 900 months. Unilateral patients had greater mean age at both diagnosis and survey than bilateral ones but it was not significantly higher (at diagnosis: 164.1 months vs. 109.2 months, p = .27).

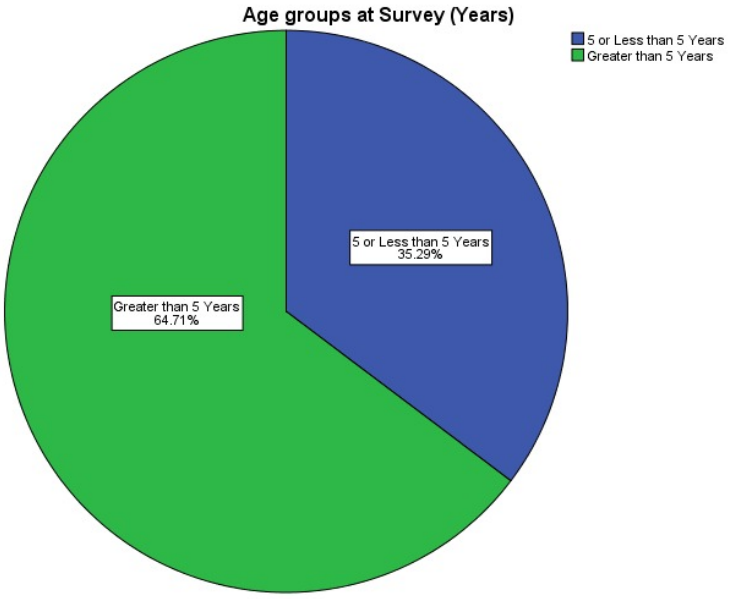

FIGURE 4.3: Age of Patients at survey in months

At survey the mean age ranged from 2 months to 929 months. Unilateral patients had greater mean age at both diagnosis and survey than bilateral ones but it was not significantly higher (175.2 months vs. 120.4 months, p= .26).

Out of these 51 patients, 13 patients belong to Rawalpindi and 38 patients belong to Islamabad.

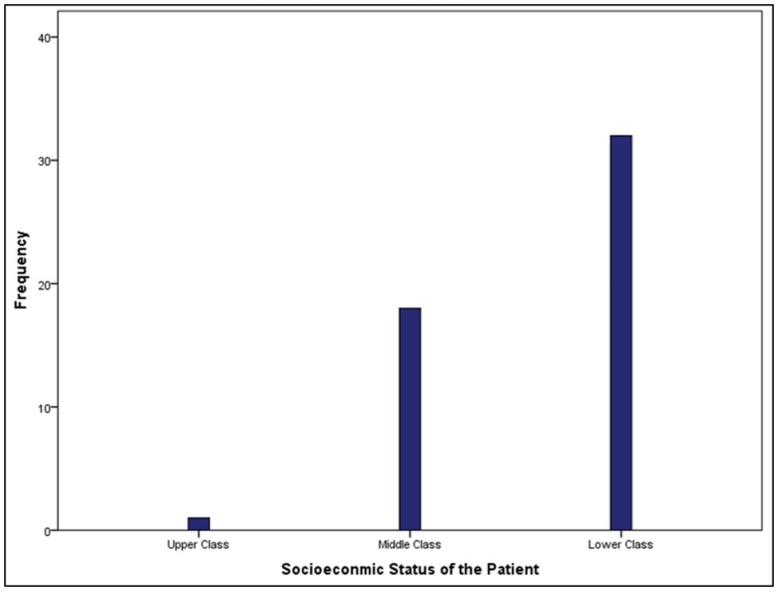

FIGURE 4.4: Region of Patients

Most of the patients (32 out of 51) belonged to low socioeconomic status, 18 belonged to middle class and 1 belonged to upper class. Parents with high and middle socioeconomic status had more knowledge as compared to those with low socioeconomic status.

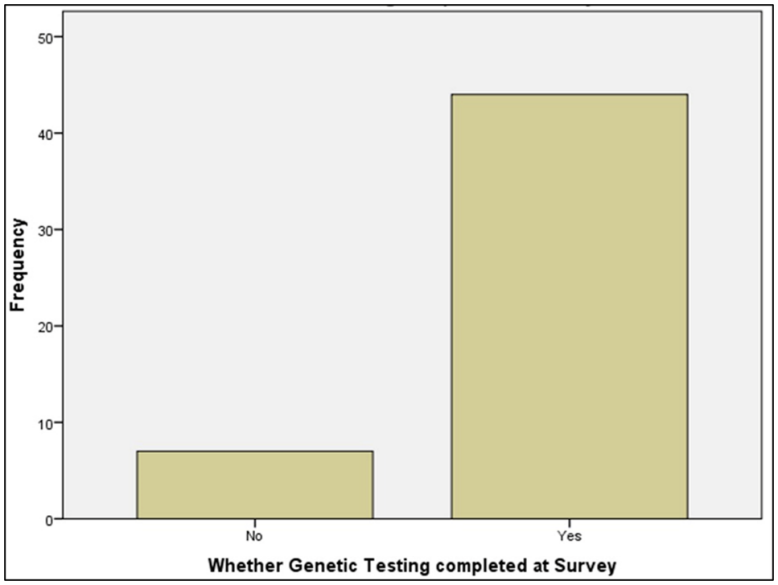

FIGURE 4.5: Genetic Testing

Most of the patients had completed genetic testing at the time of survey. Genetic testing can be more cost effective and feasible than screening programs in case of diseases with low incidence rate such as retinoblastoma. Genetic testing can be helpful to screen children with higher risk of developing retinoblastoma.

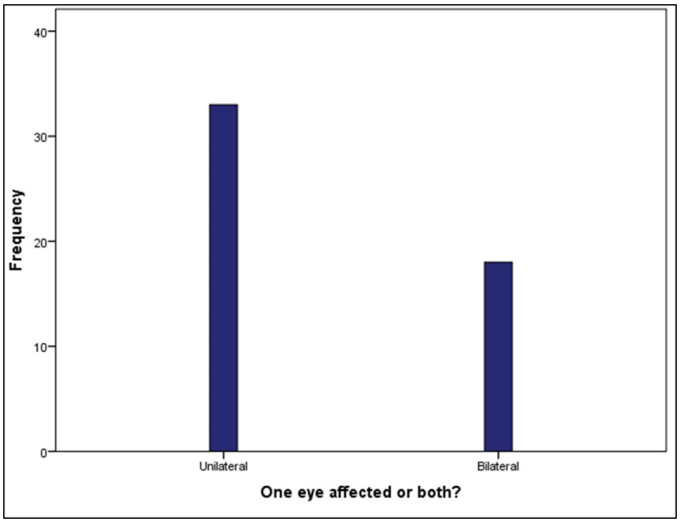

FIGURE 4.6: Organ Affected

33 patients, out of 51, had unilateral retinoblastoma while 18 patients had bilateral retinoblastoma. Out of 33 patients having unilateral retinoblastoma 25 (75.75%) were male and 8 (24.24%) were female.

TABLE 4.1: Demographic and clinical features of retinoblastoma patients by laterality

	Laterality Category			
	All (N=51)	**Unilateral (n=33)**	**Bilateral (n=18)**	**p value**
Age at diagnosis (M)	113.39	126.67	89.06	.46
Age at survey (M)	129.41	142.64	105.17	.47
Genetic Testing Completed at survey (n,%)	-	-	-	.69
Yes	44 (86.3)	28 (84.84)	16 (88.89)	-
No	7 (13.7)	5 (15.15)	2 (11.11)	-
Gender (n,%)	-	-	-	.19
Male	33 (56.9)	25 (75.75)	8 (44.44)	-
Female	18 (43.1)	8 (24.24)	10 (55.55)	-

A total of 51 patients were diagnosed with retinoblastoma in Islamabad and Rawalpindi from 1st January 2021 to 31st December 2023. Out of these 51 patients, 33 were males and 18 were females. Overall mean age at diagnosis ranged from 2 months to 900 months and at survey it ranged from 2 months to 929 months. 24 patients aged 5 or under 5 while 27 patients were above the age of 5.

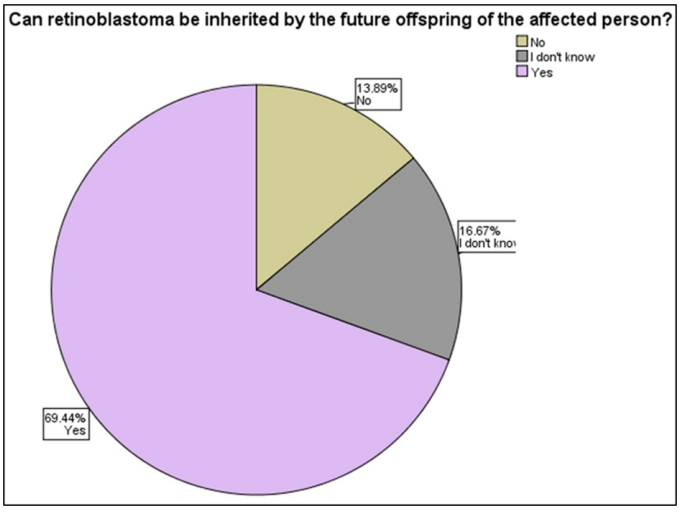

FIGURE 4.7

Most of the participants answered "Yes" to the question "Can retinoblastoma be inherited by the future offspring of the affected person?" Only 13.89% participants

answered "No" and 16.67% participants answered "I don't know". The correct answer for this question was "Yes". 25 (69.44%) out of 36 participants correctly answered the first question. 5 participants answered incorrectly.

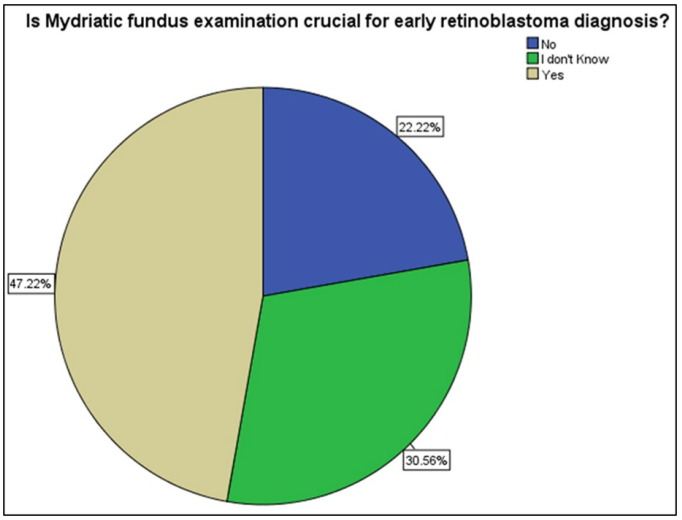

FIGURE 4.8

The second question in the questionnaire was "Is mydriatric fundus examination crucial for early retinoblastoma for early retinoblastoma detection?" Participants were given explanation about mydriatic fundus. The correct answer for this question was "Yes". A total of 17 (47.1%) participants answered this question correctly. 11 (30.56%) participants replied "I don't know" and 8 (22.22%) participants answered "No".

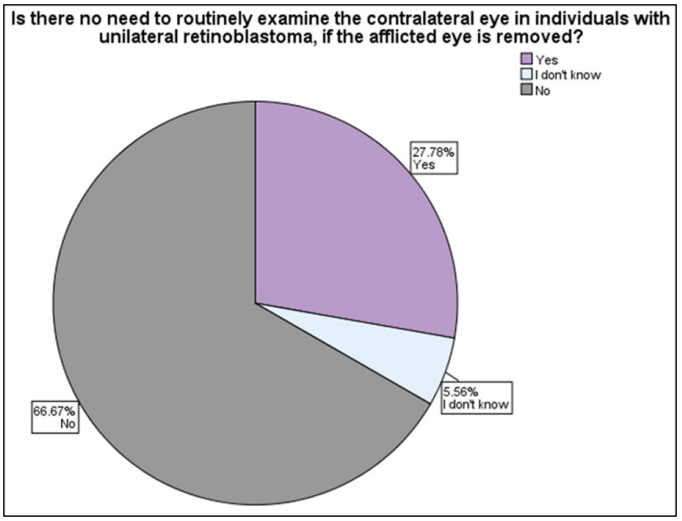

FIGURE 4.9

Next question was "Is there no need to routinely examine the contralateral eye in individuals with unilateral retinoblastoma, if the afflicted eye is removed?" Parents were given explanation about the question. The correct answer for this question was "Yes". 24 out of 51 (66.7%) patients correctly answered this question. Only 2 (5.56%) respondents responded "I don't know".

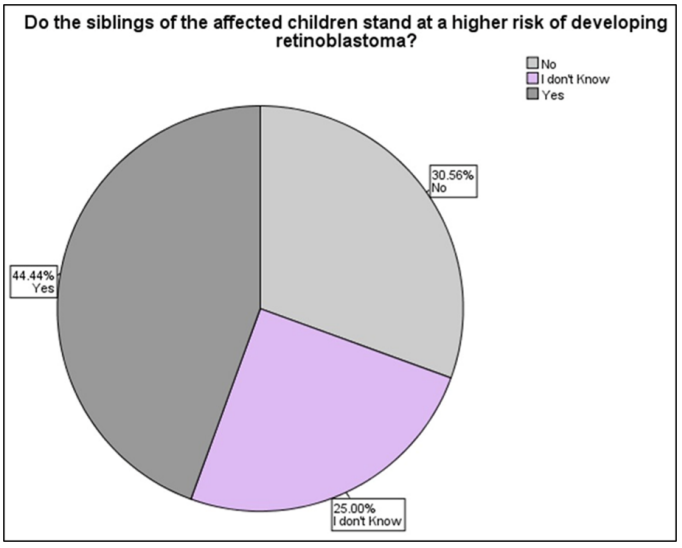

FIGURE 4.10

Fourth item in the questionnaire was "Do the siblings of the affected children stand at a higher risk of developing retinoblastoma?" The correct answer for this question was "Yes". 16 out of 36 (44.4%) respondents responded correctly. 11 respondents responded "No" while 9 respondents responded "I don't know".

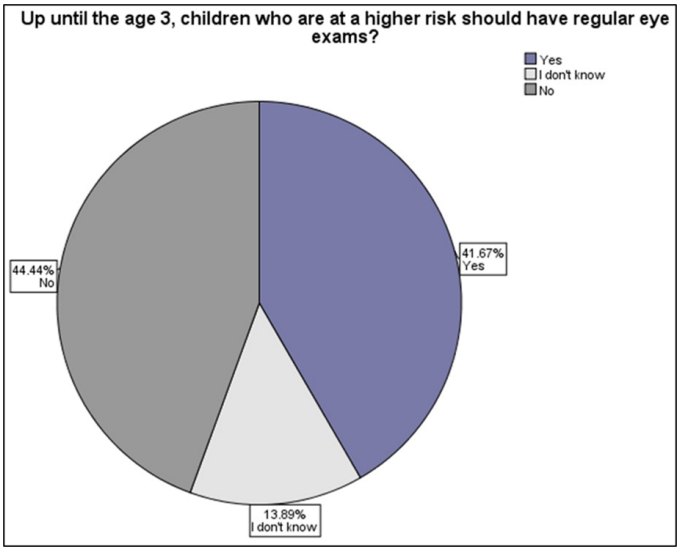

FIGURE 4.11

Next questionnaire item was "Up until the age of 3, children who are at a higher risk should have regular eye exams." Its correct answer was "No". 16 parents responded correctly. 15 (41.67) respondents answered "Yes" while 5 (13.89) responded "I don't know".

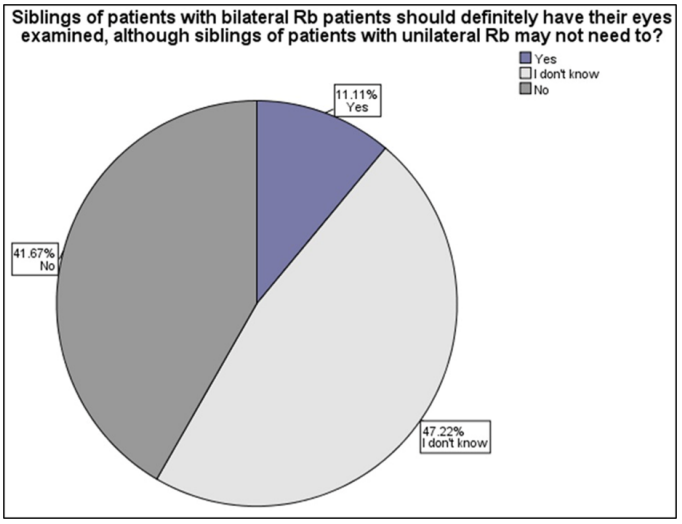

FIGURE 4.12

In the questionnaire the sixth question was "Siblings of patients with bilateral Rb patients should definitely have their eyes examined, although siblings of patients with unilateral Rb may not need to?" The correct answer for this question was "No". Out of 36, 15 (41.67%) participants answered this question correctly. 4 (11.11%) responded "Yes" while rest of the 17 (47.22%) participants responded "I don't know" to this question.

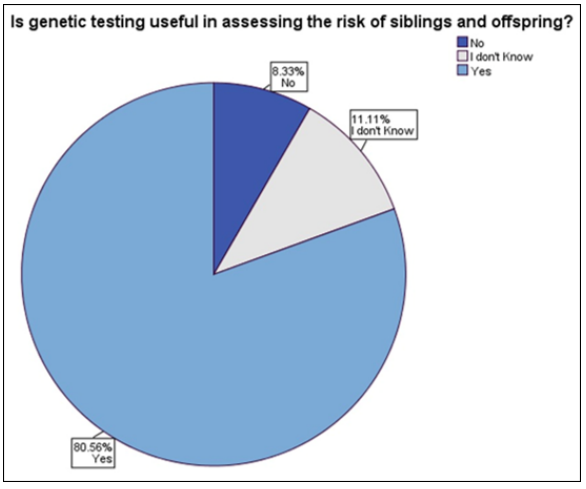

FIGURE 4.13

The seventh and last question was "Is genetic testing useful in assessing the risk of siblings and offspring?" and the correct answer was "Yes". 29 (80.56%) participants responded "Yes" to this question. 3 (8.33%) responded "No" while 4 (11.11%) participants responded "I don't know" to this question.

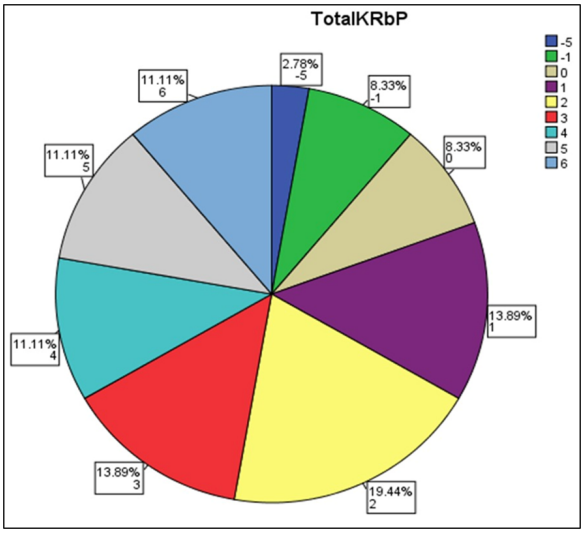

FIGURE 4.14

For every single item in the questionnaire, 41.4% to 80.5% of all parents accurately responded each question. The last question was correctly answered by most parents (80.5%), "Is genetic testing useful in assessing the risk of siblings and offspring?" The median total score of all respondents was 2 (range: -5-6). 11.11% participants scored 6, the maximum score. None of the parents correctly answered all 7 questions.

TABLE 4.2: Demographic & clinical features of retinoblastoma patients by laterality

	Laterality Category			
	All (N=73)	**Unilateral (n=47)**	**Bilateral (n=26)**	**p value**
Age at diagnosis (M)	100.05	106.4	88.58	0.63
Age at survey (M)	111.14	117.45	99.73	0.64
Genetic Testing Completed at survey (n, %)				0.32
Yes	51 (69.9)	31 (65.9)	20 (76.9)	
No	22 (30.1)	16 (34.1)	6 (23.1)	
Gender (n, %)				0.18
Male	44 (60.3)	31 (65.9)	13 (50)	
Female	29 (39.7)	16 (34.1)	13 (50)	

A total of 73 patients were enrolled during the study period. As for the patients, 47 (23.0%) were bilaterally affected, and 44 (60.3%) were males. Overall mean

age at diagnosis ranged from 2 months to 900 months and at survey it ranged from 2 months to 929 months. Unilateral patients had greater mean age at both diagnosis and survey than bilateral ones but it was not significantly higher (at diagnosis: 106.4 months vs. 88.58 months, p = .63; at survey: 117.45 months vs. 99.73 months, p= .64). More than half of the bilateral (65.9%) as well unilateral patients (76.9)% had completed genetic testing prior to our survey. No significant difference was found in gender distribution by laterality (p=.64).

Table 4.3: Knowledge of retinoblastoma among parents

Questions		Answers (n)			Correctly answered(n, %)
		Yes	No	Do not know	
Q1	Can retinoblastoma be inherited by the future offspring of the affected person?	25	5	6	25 (69.4)
Q2	Is Mydriatic fundus examination crucial for early retinoblastoma diagnosis?	17	8	11	17 (47.2)
Q3	Is there no need to routinely examine the contralateral eye in individuals with unilateral retinoblastoma, if the afflicted eye is removed?	10	24	2	24 (66.7)
Q4	Do the siblings of the affected children stand at a higher risk of developing retinoblastoma?	16	11	9	16 (44.4)
Q5	Up until the age of 3, children who are at a higher risk should have regular eye exams.	15	16	5	16 (44.4)
Q6	Siblings of patients with bilateral Rb patients should definitely have their eyes examined, although siblings of patients with unilateral Rb may not need to?	4	15	17	15 (41.67)
Q7	Is genetic testing useful in assessing the risk of siblings and offspring?	29	3	4	29 (80.5)

For every single item in the questionnaire, 41.4% to 80.5% of all parents accurately responded each question. The last question was correctly answered by most parents (80.5%), "Is genetic testing useful in assessing the risk of siblings and offspring?" The median total score of all respondents was 2 (range: -5-6). None of the parents correctly answered all 7 questions.

Chapter 5

Discussion

In general the incidence rate of retinoblastoma in my study was 3.5 patients per 100,000 in Islamabad and Rawalpindi, the twin cities of Pakistan. This study was conducted in Al Shifa Trust Eye Hospital, Rawalpindi, which is the biggest eye hospital in this region. This hospital offers many different treatment options for retinoblastoma including cryotherapy, laser therapy, plaque therapy. The hospital offers treatment in private and government categories as well as completely free treatment.

Previously, no study regarding the incidence of retinoblastoma has been conducted in these two cities. This is the first study on incidence rate of retinoblastoma in Islamabad and Rawalpindi. My study showed that the incidence rate of retinoblastoma is considerably higher in this region than rest of the world.

My study consisted of two parts. First part of the study included 51 newly diagnosed retinoblastoma patients of Islamabad and Rawalpindi. Patients diagnosed for retinoblastoma from 1st January 2021 to 31st December 2023 were identified and their medical history was viewed. Data regarding some of the demographic and clinical features of the retinoblastoma patients was obtained from the electronic media of Al Shifa Trust Eye Hospital, Rawalpindi.

Out of 51 newly diagnosed cases, 29 were male and 22 were female. The p value for gender was calculated as 0.19. It shows that the difference of gender was

insignificant with respect to the incidence of retinoblastoma. Both genders can be affected by retinoblastoma equally.

33 (64.7%) patients had unilateral retinoblastoma while 18 (35.29%) patients had bilateral retinoblastoma. Out of 33 patients having unilateral retinoblastoma 25 (75.75%) were male and 8 (24.24%) were female While globally retinoblastoma is unilateral in 60% of the cases and bilateral in 40% cases [6].

The patients included in this study belong to different age groups. Although retinoblastoma is usually referred to as childhood cancer, 24 (47.06%) patients included in this study were under 5 or 5 while 27 (52.94%) patients were above the age of 5. The mean age of the patients at diagnosis was calculated to be 113.9 months. The mean age was 126.67 months for unilateral and 89.06 for bilateral retinoblastoma patients. Value of significance was calculated as 0.46. Overall mean age of patients at survey was 129.41 months, 142.64 months for patients with unilateral retinoblastoma and 105.17 months for retinoblastoma patients with bilateral retinoblastoma. Value of significance was calculated to be 0.47.

Out of these 51 newly diagnosed retinoblastoma patients 13 patients belonged to Islamabad and rest of the 38 retinoblastoma patients belonged to Rawalpindi region. This difference in the number of cases between Islamabad and Rawalpindi can be attributed to the population difference. The population of Islamabad is 1.2 million [78] and the population of Rawalpindi is 2.37 million [79].

During the study period, a total of 73 patients were enrolled. As for the patients, 47 (23.0%) were bilaterally affected, and 44 (60.3%) were males. Overall mean age at diagnosis ranged from 2 months to 900 months and at survey it ranged from 2 months to 929 months. Unilateral patients had greater mean age at both diagnosis and survey than bilateral ones but it was not significantly higher (at diagnosis: 106.4 months vs. 88.58 months, $p = .63$; at survey: 117.45 months vs. 99.73 months, $p = .64$). More than half of the bilateral (65.9%) as well as unilateral patients (76.9) % have completed genetic testing prior to our survey. No significant difference was found in gender distribution by laterality ($p=.18$).

In the second part of the study, a total of 73 subjects did participate. In this part a questionnaire derived from *Xiao et. al 2020* [78] consisting of 7 close ended questions about genetic and clinical testing in retinoblastoma was used. 41.4% to 80.5% of parents correctly answered each and every question on the questionnaire. The majority of parents (80.5%) correctly answered the final question: "Is genetic testing useful in assessing the risk of siblings and offspring?" Across all responders, the median overall score was 2 (range: -5-6). Not a single parent gave a right response to all seven questions.

Chapter 6

Conclusion and Recommendations

6.1 Conclusion

Retinoblastoma is a rare cancer. Researches reveal that the global incidence of retinoblastoma is 1 patient per 100,000 individuals while a very limited data is available for incidence rate of retinoblastoma in Pakistan. So, it was necessary to study the incidence rate of retinoblastoma in Pakistan.

The present study was done in the direction to estimate the rate of incidence of retinoblastoma in Islamabad, the capital city of Pakistan and Rawalpindi. The study concluded that the incidence rate of retinoblastoma in Pakistan is remarkably higher than rest of the world. The study also concluded that the incidence of retinoblastoma in Islamabad and Rawalpindi, the twin cities of Pakistan is 3.5 patients per 100,000 individuals. Previously, incidence rate of retinoblastoma in Pakistan was reported only for Karachi (as 4 per 100,000).

In the first part of this study, the electronic medical record of ocular oncology department of Al Shifa Trust Eye Hospital, Rawalpindi, from 1st January 2021 to 31st December 2023, was explored. Medical histories of all the retinoblastoma

patients were viewed and the data was obtained for those patients who were permanent residents of Islamabad and Rawalpindi and were diagnosed for retinoblastoma from 1^{st} January 2021 to 31^{st} December 2023.

A total of 51 new cases of retinoblastoma were diagnosed from 1^{st} January 2021 to 31^{st} December 2023 in Islamabad and Rawalpindi. Out of these 51 new cases, 29 cases (56.86%) were male and 22 cases (43.14%) were female. 33 (64.7%) patients had unilateral retinoblastoma while 18 (35.29%) patients had bilateral retinoblastoma. 24 (47.06%) patients had ages under or equal to 5 while 27 (52.94%) patients were above the age of 5. A total of 20, 12 and 19 new cases of retinoblastoma were diagnosed in 2021, 2022 and 2023 respectively.

In the second part of this study, the knowledge of parents of retinoblastoma patients about genetic and clinical testing was assessed. For this purpose, a short and simple questionnaire consisting of 7 close ended questions about genetic and clinical testing in retinoblastoma was used. Retinoblastoma patients belonging to different parts of the country, including Islamabad and Rawalpindi, voluntarily participated in this part of the study. The parents of those patients who were diagnosed and hospitalized during the survey time were included. A total of 36 patients' parents participated in head-to-head question answer session. Overall parents' knowledge about genetic and clinical testing in retinoblastoma was low. Lack of awareness among the community can also be a factor of higher incidence rate of retinoblastoma in Pakistan. Parents belonging to middle and higher socioeconomic status had more knowledge as compared to those belonging to lower socioeconomic status.

6.2 Recommendations

1. Increasing public knowledge about factors and signs and symptoms of retino blastoma and the importance of early diagnosis can encourage prompt medical attention and timely intervention.

2. Continued funding and support for research efforts are crucial for advancing scientific understanding and developing effective strategies for prevention, diagnosis and treatment of retinoblastoma.

3. Implementing newborn screening programs utilizing genetic or ocular techniques could enable diagnosis at pre-symptomatic stages, leading to earlier intervention and improved prognosis.

4. Refining genetic testing methods for germline and somatic mutations in the RB1 gene can facilitate identification of carriers and families at risk.

5. Offering genetic counseling to families with a history of retinoblastoma or identified mutations can empower them with informed reproductive choices and risk management strategies.

6. Research into RB1 protein-specific inhibitors and other targeted therapies has the potential to combat retinoblastoma more effectively with fewer side effects, compared to conventional radiotherapy and chemotherapy.

7. Precision medicine approaches: Integrating genetic and molecular profiling of tumours could pave the way for personalized treatment plans tailored to individual tumour characteristics and vulnerabilities.

8. Exploring and refining minimally invasive techniques like laser therapy, cryo therapy, and brachytherapy might preserve vision and function while effectively controlling tumours.

9. While evidence is limited, studies exploring the role of prenatal exposures, maternal nutrition, or early childhood environmental factors could potentially offer preventive strategies or early warning signs.

10. Although no concrete causal links have been identified, promoting healthy lifestyle habits, including balanced diets and adequate sleep, might support overall health and potentially modulate genetic susceptibility.

11. By focusing on these avenues, the scientific community can strive to significantly reduce the burden of retinoblastoma for children and families worldwide, even if complete eradication remains a distant goal.

12. Incidence rate of retinoblastoma may vary with race or ethnicity. Therefore, studies regarding the role of race or ethnicity must be conducted.

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An Appendix

Questionnaire

I, Rafia Jabeen, am doing MS Biosciences at Capital University of Science and Technology, Islamabad. Currently, I am doing my research work. The purpose of my research is to study the incidence of retinoblastoma in Islamabad and Rawalpindi population and to know parent knowledge of screening and genetic testing in retinoblastoma.

I assure that all the information, collected by me, will only be used for research purpose and the identity of patients will be kept confidential.

Part A

Demographic and clinical features of retinoblastoma patients

Patient Name _____ **Diagnosis date** _____

1. **Laterality category**
   a) Unilateral
   b) Bilateral
2. **Age of the patient**
   a) Age at diagnosis (months)
   b) Age at survey (months)
3. **Genetic testing completed at survey**
   a) Yes
   b) No
4. **Gender (n, %)**
   a) Boy
   b) Girl
5. **Region**

   _____

6. **Socioeconomic status**

a) High
b) Middle
c) Low

Part B

Knowledge of retinoblastoma among parents

	Questions	**Yes**	**No**	I don't know
Q1.	**Can retinoblastoma be inherited by the future offspring of the affected person?**			
Q2.	**Is mydriatic fundus examination crucial for retinoblastoma early diagnosis?**			
Q3.	**Is there no need to routinely examine the contralateral eye in individuals with unilateral retinoblastoma, if the afflicted eye is removed?**			
Q4.	**Do the siblings of the affected children stand at a higher risk of developing retinoblastoma?**			
Q5.	**Up until the age of three, children who are at a higher risk should have regular eye exams.**			
Q6.	**Siblings of patients with bilateral Rb should definitely have their eyes examined, although siblings of patients with unilateral Rb may not need to.**			
Q7.	**Is genetic testing useful in assessing the risk of siblings and offspring?**			