INTRODUCTION

Retinoblastoma represents the most common cause of intraocular tumors in children, representing over 80% of cases under the age of 15 years ***(Balmer and Munier, 2001)***. It also represents a significant percentage of all childhood neoplasms, accounting for 2-4 % of cases in Europe, North America, Asia and Australia and 10-15% of total tumors in African children. Retinoblastoma has the lowest median age of all childhood malignancies, approximately 15 months ***(Yeole and Advani, 2002)***.

Sixty percent of retinoblastomas are unilateral; most of these forms are not hereditary and the median age at diagnosis is two years. However, 40% of retinoblastomas are bilateral with median age at diagnosis of one year. All bilateral and multifocal unilateral forms are hereditary ***(Aerts et al., 2006)***.

A 2-mutation hypothesis to explain the occurrence of retinoblastoma in both hereditary and sporadic forms has been proposed ***(Yeole and Advani, 2002)***. In the hereditary setting, mutation of the retinoblastoma gene (RB1 gene.) on chromosome 13q14 is present in all germ line cells; however it requires a mandatory second "hit" or mutation of the remaining allele for retinoblastoma to develop ***(Ray et al., 2012)***. The penetrance is 95%, as in approximately 5% of the mutation carriers, no second somatic mutation occurs in one of their retina cells during embryonic development ***(Scheffer et al.,***

2001). A subject constitutionally carrying an RB1 gene mutation is also at increased risk of developing other types of cancers ***(Aerts et al., 2006)***. The non-hereditary form arises from spontaneous mutation affecting both alleles of RB1 gene in a somatic cell of the retina ***(Ray et al., 2012)***. However, ***Mastrangelo et al. (2007)*** stated that this is a rather outdated view of cancer aetiology, and proposed that epigenetic factors and aneuploidy play central roles in the disease.

Management of patients with retinoblastoma must take into account the various aspects of the disease: the visual risk, the possibly hereditary nature, the life-threatening risk. Enucleation is still often necessary in unilateral disease; the decision for adjuvant treatment is taken according to the histological risk factors ***(Aerts et al., 2006)***, which include: optic nerve and deep choroidal invasion, orbital extension, and metastatic disease ***(Li et al., 2012)***. Conservative treatment for at least one eye is possible in most of the bilateral cases. It includes laser alone or combined with chemotherapy, cryotherapy and brachytherapy. Long term follow-up and early counseling regarding the risk of second primary tumors and transmission should be offered ***(Aerts et al., 2006)***.

CD24 is a glycosylphosphatidylinositol-anchored membrane protein ***(Schabath et al., 2006)***; complexed to protein tyrosine kinases responsible for signaling ***(Salamone et al., 2001).*** CD24 is expressed in hematopoietic cell subpopulations, especially B lymphocytes ***(Salamone et al., 2001)***. It is also

present in certain epithelial cells such as keratinocytes and renal tubular epithelium ***(Baumann et al., 2005)***, and the developing brain ***(Schabath et al., 2006)***.

CD24 is thought to function as an adhesion molecule. It is known to bind to P-selectin, a protein expressed on thrombin-activated platelets and endothelial cells, allowing adhesion of monocytes or neutrophils to them ***(Ju et al., 2011)***, and to L1, a member of the immunoglobulin superfamily that is expressed on neural and lymphoid cells ***(Baumann et al., 2005)***. It, also, has a role in stimulating proliferation and maturation of pre-B lymphocytes within the bone marrow ***(Salamone et al., 2001)***.

An expanding body of literature points to a role for CD24 in the tumorigenesis and progression of a number of types of cancer. CD24 expression is a prognostic indicator of poor survival, in particular, in breast cancer and, also, in non–small cell lung carcinomas, epithelial ovarian, colorectal and prostate tumors. Enhanced CD24 expression in comparison to matched nonmalignant tissue has also been reported for a number of other types of cancer, including B-cell lymphoma, renal cell carcinoma, small cell lung carcinoma, nasopharyngeal carcinoma, hepatocellular carcinoma, Merkel cell carcinoma, pancreatic carcinoma, glioma, tumours of neuroectodermal origin, bladder carcinoma,choriocarcinoma, and cholangiocarcinoma ***(Salamone et al., 2001; Baumann et al., 2005; Schabath et al., 2006; Li et al., 2012; Deng et al., 2012; Seo et al., 2015).***

It has been proposed that CD24-mediated binding to P-selectin on endothelial cells and platelets could facilitate the exit of tumour cells from the bloodstream and hence favour metastasis ***(Schabath et al., 2006)***. Furthermore, CD24 expression increases tumor cell proliferation and also indirectly stimulates cell adhesion to fibronectin, collagens I and IV, and laminin through the activation of α3β1 and α4β1 integrin. Moreover, CD24 expression supports rapid cell spreading and strongly induces cell motility and invasion. CD24-induced proliferation and motility are integrin independent ***(Baumann et al., 2005)***.

Very few studies were performed to assess CD24 expression and its prognostic significance in retinoblastoma, therefore, further studies are required to be conducted in this field.

Aim of the Work

The aim of the study was to evaluate the immunohistochemical expression of CD24 in retinoblastoma and to correlate it with different histopathological parameters aiming at identifying the potential prognostic significance of CD24 in retinoblastoma.

Chapter 1
THE ANATOMY AND HISTOLOGY OF THE EYE

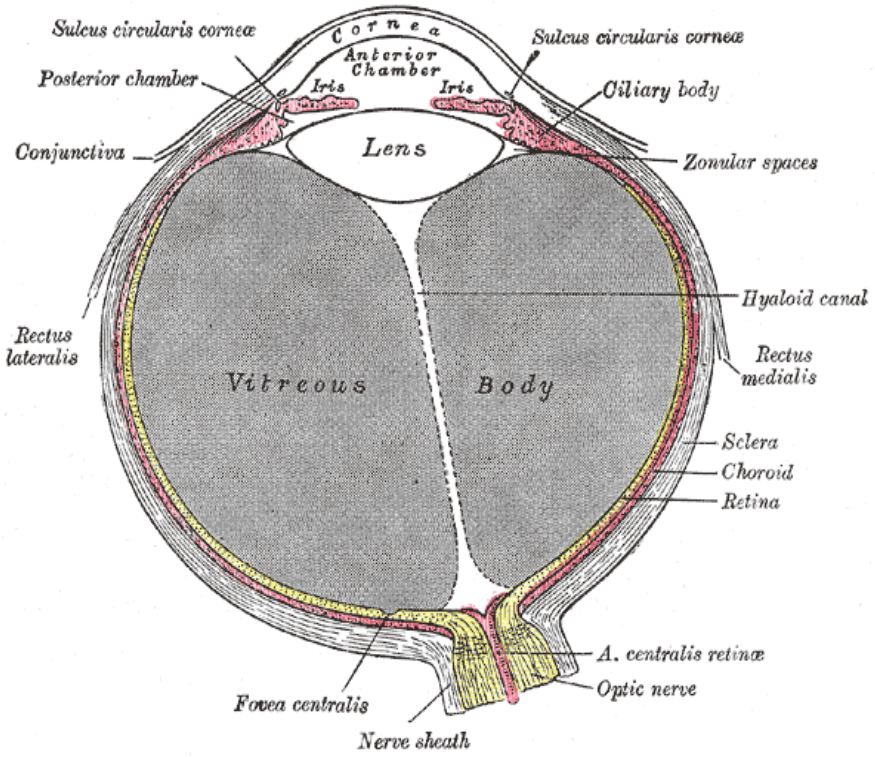

Figure (1): Anatomy of the eye ***(Clemente and Gray, 1985).***

The eye is a sphere whose wall is made up of three layers. The outermost layer, the **sclera**, is composed of tough connective tissue which protects the eyes and provides for extraocular muscle insertion. Posteriorly, the sclera is perforated by the optic nerve at the lamina cribrosa. The **cornea** is the anterior transparent part of this outermost layer and is the eye's main refracting surface. The **conjunctiva** is the mucous

membrane lining the inner surface of the eyelids and the anterior part of the sclera ***(Clemente and Gray, 1985).***

The second layer is the **choroid**. Its black color allows it to absorb light so that it does not reflect in the eye and impair vision.

It has the following 3 layers:

1. The epichoroid layer bridges the space between the sclera and choroid
2. The vessels layer forms the bulk of the choroid layer and contains melanocytes
3. The choriocapillaris is a layer of capillaries lined by fenestrated type II endothelium that supplies nutrition to the outer portion of the retina ***(Clemente and Gray, 1985).***

The **ciliary body** consists of smooth muscles which alter the tension on the capsule of the lens by contracting and relaxing the zonule fibers ***(Clemente and Gray, 1985).***

The iris, the most anterior part of the uvea, is the colored part of the eye consisting of smooth muscle that surrounds the **pupil** and regulates the amount of light that enters ***(Rizzo, 2015).***

The retina is the innermost layer of the eye (figure 2). Its layers are as follows:

- Pigment epithelium (layer closest to the choroid layer) (RPE)
- Layer of rods and cones- outer segment (OS) and inner segment (IS)
- External limiting membrane
- Outer nuclear layer (ONL)
- Outer plexiform layer (OPL)
- Inner nuclear layer (INL)
- Inner plexiform layer (IPL)
- Ganglion cell layer (GCL)
- Optic nerve fiber layer (NFL)
- Internal limiting membrane (layer closest to the vitreous body) ***(Clemente and Gray, 1985).***

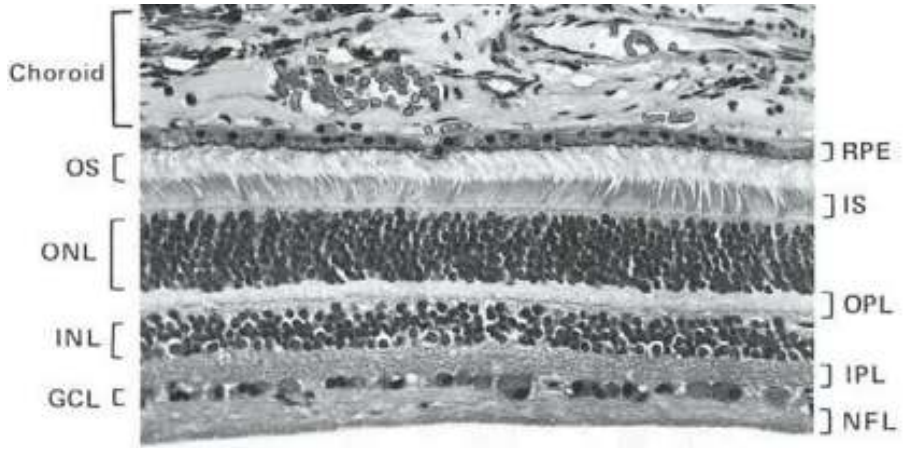

Figure (2): Microscopic layers of the retina ***(Mills, 2012).***

The retinal pigment epithelium (RPE) helps keep light from being reflected back into the eye. It is composed of a single layer of cuboidal cells. The apical aspect of these cells is intimately related to photoreceptor outer segments and is involved in phagocytosis of their distal tips essential for continued renewal. Any recyclable molecules (eg, retinal or docosahexaenoic acid) are returned to photoreceptors. It also delivers essential nutrients from the blood to the photoreceptors. These include glucose and vitamin A ***(Zeiss, 2010).***

The basal aspect of these cells rest upon the Bruch's membrane, a three layered structure consisting of the basement membrane of RPE cells, an intervening elastocollagen layer, and an outer layer derived from the basal layer of the choriocapillaris ***(Zeiss, 2010).***

Rods are thin, cylindrical cells and number about 130 x 10^6 ***(Clemente and Gray, 1985)***. They function in dim light and contain the pigment called **rhodopsin** ***(Rizzo, 2015).***

Cones have a flask shape and number about 67 x 10^6. Nuclei in the cones are larger and less dense than those in the rods ***(Clemente and Gray, 1985)***. They contain pigments that are sensitive to blue, green, or red light and therefore, produce color vision. Combinations of these colors produce all the other colors we see ***(Rizzo, 2015).***

The yellowish cone-dense region in the center of the retina is called the macula lutea and mediates high-acuity central vision,

with maximal visual acuity produced by the cone-dominated avascular region at its center called the fovea ***(Zeiss, 2010).***

Medial to the fovea centralis is the **optic disk** where nerve fibers leave the eye as the optic nerve. The optic disk has no receptors and is, thus, called the blind spot ***(Rizzo, 2015).***

The external limiting membrane supports the photoreceptor cells; and the internal limiting membrane is the basal lamina of the Muller cells, separating the retina from the vitreous cavity ***(Clemente and Gray, 1985).***

The outer nuclear layer contains the nucleated portions of the photoreceptors, while the inner nuclear layer contains the cell bodies of the bipolar neurons as well as Muller cells ***(Clemente and Gray, 1985).***

The outer and inner plexiform layers contain synapses made between: the photoreceptors and the bipolar cells and the bipolar neurons and the ganglionic cells, respectively ***(Clemente and Gray, 1985).***

The ganglion cell layer contains cell bodies of the ganglion cells. The optic nerve fibers are the axons of the ganglion cells that pass radially to form the optic nerve ***(Clemente and Gray, 1985).***

The eye is divided into two compartments by the lens. In front of the lens is the anterior compartment that is filled with the **aqueous humor**. The posterior compartment is filled with **vitreous humor** ***(Rizzo, 2015).***

Chapter 2

RETINOBLASTOMA

Epidemiology

Retinoblastoma is a primitive neuroectodermal tumor occurring in early childhood. The oncogenic mutation can develop anytime between the start of the third intrauterine month (start of retinal formation in early embryonal development) and the age of 4 years (the age of final maturity of retinoblasts) ***(Balmer et al., 2006).***

Incidence:

Although rare, it represents 80% of all primary ocular malignancies in children up to 15 years old and rates third highest of all intraocular tumors in all ages ***(Balmer et al., 2006).***

It has the lowest median age of all childhood cancers, approximately 15 months ***(Yeole et al., 2002).*** Two-thirds of all cases of retinoblastoma are diagnosed before age 2 years, and 95% of cases are diagnosed before age 5 years ***(Ries et al., 1999).*** The median age of diagnosis differs according to disease laterality: 24 months in unilateral cases versus 9–12 months in bilateral cases ***(Butros et al., 2002).*** Later age at diagnosis is generally reported in regions where there is decreased access to medical care, but diagnosis in children older than 5 years is rare ***(De Aguirre Neto et al., 2007)***. Incidence rates are thus

commonly better expressed as "per million children 0–4 years of age," rather than as "per million children 0–14 years of age," as is common for other childhood cancers ***(Rodrigeuz-Galindo and Wilson, 2010).***

Regarding Egypt, the expected number of new retinoblastoma cases is 100-120 cases/ year, given that the incidence of retinoblastoma is one in 15000-18000 live births and according to national census and WHO statistics in 2008, the Egyptian population approximated 80 million with 1.8 million live births /year ***(Othman et al., 2011).***

Geographic variation:

Global incidence data for retinoblastoma, unlike most pediatric tumors, show an approximate 50-fold variation, which is comparable to the degree of variation seen in adult malignancy, such as cervical, gastric, and colon cancer, in which variations in environmental exposures – such as infectious agents and diet – are known to play a role. Other pediatric tumors with widely varying incidence rates, such as Hodgkin and non-Hodgkin lymphomas, are tumors in which infectious agents are known to play a role ***(Rodrigeuz-Galindo and Wilson, 2010).***

This variation appears largely restricted to unilateral disease. Its incidence being higher in several less developed countries, suggests that environmental exposures associated

with poor living conditions may increase the risk of mutagenesis in retinal cells ***(Stiller and Parkin, 1996).***

Incidence rates are generally similar in North America, Europe, and Australia (with retinoblastoma representing 2-4 % of all childhood neoplasms); somewhat higher rates are observed in Central and South America; a wide range of rates are reported in Asia with the highest in one region in India (Chennai); and generally the highest rates are clearly in Africa (with retinoblastoma representing 10-15% of total pediatric tumors) ***(Yeole et al., 2002).***

Incidence rates among African countries are also extremely variable, with population based registries in Algeria and Egypt reporting rates of 4–6 per million <5 years, while incidence rates for some sub-Saharan African countries, such as Mali, Uganda, and Zimbabwe, are amongst the highest worldwide ***(Rodrigeuz-Galindo and Wilson, 2010).***

Some populations with apparently higher rates of retinoblastoma have relatively lower rates of neuroblastoma. This may indicate a possible shared underlying mechanism of primitive neuroectodermal tumor formation in which variable secondary factors lead to development of neuroblastoma in one population, and retinoblastoma in another. Alternatively, the factors that lead to development of retinoblastoma may coexist with factors that protect against neuroblastoma ***(Rodrigeuz-Galindo and Wilson, 2010).***

Ethnic differences in the frequencies of unilateral versus bilateral retinoblastomas are observed. In a british series of cases, 40% of retinoblastomas were bilateral while in two African series in which laterality was reported, no bilateral cases were observed. The male-to-female ratio of retinoblastoma fluctuates around unity in most of the populations (reviewed by ***Yeole et al., 2002).***

Associations and risk factors:

Parental occupation:

Bunin et al. (1990) found that paternal employment in the military or in the metal industry was associated with having a child with bilateral disease, and paternal employment as a welder or machinist was associated with having a child with unilateral disease. Interestingly, this study also examined potential transgenerational exposures and found that maternal grandparental employment in farming was also associated with unilateral disease. Also another case–control study in Mexico found increased risk with paternal occupation as a farm worker for bilateral disease ***(Orjuela et al., 2000a)***; these trends are comparable to findings in epidemiologic studies of other PNETs ***(Olshan et al., 1999).***

Parental age:

Higher parental age (of both fathers and mothers) has been associated with greater risk for bilateral retinoblastoma ***(Moll et al., 1996).***

Increased paternal age is hypothesized to be associated with an increased risk of new germ cell mutations due to increased probability of mutagenesis in dividing spermatocytes. This is supported by the fact that new germline mutations occur preferentially on the paternal allele ***(Rodrigeuz-Galindo and Wilson, 2010)***.

However, ***Yip et al., 2006*** found that for children <5 years of age, advanced *maternal* age (after adjustment for paternal age) was associated with increased risk of retinoblastoma (when women older than 40 years at childbirth were compared with women younger than 25 years at childbirth).

Perinatal factors:

Risk for the development of *unilateral* retinoblastoma was significantly increased with factors related to maternal poverty during pregnancy, including poor nutrition, lack of prenatal care, delivery at home, low level of maternal education (not finishing secondary school), and exclusive breast feeding for longer than 6 months. However, none of these risk factors appeared to be associated with increased risk of bilateral disease ***(Orjuela et al., 2000a).***

One of the most important of these factors is gestational nutrient intake. Indeed, gestational maternal diets low in nutrients necessary for DNA methylation and synthesis, as well