CHAPTER II

LITERATURE REVIEW

1. Anatomy of the Eye

The eye is made up of three layers, enclosing three transparent structures.

- ***The outermost layer***; is composed of the cornea and sclera.
- ***The middle layer (uvea)***; consists of the choroid, ciliary body and iris.
- ***The innermost is the retina***, which gets its circulation from the vessels of the choroid as well as the retinal vessels, which can be seen in an ophthalmoscope.

Within these layers are the aqueous humor, the vitreous body, and the flexible lens. The aqueous humor is a clear fluid that is contained in two areas: the anterior chamber between the cornea and the iris and exposed area of the lens; and the posterior chamber, behind the iris and the rest. The lens is suspended to the ciliary body by the suspensory ligament (Zonule of Zinn), made up of fine transparent fibers. The vitreous body is a clear jelly that is much larger than the aqueous humor, and is bordered by the sclera, zonule, and lens. They are connected via the pupil.

The main compartments of the eye are the following; (Riordan-Eva P, 2008)

- ***Cornea***: the cornea is the transparent front part of the eye that covers the iris, pupil and anterior chamber. It is a fibrous, transparent tissue that extends over the pupil and colored portion of the eye. The function of the cornea is to bend or reflect

the rays of light, so they are focused properly on the sensitive receptor cells in the posterior region of the eye. The cornea has unmyelinated nerve endings sensitive to touch, temperature and chemicals; a touch of the cornea causes an involuntary reflex to close the eyelid. Because transparency is of prime importance the cornea does not have blood vessels; it receives nutrients and oxygen via diffusion from the tear fluid at the outside and the aqueous humour at the inside and also from neurotrophins supplied by nerve fibres that innervate it.

- ***Conjunctiva***: the conjunctiva is the thin transparent mucous membrane consisting of cells and rare stratified columnar epithelium that underly the basement membrane. This compartment covers the outer surface of the eye. It begins at the outer edge of the cornea, covering the visible part of the sclera, and lining the inside of the eyelids. It is nourished by tiny blood vessels that are nearly invisible to the naked eye. The conjunctiva also secretes oils and mucous that moistens and lubricate the eye.

- ***Pupil***: the pupil is a hole located in the center of the iris of the eye that allows light to enter the retina. It appears black because most of the light entering the pupil is absorbed by the tissues inside the eye. In humans the pupil is round in shape.

- ***Sclera***: the sclera, also known as the white or white of the eye. It is tough, fibrous, supportive, connective tissue that extends from the cornea on the anterior surface of the eyeball to the optic nerve in the back of the eye. The sclera forms the posterior five-sixths of the connective tissue layer of the eyeball. It is continuous with

the dura mater and the cornea, and maintains the shape of the eyeball, offering resistance to internal and external forces, and provides an attachment for the extraocular muscle insertions.

- ***Choriod***: the choroid, also known as the choroidea or choroid coat, is the vascular layer of the eye, containing connective tissue, and lying between the retina and the sclera. It is a dark brown membrane inside the sclera. It contains many blood vessels that supply nutrients to the eye. The choroid is continuous with the pigment-containing iris and the ciliary body on the anterior surface of the eye. The human choroid is thickest at the far extreme rear of the eye (at 0.2 mm), while in the outlying areas it narrows to 0.1 mm. The choroid provides oxygen and nourishment to the outer layers of the retina. Along with the ciliary body and iris, the choroid forms the uveal tract.

The structure of the choroid is generally divided into four layers:

♦ Haller's layer - outermost layer of the choroid consisting of larger diameter blood vessels.

♦ Sattler's layer - layer of medium diameter blood vessels.

♦ Choriocapillaris - layer of capillaries.

♦ Bruch's membrane (synonyms: Lamina basalis, Complexus basalis, Lamina vitra) - innermost layer of the choroid.

- ***Iris*:** the colored part of the eye is called the iris. It is a thin diaphragm composed mostly of connective tissue and smooth muscle fibers. It is situated between the cornea and the crystalline lens. The iris is composed of 3 layers; endothelium, stroma and epithelium. The iris is flat and divides the front of the eye (anterior chamber; between the cornea and the iris) from the back of the eye (posterior chamber; between the iris and the lens). Its color comes from microscopic pigment cells containing melanin. The color, texture, and patterns of each person's iris are as unique as a fingerprint.

- ***Ciliary body:*** the ciliary body is the circumferential tissue inside the eye composed of the ciliary muscle and ciliary processes. It is triangular in horizontal section and is coated by a double layer, the ciliary epithelium. This epithelium produces the aqueous humor. The inner layer is transparent and covers the vitreous body, and is continuous from the neural tissue of the retina. The outer layer is highly pigmented, continuous with the retinal pigment epithelium, and constitutes the cells of the dilator muscle. This double membrane is often regarded to be continuous with the retina and a rudiment of the embryological correspondent to the retina. The inner layer is unpigmented until it reaches the iris, where it takes on pigment.

The ciliary body has 3 functions: accommodation, aqueous humor production and the production and maintenance of the lens zonules. It also anchors the lens in place. Accommodation essentially means that when the ciliary muscle contracts, the lens becomes more convex, generally improving the focus for closer objects. When it relaxes,it flattens the lens, generally improving the focus for farther objects. One of the essential roles of the ciliary body is also the production of the aqueous humor,

which is responsible for providing most of the nutrients for the lens and the cornea and involved in waste management of these areas. Ciliary body has 2 parts, the anterior part with muliple folds and posterior flat part (pars plana)

- ***Lens***: the lens is part of the anterior segment of the eye. Anterior to the lens is the iris, which regulates the amount of light entering into the eye. The lens is suspended in place by the suspensory ligament of the lens, a ring of fibrous tissue that attaches to the lens at its equator and connects it to the ciliary body. Posterior to the lens is the vitreous body, which, along with the aqueous humor on the anterior surface, bathes the lens. The lens has an ellipsoid, biconvex shape. The anterior surface is less curved than the posterior. In the adult, the lens is typically circa 10 mm in diameter and has an axial length of about 4 mm, though it is important to note that the size and shape can change due to accommodation and because the lens continues to grow throughout a person's lifetime. The lens is also known as the *aquula* (Latin, *a little stream*, dim. of *aqua*, *water*) or *crystalline lens*. In humans, the refractive power of the lens in its natural environment is approximately 18 dioptres, roughly one-third of the eye's total power.

The crystalline lens is a transparent, biconvex structure in the eye that, along with the cornea, helps to refract light to be focused on the retina. The lens, by changing shape, functions to change the focal distance of the eye so that it can focus on objects at various distances, thus allowing a sharp real image of the object of interest to be formed on the retina. This adjustment of the lens is known as accommodation. It is similar to the focusing of a photographic camera via movement of its lenses. The lens is flatter on its anterior side.

- ***Retina:*** The retina is a layered structure with several layers of neurons interconnected by synapses. The only neurons that are directly sensitive to light are the photoreceptor cells. These are mainly of two types: the rods and cones. Rods function mainly in dim light and provide black-and-white vision, while cones support daytime vision and the perception of colour. A third, much rarer type of photoreceptor, the photosensitive ganglion cell, is important for reflexive responses to bright daylight. Neural signals from the rods and cones undergo processing by other neurons of the retina. The output takes the form of action potentials in retinal ganglion cells whose axons form the optic nerve. Several important features of visual perception can be traced to the retinal encoding and processing of light.

- ***Macula:*** the macula is an oval-shaped highly pigmented yellow spot located roughly in the center of the retina, temporal to the optic nerve. It has a diameter of around 5 mm and is often histologically defined as having two or more layers of ganglion cells. It is a small and highly sensitive part of the retina responsible for detailed central vision. The fovea is the very center of the macula. The macula allows us to appreciate detail and perform tasks that require central vision such as reading. Structures in the macula are specialized for high acuity vision. Within the macula are the fovea and foveola which contain a high density of cones. Because the macula is yellow in colour it absorbs excess blue and ultraviolet light that enter the eye, and acts as a natural sunblock (analogous to sunglasses) for this area of the retina. The yellow colour comes from its content of lutein and zeaxanthin, which are yellow xanthophyll carotenoids, derived from the diet. Zeaxanthin predominates at the macula, while

lutein predominates elsewhere in the retina. There is some evidence that these carotenoids protect the pigmented region from some types of macular degeneration.

- ***Optic nerve:*** the optic nerve is the second of twelve paired cranial nerves but is considered to be part of the central nervous system. The name "optic nerve" is, in the technical sense, a misnomer, as the optic system lies within the central nervous system and therefore should be named the "optic tract," as nerves exist only, by definition, within the peripheral nervous system. The optic nerve is composed of retinal ganglion cell axons and support cells. It leaves the orbit (eye) via the optic canal, running postero-medially towards the optic chiasm, where there is a partial decussation (crossing) of fibres from the nasal visual fields of both eyes. The optic nerve is ensheathed in all three meningeal layers (dura, arachnoid, and pia mater) rather than the epineurium, perineurium, and endoneurium found in peripheral nerves. Fibre tracks of the mammalian central nervous system (as opposed to the peripheral nervous system) are incapable of regeneration, and, hence, optic nerve damage produces irreversible blindness. The fibres from the retina run along the optic nerve to nine primary visual nuclei in the brain, whence a major relay inputs into the primary visual cortex.

- ***Optic disc:*** the optic disc or optic nerve head is the location where ganglion cell axons exit the eye to form the optic nerve. There are no light sensitive rods or cones to respond to a light stimulus at this point. This causes a break in the visual field called "the blind spot" or the "physiological blind spot". The Optic Disc represents the beginning of the optic nerve (second cranial nerve) and is the point where the axons of retinal ganglion cells come together. The Optic Disk is also the

entry point for the major blood vessels that supply the retina. The optic nerve head in a normal human eye carries from 1 to 1.2 million neurons from the eye towards the brain.

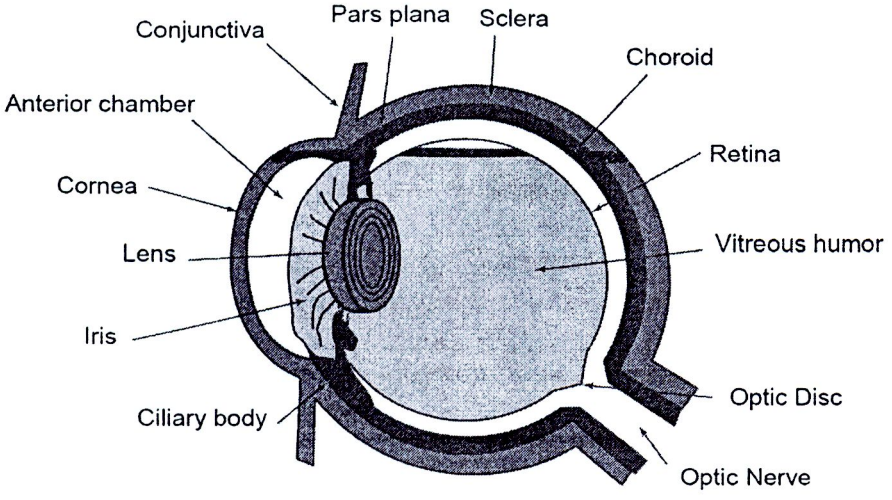
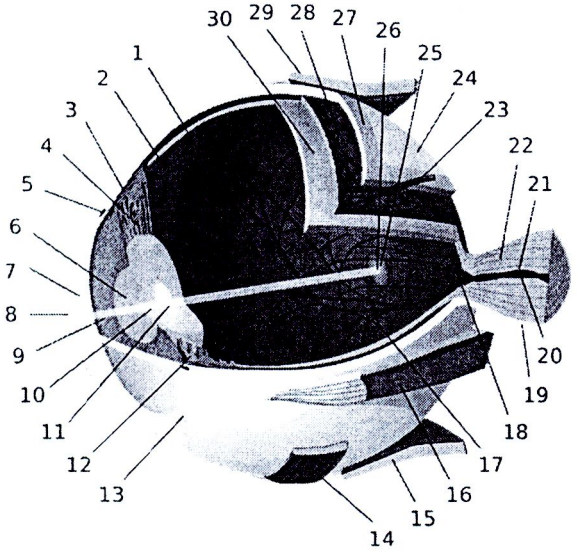


Figure 1 The eye compartments

(Modified from Figure 1 of Munoz-Fernandez *et al.*, 2006*)*



1. posterior chamber	2. ora serrata	3. ciliary muscle	4. ciliary zonules
5. canal of Schlemm	6. pupil	7. anterior chamber	8. cornea
9. iris	10. lens cortex	11. lens nucleus	12. ciliary process
13. conjunctiva	14. inferior oblique muscle		15. inferior rectus muscle
16. medial rectus muscle	17. retinal arteries and veins		18. optic disc
19. dura mater	20. central retinal artery		21. central retinal vein
22. optic nerve	23. vorticose vein		24. bulbar sheath
25. macula	26. fovea	27. sclera	28. choroid
29. superior rectus muscle	30. retina		

Figure 2 Anatomy of human eye

(Modified from the picture of the human eye from http://en.wikipedia.org/wiki/Human_eye)

2. Uveitis (Cunningham and Shetlar, 2008)

The term "Uveitis", by definition is an inflammation of the uveal tract, which is the middle layer of the eye located between the sclera, conjunctiva and anterior chamber on the outside and the retina on the inside.

The uveal tract consists of the choroid, ciliary body and iris. Inflammation of uveal tract and tumors together comprise the vast majority of diseases affecting these structures. Many inflammatory and neoplastic disorders of the uveal tract are associated with systemic diseases, some of which can be life-threatening if unrecognized. The term uveitis is now used to describe many forms of intraocular inflammation involving not only the uveal tract, but also adjacent structures.

2.1 Uveitis Classification

The International Uveitis Study Group classification system is widely used for classification. Uveitis can be classified on the basis of (A) anatomy or location, (B) clinical course and (C) laterality In addition, uveitis also has been categorized due to the (D) etiology. (Munoz-Fernandez e*t al.*, 2006).

(A) <u>Anatomical classification</u>

(Standardization of uveitis nomenclature working group, 2005)

- **Anterior uveitis** can be subdivided into:

  - ***Iritis:*** the inflammation that predominantly affects the iris.

  - ***Iridocyclitis:*** the inflammation which involves both of the iris and the anterior part of the ciliary body (pars plicata).

- **Intermediate uveitis**

Intermediate uveitis is characterized by involvement predominantly of the posterior part of the ciliary body (pars plana), the extreme periphery of the retina and the underlying choroid.

- **Posterior uveitis**

Posterior uveitis refers to the inflammation that affects the choroid or, by extension, and the retina posterior to the vitreous base (choroiditis or retinochoroiditis). Posterior uveitis may or may not be accompanied by retinal vasculitis.

- **Panuvetis**

Panuveitis implies involvement of the entire uveal tract.

(B) <u>Clinical course classification</u>

According to the mode of onset and duration, uveitis can be classified into 3 types

- **Acute uveitis**

Acute uveitis usually has a sudden, symptomatic onset and persists for up to 3 months. If the inflammation recurs following the initial attack, it is referred to as recurrent acute.

- **Chronic uveitis**

Chronic uveitis persists for longer than 3 months. The onset is frequently insidious and may be asymptomatic, although acute or subacute exacerbations may occur.

- **Recurrent uveitis**

Recuurent uveitis is defined when acute flare appears after complete resolution of a previous episode.

(C) Classification by laterality

With regard to laterality, uveitis is defined as:

1. **Unilateral uveitis**, when only one eye is affected at a given time, although recurrences may occur in the contraleteral eye.
2. **Bilateral uveitis**, when both eyes are affected simultaneously.

(D) Etiological classification

Etiological agents that cause uveitis may be classified to exogenous or endogenous agents. Exogenous cause refers to the inflammation that involves external injury to the uveal tract or invasion by micro-organisms from outside. In contrast, endogenous cause means uveitis which is associated with micro-organisms or other causes from inside the patient's body. The main types are the following;

- **Associated with systemic disease:** such as sarcoidosis.
- **Infections** with bacteria (e.g. tuberculosis), fungi (e.g. candidiasis) and viruses (e.g. herpes zoster).

- **Infestations** with protozoa (e.g. toxoplasmosis) or nematodes (e.g. toxocariasis).
- **Idiopathic specific uveitis entities** are a group of unrelated disorders unassociated with underlying systemic disease but with special characteristics of their own warranting independent description (e.g. Fuchs uveitis syndrome).
- **Idiopathic non-specific uveitis entities** which do not fall into any of the above categories constitute about 20% of cases.

2.2 Clinical features (Kanski JJ, 2003; Hajj-Ali *et al.*, 2005)

Inflammation of the uveal tract has many causes and may involve one or more regions of the eye simultaneously.

- **Anterior uveitis (AU)**

Anterior uveitis is most common and is usually unilateral and acute in onset.

Symptoms

1. Acute anterior uveitis is characterized by photophobia, pain, redness, decreased vision and lacrimation.

2. Chronic anterior uveitis may be asymptomatic or give rise to mild redness and the perception of floaters.

Typical symptoms of AU include pain, photophobia and blurred vision. Examination usually reveals circumcorneal redness with minimal palpebral conjunctival injection or discharge. The pupil may be small (miosis) or irregular due to the formation of posterior synchiae. Inflammation limited to the anterior chamber is

called "iritis", whereas inflammation involving both the anterior chamber and the anterior vitreous is often refered to as "iridoclyclitis". Decreased sensation occurs in herpes simplex or herpes zoster infection or leprosy. Whereas increased intraocular pressure can occur with herpes simplex virus (HSV), varicella zoster virus (VZV), toxoplasmosis, syphilis, sarcoidosis or an uncommon form of iridocyclitis called glaucomatocyclitic crisis (also known as the Posner-Schlossman syndrome; PSS). Stellate keratic precipitates are usually distributed evenly over the entire corneal endothelium and may be seen in uveitis due to HSV, VZV, toxoplasmosis, Fuchs' heterochromic iridocyclitis (FHI) and sarcoidosis.

- **Intermediate uveitis**

Intermediate uveitis also called cyclitis, peripheral uveitis or pars planitis, is the least common anatomical type of intraocular inflammation. The hallmark of intermediate uveitis is vitreous inflammation. It is typically bilateral and tends to affect patients in their late teens or early adult years. Men are affected more commonly than women.

Symptoms

Symptoms of intermediate uveitis are initially floaters and later impairment of visual acuity due to macular edema. Typical symptoms include floaters and blurred vision. Pain, photophobia and redness are usually absent or minimal although these symptoms may be more prominent at onset.

The cause of intermediate uveitis is unknown in the vast majority of patients, although sarcoidosis and multiple sclerosis account for 10-20% of cases.

Syphilis and tuberculosis, although uncommon should be excluded in all patients. The most common complications of intermediate uveitis include cystoid macular edema, retinal vasculitis and neovacularization of the optic disc.

- **Posterior uveitis**

Posterior uveitis includes retinitis, choroiditis, retinal vaculitis and papillitis, which may occur alone or combination.

Symptoms

Symptoms typically include floaters, loss of visual field or scotomas, or decreased vision. Retinal detachment, although infrequent, occurs most commonly in posterior uveitis and may be tractional, rhegmatogenous or exudative in nature. Eye redness is uncommon in strictly posterior uveitis but can be seen in diffuse uveitis. Pain is atypical in posterior but can occur in endophthalmitis, posterior scleritis or optic neuritis, particularly when caused by multiple sclerosis.

- **Panuveitis**

Panuveitis also term as "diffuse uveitis" that denote a more or less uniform cellular infiltration of both the anterior and posterior segment. Associated findings, such as retinitis, vaculitis or choroiditis can occur and often prompt further diagnostic testing. Tuberculosis, sarcoidosis and syphilis should always be considered in patients with diffuse uveitis.

2.3 Causes of uveitis

Uveitis, a complex intraocular inflammatory disease, results from multiple etiological entities. Causes of uveitis are known to vary in different populations depending on the ecological, racial and socioeconomic variation of the population studied. Uveitis affects most commonly young adults. Around 60-80% of uveitis patients were in the third through sixth decade of life with the mean age at presentation most often between 35 and 45 years of age. (U.S. National Library of Medicine.Uveitis, 2006; Wakefield *et al.*, 2005). Various different mechanisms and diseases may cause uveitis and present the frequent associated with the diagnosis of this condition as showed in Table 1, 2, 3 and 4.

Table 1 Causes of Anterior uveitis.

Autoimmune (presumed)	**Infections**	**Malignancy**	**Other**
- Juvenile idiopathic arthritis (JIA) - Ankylosing spondylitis - Reiter's syndrome - Ulcerative colitis - Lens-induced uveitis - Sarcoidosis - Crohn's disease - Psoriasis	- Syphilis - Tuberculosis - Leprosy (Hansen's disease) - Varicella zoster virus (VZV) - Herpes simplex - Onchocerciasis - Leptospirosis - Cytomegalovirus - Rubella	- Masquerade syndrome - Leukemia - Malignant Melanoma of iris	- Idiopathic - Traumatic uveitis - Retinal detachment - Fuchs' heterochromic iridocyclitis (FHI)* - Glaucomatocyclitis crisis (Posner-Schlossman syndrome; PSS)*

(Modified from Table 7-3 of Cunningham and Shetler, 2008)

**: recently attributed to rubella and/or CMV*

Table 2 Causes of Posterior uveitis.

Infectious disorders	Non-infectious disorders
1. Viruses - Cytomegalovirus (CMV) - Herpes simplex virus (HSV) - Varicella zoster virus (VZV) - Rubella virus - Rubeola virus	**1. Autoimmune disorders** - Behcet's disease - Vogt-Koyanagi-Harada syndrome - Systemic lupus erythematosus - Wegener's granulomatosis - Sympathetic ophthalmia - Retinal vasculitis
2. Bacteria - *Mycobacterium tuberculosis* - *Brucella* sp. - *Treponema pallidum* - *Borrelia* sp. (Lyme disease) - various hematogenously spread gram-positive and gram-negative baceria	**2. Malignancies** - Intraocular lymphoma - Malignant melanoma - Leukemia - Metastatic lesions

Table 2 Causes of Posterior uveitis (continued)

Infectious disorders	Non-infectious disorders
3. Fungi - *Candida* sp. - *Histoplasma* sp. - *Cryptococcus* sp. - *Aspergillus* sp.	**3. Unknown etiology** - Sarcoidosis - Serpiginous choroiditis - Acute multifocal placoid pigment epitheliopathy - Birdshot retinochoroidopathy - Retinal pigment epitheliopathy - Multiple evanescent white dot syndrome
4. Parasite - *Toxoplasma* sp. - *Toxocara* sp. - *Onchocerca* sp.	

(Modified from Table 7-4 of Cunningham and Shetler, 2008)

Table 3 Causes of Panuveitis.

Infectious disorders	Non-infectious disorders
1. Bacterial infection - Tuberculosis - Syphilis - Leptospirosis - Brucellosis	**1. Autoimmune disorders** - Behcet's disease - Vogt-Koyanagi-Harada syndrome - Sympathetic ophthalmia - Multiple sclerosis
2. Parasitic infastation - Ochocerciasis - Cysticerosis	**2. Malignancies** - Leukemia - Retinoblastoma
	3. Unknown etiology - Sarcoidosis - Retinal intraocular foreign body

(Modified from Table 7-6 of Cunningham and Shetler, 2008)

2.3.1 Non-infectious uveitis

Uveitis can be presented in patients following a huge spectrum of diseases. Genetic, infectious, environmental, systemic and immunologic diseases are some of the predisposing factors associated with uveitis. Most cases of uveitis are associated with varities of disorders including autoimmune disease, malignancies and unidentified eitiolgy (Chams H *et al.*, 2009). Particular forms of uveitis may affect certain age groups more frequently. In JIA-associated chronic anterior uveitis in children; HLA-B27-associated acute anterior uveitis (AAU) predominate effecting

young adults (mean age of about 35 years); birdshot retinochoroidopathy in older adults (mean age of about 50 years); and uveitis masquerade syndromes, such as intraocular lymphoma, affecting the elderly population (Wakefield *et al.*, 2005).

Studies of the pattern of uveitis in childhood in the USA showed that juvenile rheumatoid arthritis (JRA) was the most common (41.5%) followed by idiopathic uveitis (21.5%) and pars planitis (15.3%) (Tugal-Tutkun *et al.*, 1996). A report from Switzerland stated that uveitis in childhood can be due to a wide spectrum of non-infectious diseases with juvenile idiopathic arthritis being the leading cause (Stoffel *et al.*, 2000). Most of Italian children with non-infectious uveitis represented association with systemic diseases (27.8%) mostly due to JRA, while idiopathic uveitis was found in 12.8% (Paroli *et al.*, 2009).

In the elderly, uveitis can present *de novo* after the age of 60 years or may represent a process initiated earlier in life continuing after the age of 60 years, although many cases will have become quiescent by that time. More recent studies suggest that uveitis presenting after 60 years of age is more common than previously believed. Most cases of uveitis are of unknown etiology and are classed as idiopathic, although sarcoidosis, ocular ischaemia and birdshot chorioretinopathy are recognised non-infectious causes of uveitis in the elderly. Systemic immunosuppression, with its well known complications, may be required to preserve vision. In this age group, one should always have high suspicion of a masquerade syndrome, particularly a primary CNS non-Hodgkin's lymphoma (Kirsch *et al.*, 2003; Gupta *et al.*, 2006).

As in the Western world, up to 51.2% of uveitis cases in Asian countries were idiopathic. In most cases this was a non-infectious form (45-95%)

(Chams *et al.*, 2009). However, the trend and prevalence of non-infectious uveitis in Asia has not greatly changed during the last several decades but widely differs among different countries (Pathanapitoon *et al.*, 2008; Chams *et al.*, 2009). According to a report from Japan the prevalence of VHK disease-associated ocular inflammation has remained unchanged (Wakabayashi *et al.*, 2003). Meanwhile, Bechets disease related uveitis has decreased in prevalence and severity (Wakabayashi *et al.*, 2003; Chams *et al.*, 2008; Islam *et al.*, 2002; Kazokoglu *et al.*, 2008). However, the upward trend of idiopathic uveitis that shows an increasing incidence of immunologically related uveitis (Wakabayashi *et al.*, 2003; Chams *et al.*, 2008).

2.3.2 Infectious uveitis

Table 4 : Causes of infectious uveitis.

Causes of infectious uveitis		
More Common	**Less Common**	**Rare**
- Cytomegalovirus* - Herpes viruses - *Pneumocystis jiroveci* (formerly *P.carinii*)* - Toxoplasmosis - Rubella	- Histoplasmosis - Lyme disease - Syphilis - Toxocariasis - Tuberculosis	- Aspergillus - Candida - Coccidioidomyosis - Cryptococcus - Cysticercosis - Leprosy - Leptospirosis - *Tropheryma whippelii*
*In patients with AIDS.		

Note: Table 4 was modified from http://www.merck.com/mmpe/sec09/ch105/ch105a.html

Major causes of infectious uveitis

Infectious etiology was documented in at least 20-30% of all uveitis cases (Gritz *et al.*, 2004). The common causes of infectious uveitis in the western world include toxoplasmosis and herpesviruses. Less frequent are syphilis, Lyme disease and tuberculosis. In the immunocompromised person, the most frequent cause is CMV, followed by *Pneumocystis jiroveci* (formerly *P. carinii*) and toxoplasmosis, respectively (Rathinam *et al.*, 2007).

- ***Herpesviruses***

However, EBV does not seem to be a frequent cause of uveitis (Ongkosuwito *et al.*, 1998). Ocular HSV and VZV infections show various clinical presentations including blepharitis, conjunctivitis, scleritis, keratitis, anterior uveitis, necrotizing retinitis, choroiditis, and optic neuritis. In a study of anterior chamber paracentesis on a large, clinically-based cohort of patients from the Netherlands, ocular fluid obtained was analyzed for elevated concentrations of anti-HSV or anti-VZV antibodies and for the presence of HSV, VZV, or CMV DNA using polymerase chain reaction–based amplification assays. Important findings reported in this study included: 1) evidence of either HSV or VZV, infection was found in every patient tested who had both active anterior uveitis and sectoral iris atrophy; 2) HSV accounted for more than 80% of cases of anterior herpetic uveitis, even in patients with sectoral iris atrophy. Of note, however, VZV-induced iritis was more common in patients 50 years of age and older, accounting for approximately 60% of cases in this age group; and 3) both VZV- and HSV-associated anterior uveitis tended to recur on average once per year (Cunningham *et al.*, 2000). Although the prevalence of CMV retinitis is decreasing in

industrialized countries because of the widespread availability of highly active antiretroviral therapy (HAART), between 10% and 20% of HIV-infected patients worldwide can be expected to lose vision in one or both eyes as a result of ocular CMV infection (Kestelyn and Cunningham, 2001). In Africa, the CMV retinitis appears to be less severe. Prevalence rates found in the cross-sectional surveys ranged from 0%–8.5% (Heiden *et al.*, 2007).

Moreover, among eyes of AIDS patients with immune recovery, the prevalence of immune recovery uveitis (IRU) is substantial. CMV retinitis showed high risk in immune reconstitution induction and contributions to the pathogenesis progression of IRU which lead to a high risk of additional morbidity of patients with AIDS (Kempen *et al.*, 2006; Schrier *et al.*, 2006). Less frequent but important causes of bilateral vision loss in patients with HIV/AIDS include VZV and HSV retinitis, HIV-related ischemic drug reactions (Kestelyn and Cunningham, 2001). In immunosuppressive patients, dermatitis and ocular inflammatory disease are more prolonged and complications prevention is more difficult. In addition, patients with HIV/AIDS have a 15-25 times greater prevalence of herpes zoster reactivation compared to the general population. Thus, herpes zoster ophthalmicus may be the initial clinical manifestation of HIV infection and is influenced by the age of the patient. The highest rise in herpes zoster ophthalmicus prevalence is in the fifth decade of life (Wiafe *et al.*, 2003).

- ***Mycobacterium tuberculosis***

In the era of HIV/AIDS, the incidence of tuberculosis is re-emerging. This was found to be associated with uveitis worldwide, especially in Africa, West Pacific and

Eastern Europe. Mycobacterium tuberculosis can affect any structure in the eye and typically presents as granulomatous process (WebMD LLC: 1994-2011. Tuberculosis, 2006). In addition, tuberculosis (TB)-related uveitis is being increasingly reported from Southeast Asia, Western Pacific and Eastern Mediterranean regions (Singh *et al.*, 2004; Wakabayashi *et al.*, 2003; Islam *et al.*, 2002). Because 60% of patients with extrapulmonary TB have no evidence of pulmonary TB, the patients in whom absence of clinical evident pulmonary TB could not be ruled out have the possibility of ocular TB. However, the exact association of latent TB with uveitis is not known. Furthermore, the long-term outcome of patients with uveitis who receive the full course of anti-tubercular therapy for latent TB is also not known (Muccioli *et al.*, 1996; Reny *et al.*, 1996; Gupta *et al.*, 2005). Recent reports indicate that additional anti-tubercular therapy with corticosteroids in uveitis patients with latent/manifest TB led to significant reduction in recurrences of uveitis (CDC, 2003; Bansal *et al.*, 2008).

- ***Toxocara* sp.**

Toxocariasis is a world-wide zoonotic infection caused by the ascarid nematodes *Toxocara canis* and *Toxocara cati*. The migration of these nematodes in the eye, can present as strabismus, pars planitis, endophthalmitis, uveitis, retinal granuloma and retinal detachment leading to loss of visual acuity. The diagnosis of human toxocariasis currently depends on immunological examinations because it is extremely difficult to detect an infective *Toxocara* larva in biopsy samples (Snyder *et al.*, 1994). Seroprevalence of Toxocara is often lower in developed than in developing countries (Stewart *et al.*, 2005). Ocular toxocariasis is an uncommon (occurred in 1.0%) cause of uveitis that mainly affects younger patients in San Francisco (Stewart

et al., 2005). In France, 2–5% of apparently healthy adults from urban areas were *Toxocara* seropositive compared to 14.2–37% of adults from rural areas (Noordin *et al.*, 2005). The high seroprevalence of *Toxocara* as about 38% was reported from Northeast Brazil (Agular-Santos *et al.*, 2004). Whereas, 63.2% *Toxocara* seropositivity was reported in Bali and 20% in Malaysia (Noordin *et al.*, 2005). However, the frequency analysis of anti-*Toxocara* antibodies in a population survey from tropical areas with serious sanitary and socioeconomic deficiency is complicated by potential cross reactivity between a large variety of other parasites to which a given individual may have been exposed. This could lead to false-positive results (Lynch *et al.*, 1988; Fernando *et al.* , 2007).

- ***Toxoplasma gondii***

Toxoplasma gondii (*T. gondii*) leading cause of PU in the West is the protozoan parasite distributed throughout the world. The seroprevalence of toxoplasmosis differs widely in different geographic areas, but Toxoplasma infection is asymptomatic in almost all cases. Toxoplasma encephalitis and pneumonia cases have been increasing in patients with AIDS (Terazawa *et al.*, 2003; U.S. National Library of Medicine. NIH. Toxoplasmosis, 2006). The high risk of disseminated toxoplasmosis is due to immunosuppressive condition of patient as a result of immunosuppressive therapy, malignancy or AIDS. It has been estimated that 1-3% of ocular infection in AIDS patients are resulting from *T.gondii* infection (Cochereau *et al.* ,1992; Moorthy *et al.* ,1993).

3. Ocular infection and infectious uveitis in Thailand and Southeast Asia

The causes of infectious uveitis reported in South East Asia are *Mycobacterium tuberculosis*, *Toxoplasma* sp., *Histoplasma* sp., *Leptospira* sp., herpesvirues and dengue virus. However, the prevalence and outbreak of each pathogen differed among countries of South East Asia (Rathinam *et al.*, 2007).

Prior the era of highly active antiretroviral therapy (HAART), CMV was a common cause of blindness and death in patients with advanced AIDS in Western countries. CMV retinitis could be found in about one-third of AIDS patients and caused of 90% of HIV-related blindness cases (Holbrook *et al.*, 2003). In developing countries, most cases of CMV retinitis were neglected unless a patient had damaged vision. It has been estimated that 5% to 25% of all HIV-infected patients in the developing world develop blinding disorder (Kestelyn and Cunningham, 2001). In 2003, CMV retinitis was reported to be the most common sight-threatening complication in AIDS patients attending Chiang Mai University Hospital. Other ocular complications including cotton wool spot (8%), uveitis (4%), opticneuropathy (3%), and keratoconjunctivitis sicca (2%) were also presented in AIDS patients. Among infectious uveitis in the HIV-infected group, CMV retinitis is most common in patients with bilateral blindness (Ausayakhun *et al.*, 2003). In a study of the spectrum of uveitis in northern Thailand in 2008, HIV-associated uveitis was noted in 31% and it included mostly CMV retinitis. Meanwhile HSV and VZV were detected at low rates in HIV positive patients (Pathanapitoon *et al.*, 2007; Pathanapitoon *et al.*, 2008). Prevalence of CMV retinitis in HIV vary in different geographic areas such as 33% in Thailand and 17-21.4% in India (Pathanapitoon *et al.*, 2008; Rathinam *et al.*,

2007). Meanwhile, reports from other Southeast Asian countries have shown lower figures in the association of CMV infection and ocular disease (Chams *et al.*, 2009).

The Departments of Epidemiology and Statistics of the Division of Epidemiology, Ministry of Public health, Thailand reported that among of 312,429 AIDS patients who registered since 1984-2003, most were at risk to five common opportunistic infections. Those included *Mycobacterium tuberculosis* (26%), *Pneumocystis carinii* (19%), Crytoccocus (15%) and Candida (5%) (Annual epidemiological surveillance report, 2006). In addition, the investigation based on Mantoux testing and radiological chest examination in Northern Thai uveitis patients showed that tubercular uveitis was found in 4% of HIV-associated uveitis population and about 2.2% of non-HIV patients with uveitis were presumed ocular tuberculosis (Pathanapitoon *et al.*, 2008).

Southern and Eastern Asia has one of the fastest growing HIV/AIDS populations in the world. The reported prevalence of antibody to toxoplasmosis in humans and animals ranges from 2% to 75% in Southeast Asia countries including Bangladesh, Laos, Malaysia, Singapore, Thailand, Vietnam and Indonesia. However relatively low overall prevalences below 20% were found in both urban and rural areas of Japan. In the Philippines, high prevalence of 30% to 60% was found in rural settings, whereas urban areas showed low prevalence of around 10%. However, in Indonesia, an overall prevalence of 58% was found in the urban area whereas in Jakarta, the seroprevalence rate was 70% without any significant differences between males and females (Terazawa *et al.*, 2003).

It is estimated that at least 10% of adults in northern temperate countries and more than half of adults in Mediterranean and tropical countries might be infected by

T.gondii (Stanford *et al.*, 2003; Arevalo *et al.*, 2010). Up to 10% of *T.gondii* infected individuals present with retinal lesions, and this infection accounts for one-third of the world's population and is responsible for the majority of infectious uveitis cases (Zamora *et al.*, 2008; Vallochi *et al.*, 2002; Arevalo *et al.*, 2010). In some countries, up to 50% of all posterior uveitis cases in a given population are attributable to toxoplasmosis (Commondaro *et al.*, 2009; Soheilian *et al.*, 2004; Vallochi *et al.*, 2005). Ocular toxoplasmosis is characterized by recurrent attacks of chorioretinitis in usually otherwise healthy young adults. Ocular toxoplasmosis in immunocompromised patients is a very severe disease with atypical clinical manifestations and requires prolonged treatment. In 2005, in Maharaj Nakorn Chiang Mai Hospital, the Toxoplasma IgG antibody was detected in 26.0% of uveitis patients, 19.0% of uveitis patients with HIV and in 15.0% of non-uveitis controls (Wongboonma, 2005; Sirirungsi *et al.*, 2009). A year later, seroprevalence of *T.gondii* and *T.pallidum* antibody in 50 uveitis patients who attended the Ophthalmology Clinic, Maharaj Nakorn Chiang Mai Hospital was studied by indirect ELISA and immunochromatography, respectively. In this study, sera from 100 normal persons were also tested. Seroprevalence of *T.gondii* specific IgG antibody was 34% and was 18% in the normal group. Seroprevalence of *T.pallidum* antibody in uveitis patients and normal persons were similar at a low prevalence of 2%. Since the seroprevalence of *T. gondii* was shown to be significantly higher in uveitis patients than in the normal group ($p<0.05$), *T.gondii* could possibly be a major cause of uveitis in the Northern Thai population (Daidee, 2006; Sirirungsi *et al.*, 2009).

4. Laboratory investigation: Diagnosis of ocular infection

Patients with uveitis underwent an ophthalmic examination including visual acuity measurements, ophthalmic pressure measurement, slit lamp examination and fundus examination as well as photography. Those results were used for ophthalmic characterization and determination of possible cause. Although most of uveitis patients showed the typical lesion on their eyes, some had to be confirmed by using other diagnostic tools such as serological tests and molecular examination. Rapid discrimination of the infectious from the non-infectious type of intraocular inflammation is a major importance for patient management because these two conditions have entirely different treatment regimens and visual prognosis (de Groot-Mijnes *et al.*, 2006).

4.1 Serological Method

Detection of specific antibody in blood circulation is the one of the serologic tests that has clinical value for diagnosis. However, for some infections such as toxoplasma, toxocara and herpesviruses infection, the presence of circulating specific antibody is not of great importance in view of the fact that many healthy individuals also have these antibodies resulting in a low specific diagnostic value for ophthalmic diagnosis. Therefore, an analysis of intraocular fluid is required. Furthermore, active retinitis may lead to hemorrhage and sheathing of blood vessels so the circulating specific antibody may leak into the intraocular section. As a result, intraocular specific antibodies may be present. For effective ophthalmic diagnosis, the combination of the Goldmann-Witmer coefficient (GWC) and the polymerase chain

reaction (PCR) technique was proven to be of the most informative (de Groot-Mijnes *et al.*, 2006).

Goldmann-Witmer Coefficient (GWC)

GWC is the ratio of specific immunoglobin G (IgG) and total IgG that is present in intraocular fluid and in serum. This value affords a quantitative estimate of local specific antibody production. It was calculated for antibodies of the IgG type by the equation;

$$\frac{\text{(IgG in intraocular fluid / IgG in serum)}}{\text{(total IgG in serum / total IgG in intraocular fluid)}}$$

A GWC value equal to or greater than 3 is a positive for the Goldmann-Witmer analysis. This result implies that the intraocular inflammation was caused from the organism which induced that specific antibody (de Groot-Mijnes *et al.*, 2006; Kijlstra *et al.*, 1989).

4.2 Molecular Methods

4.2.1 Conventional Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) has been used as the new gold standard for a wide variety of template detection. It has been an effective tool across a wide range of scientific specialties, including microbiology. Molecular methods, essentially based on PCR, have been an indispensable tool in the diagnosis of infectious disease. PCR uses a pair of synthetic oligonucleotides or primers, each hybridizing to one strand of a double-stranded DNA (dsDNA) target, with the pair spanning a region that will be exponentially reproduced. The hybridized primer acts as a substrate for a DNA

polymerase (most commonly derived from the thermophilic bacterium *Thermus aquaticus* and called *Taq*), which generates a complementary strand by sequential addition of deoxynucleotides. The PCR process can be summarized in three steps: (1) dsDNA separation at temperature > 90°C, (2) primer annealing at 50-75°C, and (3) optimal extension at 72-78°C (Mackay *et al.*, 2002; Mackay *et al.*, 2004).

4.2.2 Real-time Polymerase Chain Reaction (PCR) technique

Recently, real-time PCR has proven to be valuable in laboratories around the world. In contrast to conventional PCR, the amplicon detection can be visualized as soon as the amplification progresses. The accumulating amplicon in real-time PCR is continuously monitored by the fluorogenic signaling detection of labeled primers, probes or amplicons. Fluorescence based detection has clear advantages over radiogenic oligoprobes; these include an avoidance of radioactive emissions, ease of disposal and extended half-life (Mackay IM. *et al.*, 2002; TIB MOLBIOL GmbH: 2009. Real-time PCR principle, 2007; Mackay *et al.*, 2004).

. In addition, real-time PCR is an important and significant step toward quantification of DNA targets. The endpoints of the PCR assay are not applicable for routine diagnosis. This technique can generate very accurate results and high standards of assay precision. However, differences in assay precision make it difficult to compare data from different laboratories without defined standards (de Boer JH. *et al.*, 1996). At present, there are several major protocols in use. These can be classified into two groups, amplicon sequencing specific and non-specific methods of real-time PCR detection. In this study, we will focus only on the hydrolysis probes or 5'-

nuclease oligoprobes, which are classified as amplicon sequence specific real-time PCR detection.

Hydrolysis probes are single-stranded oligonucleotides that can bind to the amplicon and are modified to contain a fluorophore and quencher 3 to 30 bases apart. During the amplification, the probe will be hydrolyzed by the double-stranded specific activity of Taq Polymerase resulting in a dissociation of reporter from quencher, thus the accumulating fluorescence signal is increased (TIB MOLBIOL GmbH: 2009. Real-time PCR principle, 2007; Mackay *et al.*, 2004).

Several considerations for nuclease oligoprobe design are applicable to the other linear oligoprobes to ensure that the oligoprobe has bound to the template before extension of the primers can occur, these include:

- a length of 20-40 nucleotides
- the GC content of 40-60%
- no repeat of a single nucleotide, particularly, G
- no repeated sequence motif
- an absence of hybridization or overlap with the forward or reverse primer
- the Tm at least 5°C higher than that of the primers

The fluorescence signal mirror progression of the reaction above the background noise was used as an indicator of successful target amplification. This threshold cycle (C_T) is inversely proportional to the number of target copies present in the sample, a higher number of copies of the target will result in a low C_T of positive signal detection.

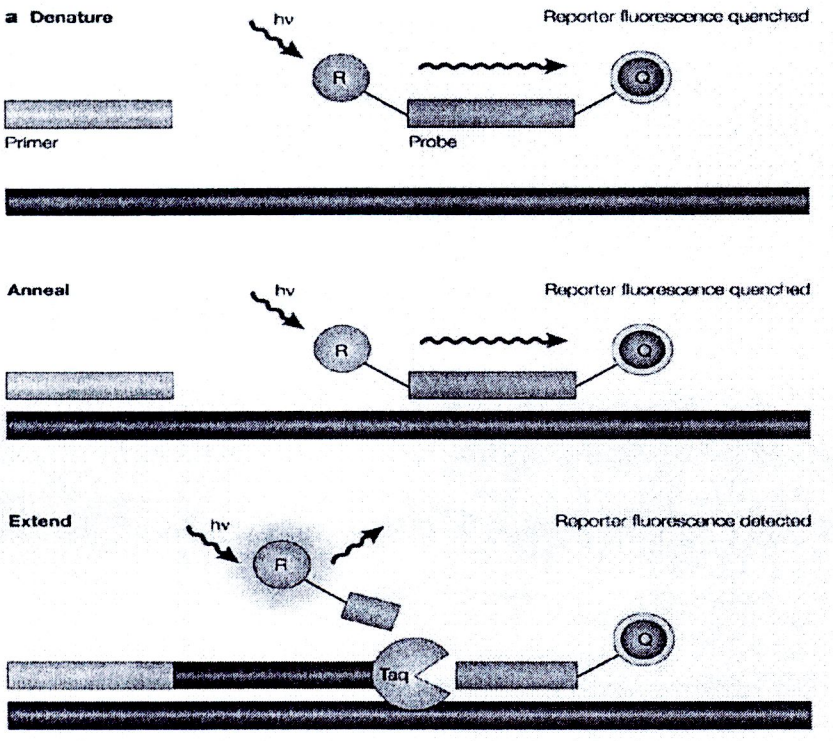


Figure 3 TaqMan hydrolysis probe principles. The 5'-nuclease activity of thermostable polymerases used in the polymerase chain reaction (PCR) cleaves hydrolysis probes during the amplicon extension step, which separates the detectable reporter fluorophore (R) from a quencher (Q). Fluorescence emitted when excited by an external light source (hν) at each PCR cycle is proportional to the amount of product formed.

Modified from Koch WH. Nature Reviews Drug Discovery 2004; 3: 749-61.

(http://www.nature.com/nrd/journal/v3/n9/images/nrd1496-f1.jpg)

Immunocompromised patients with infectious uveitis frequently have atypical clinical manifestations and require a quick and adequate antimicrobial treatment. Aqueous humor analysis using PCR and GWC analysis play a prominent role in the diagnosis of viral and *Toxoplasma* ocular infections, especially in

immunocompetent patients. These two techniques provided information on the infectious etiology and allowed early therapeutic intervention in the majority of cases (Westeneng *et al.*, 2007).

5. Treatment

Corticosteroids and cycloplegic/mydriatic agents are the mainstays of therapy for uveitis. Care should be taken to rule out an epithelial defect and ruptured eyeball when a history of trauma is elicited and to check corneal sensation and intraocular pressure to rule out HSV or VZV infection. Aggressive topical therapy with 1 % prednisolone acetate usually provides good control of anterior inflammation (Kanski JJ, 2003; Awan *et al.*, 2009). For prevention of synechia formation and reduced discomfort from ciliary spasm, 2-5% homatroprine is included. (Kanski JJ, 2003).

Non-infectious intermediate, posterior and panuveitis respond best to sub-Tenon injections of triamcinolone acetonide (Kanski JJ, 2003; Leder Awan *et al.*, 2011). Corticosteroid-sparing agents such as methotrexate, azathioprine, mycophenolate mofetil, cyclosporine, tacrolimus, cyclophosphamide, or chlorambucil are often required to treat severe or chronic forms of non-infectious inflammation, particularly when there is systemic involvement. Cataract and glaucoma are the most common complications of corticosteroid therapy (Kanski JJ, 2003).

Cycloplegic agents weaken accumulation and can be particularly bothersome to patients under 45 years of age. Because oral corticosteroid or non-corticosteroid immunosuppressive agents can cause numerous systemic

complications, dosing and monitoring are best done in close collaboration with an internist, rhematologist or oncologist (Kanski JJ, 2003).

The early distinction of infectious from non-infectious causes of uveitis is crucial for their further management. The treatment approaches for infectious and non-infectious ocular inflammation are very different. It is essential for diagnosis of intraocular infection before initiation of immunosuppressive treatment. The immunosuppressive therapy in infectious uveitis patients can be harmful, especially in areas where acquired immunodeficiency syndrome (AIDS), tuberculosis and other infectious diseases are common. However, infectious uveitis treated with antibiotics may lead to improvement or even a cure of ocular disease and might also prevent further systemic involvement (Dunn *et al.*, 2004).