INTRODUCTION

Pterygium is a common ocular surface disease in subtropical countries and is a triangular fibrovascular sub-epithelial ingrowth of degenerative bulbar conjunctival tissue over the limbus onto the cornea ***(Dushku et al., 2001).***

It typically develops in patients who have been living in hot climates and may represent a response to ultraviolet exposure and to other factors such as chronic surface dryness. Pterygium histologically, shows elastotic degenerative changes in vascularized sub-epithelial stromal collagen. Pterygia encroach onto the cornea, invading the Bowman layer ***(Milner et al., 2017)***.

The pathogenesis of pterygia seems to be multifactorial, and the variable presence of human papilloma virus suggests that it is not a required factor for lesion development ***(Sjö et al., 2007).*** Epidemiological evidence suggests that chronic sunlight exposure without ultraviolet (UV) radiation protection plays a crucial role ***(Norval et al., 2007).***

A pterygium consists of three distinct parts: the cap, the head and the body/ tail. The cap or leading edge is a flat zone on the cornea that consists mainly of fibroblasts that invade and destroy Bowman membrane the head is a vascular area that lies behind the cap and is firmly attached to the cornea. The body and tail are the mobile area of the bulbar conjunctiva, which can be easily dissected from the underlying tissue. Stocker line,

which is iron deposition in the basal layer of corneal epithelium anterior to the cap, indicates that the pterygium is chronic ***(Ardalan et al., 2010).***

Impression cytology (IC) is a method for the evaluation of superficial cell layers in the diagnosis ***(Kase et al., 2007).*** Used also for treatment follow-up of several ocular surface tumors, corneal and conjunctival diseases ***(Barros et al., 2009).***

Common histological features observed included a proliferative and locally invasive front of pterygium epithelium that abruptly transitioned into corneal epithelium at the advancing edge. At the junction between the pterygium epithelium and normal cornea, the stroma was often characterized by feeder blood vessels that preceded the fibroblastic stroma. The advancing pterygium edge was demarcated by a fragmented Bowman layer Goblet cell hyperplasia was prominent in pterygium epithelium, compared with normal conjunctiva. Feeder vessels extending the length of the lesion were regularly noted, as well as sub epithelial neovascularization. Stromal elastosis and both intra- and sub epithelial and intravascular inflammation were present in 60% of cases ***(Chui et al., 2011)***.

A graded series of ocular surface changes has been described throughout the bulbar conjunctiva. The most advanced changes occurring directly over the pterygium surface, confirming that pterygium is indeed an ocular surface disorder ***(Chan et al., 2002).***

AIM OF THE WORK

The aim of this work is to correlate the findings obtained from studying the epithelium of true pterygium by impression cytology technique and microscopical picture to find the possible changes, which may occur according to the mode of clinical presentation of the pterygium.

REVIEW OF LITERATURE

Histology of Corneal Epithelium:

The corneal epithelium is composed of four to six layers of nonkeratinized, stratified squamous epithelial cells, and in humans, it measures approximately 50 μm in thickness. The most superficial two to three layers are flat and polygonal in shape **(*DelMonte and Kim, 2011)*** with apical microvilli and microplicae, and covered by a charged glycocalyx **(*Mochizuki et al., 2010),*** which maximizes surface area with the mucinous layer of the tear film. At the cell periphery, tight junctions provide a watertight seal and assist in the prevention of pathogenic organisms from entering the cornea. Directly posterior, the wing or suprabasal cells contribute a two- to three-cell thick layer and also demonstrate tight junction complexes between cells. Basal epithelial cells represent the posterior-most layer of the corneal epithelium. Perilimbal basal epithelial cells differentiate and migrate anteriorly to repopulate the cornea; microvilli appear on the surface gradually during this process of maturation. Basal epithelial cells utilize hemidesmosomes to adhere to the underlying basement membrane and underlying stroma. The hemidesmosome, anchoring fibril, and anchoring filament complex produce an anchoring complex, which represents a common link between the intracellular cytoskeleton of the basal epithelial cell and the stroma posteriorly. The epithelial basement membrane lies

posterior to the epithelium and anterior to the corneal stroma and is laid down by basal epithelial cells ***(*Eghrari *et al., 2015).***

Histology of Epithelium at the Limbus:

The non-keratinized stratified limbal epithelium can be differentiated from the conjunctival epithelium, in that it lacks goblet cells. Compared to the corneal epithelium, while the superficial epithelial layers are rather similar, the limbal epithelium contains cell layers, a large number of mature (activated) and immature epithelial dendritic cells, T lymphocytes, highly pigmented melanocytes, and subjacent blood vessels. Moreover, the basal limbal epithelial cells are unique in that they are the least differentiated cells of the ocular surface epithelium. These cells are smaller, less columnar and have more cytoplasmic organelles. A growing body of evidence over the past years supports the theory that these cells are limbal epithelial stem cells (LESC), giving rise to the more differentiated corneal epithelium **(*Holland et al., 2013)***.

Histology of Bulbar Conjunctival Epithelium:

Conjunctival epithelial cells have fewer tight intercellular junctions. Embedded within the conjunctival epithelium are goblet cells, mucous glands, engaged in electrolyte, fluid and mucus secretion **(*Dartt, 2002)*** to form the tear film. Distribution of goblet cells in different locations of conjunctiva follows a distinct order: inferior > superior > nasal > temporal

conjunctiva ***(Ma, 1992)***. The apical surface of conjunctiva shows outfoldings called microvilli and microplicae. During tear film component secretion the conjunctival apical membrane cells are disintegrated along with secretory granules and other components into the tear film. Conjunctival apical outfoldings may aid in increasing surface area, provide support or stabilize and anchor tear film ***(Gipson and Argueso, 2003)***.

Pterygium

Pterygium takes its name from the Greek word for wing and was first described by Hippocrates. Its development is unrelated to antecedent injury or inflammation and 90% of pterygia are located nasally ***(Ganeshpuri et al., 2014).***

Pterygium is characterized as invasive, proliferative fibrovascular altered conjunctival tissue and fleshy outgrow over the cornea. It is triangular shaped fibrovascular tissue on the bulbar conjunctiva grows most commonly from the nasal aspect proliferating on the naso-temporal direction. It is linked and thought to be caused by ultraviolet radiation (UVR), low humidity, and dust ***(Coroneo, 1993).***

Pterygium consists of three distinct parts: the cap, the head and the body/tail.

The cap or leading edge is a flat zone on the cornea, preceded by fine corneal opacities with occasional Stocker line that consists mainly of fibroblasts that invade and destroy

Bowman membrane.**The head** is a vascular area that lies behind the cap and is firmly attached to the cornea. **The neck** over the limbus with upper and lower fold. **The body/tail** is the stretched mobile area of the bulbar conjunctiva, which can be easily dissected from the underlying tissue. Stocker line, which is iron deposition in the basal layer of corneal epithelium anterior to the cap, indicates that the pterygium is chronic **(*Krachmer et al., 2005*)**.

Demographics

Age:

Pterygium is more common with increasing age ***(McCarty et al., 2000).*** The incidence of pterygium rises to maximum of 32% in age group of 30-39 years and then gradually declines ***(Mithal and Sood, 1991, Cameron, 2001, Fotouhi et al., 2009, Nangia et al., 2013, and Rohatgi, 2013).***

Gender:

Male preponderance of 60%, probably because they have a greater likelihood of working outdoors or in occupations with high exposure to eye irritants ***(McCarty et al., 2000, and Fotouhi et al., 2009).***

Race:

Seems to be more prevalent among non-white persons, particularly those of African origin ***(McCarty et al., 2000, and Rohatgi, 2013).***

Geography:

The prevalence of pterygium was found to be 10.2% in the world, with highest prevalence in low altitude regions. Increased incidence of pterygium is noted in the tropics and in an equatorial zone between 30° north and south latitudes. Higher incidence is associated with chronic sun exposure ***(Khoo et al., 1998; McCarty et al., 2000; Wong et al., 2001; Al-Bdour and Al-Latayfeh, 2004 Liu et al., 2013).*** Particularly increased when there is a high surface reflectance of UV ***(Mackenzie et al., 1992).*** There was a strong protective element in the wearing of regular glasses, sunglasses, or a hat **(Mackenzie et al., 1992).**

Socioeconomic status:

Associated with low socioeconomic status. Rural areas that is 72%, maximum in fishermen then farmers(40%) followed by labourers (20%), more prevalent among those who had not used glasses or any protective measures for their eyes (84%) ***(McCarty et al., 2000; Rohatgi, 2013; and Hashemi et al., 2017).***

Etiology of pterygium

The exact factors of causation of pterygium are not clearly known. Some constants however are always present and these may have some influence in its causation. They are

environmental factors, habituation factors, age incidence and sex factors ***(Aziz and Hannot, 1986).***

Two types of factors are necessary to produce a pterygium: somatic and environmental. Among the first are inheritance, nutritional deficiencies and lacrimal hypo secretion or qualitative alteration of the lacrimal secretion. Among the second are actinic radiation, air, dust and chronic microbial infections ***(Barraquer, 1981).***

The long held belief that prolonged exposure to ultraviolet radiation has a major role in the pathogenesis of pterygium would still seem to be the most credible worldwide ***(Coroneo, 1993; Detorakis and Spandidos, 2009; Sherwin et al., 2013).***

Pathogenesis and Ultraviolet radiation

Ultraviolet radiation leads to limbal epithelial cell damage and induces the pterygium progression ***(Coroneo, 1993; Kwok and Coroneo, 1994; Coroneo et al., 1999; Threlfall and English, 1999).***

After prolonged exposure to UV light, holes appear in Bowman membrane (nerve canal) beneath the areas of epithelial necrosis, and the underlying corneal stroma becomes oedematous. These peripheral corneal changes appear to stimulate invasion by blood vessels and fibroblasts from the limbus, hastened by such factors as chronic infection or dust

which increase corneal vascularity. Subsequent organization of this fibrovascular tissue causes traction which draws the characteristic wing of conjunctival tissue onto the cornea. The presence of colander degeneration of Bowman membrane and oedema of the underlying stroma at the apex of the pterygium indicate that the degenerative process is still active and that the pterygium is progressing ***(Hiscott, 1990; McCarty et al., 2000; Paula and Thorn, 2006; Rim et al., 2013).***

The focal limbal irradiation may activate stem cells in the limbus precipitating corneal invasion by pterygium ***(Di Girolamo et al., 2003 and 2004).***

Ultraviolet (UV) increases various chemokines, cytokines and growth factors like interleukin 6 and 8 (IL-6, IL-8,) and vascular endothelial growth factor (VEGF) production in pterygium ***(Di Girolamo et al., 2006).***

The pterygium angiogenesis factors may irritate the limbal basal cells and produce vessel ingrowths to facilitate the formation of pterygium ***(Coroneo, 1993; Dushku et al., 2001).***

Pterygium is a highly vascular tissue. The vascular endothelial growth factor (VEGF) is highly expressed**.** Therefore, angiogenesis is likely to play a role in pterygium ***(Marcovich et al., 2002; and Aspiotis et al., 2007).***

The extracellular matrix remodeling is involved the pathogenesis of pterygium ***(Di Girolamo et al., 2004).***

Pterygium is regarded as a fibrotic disease and tissue growth factor beta (TGFβ) is commonly implicated in such conditions ***(Coroneo et al., 1999; Verrecchia and Mauviel, 2007).*** Pterygium demonstrates the accelerated fibroblastic proliferation ***(Liu et al., 2016).*** Pterygium fibroblasts indicate a greater growth response as well as more release of growth factors compared with normal conjunctival fibroblasts in same conditions ***(Chen et al., 1994; Kria et al., 1998).***

The fibrogenic stimuli induce the transdifferentiation from fibroblasts to myofibroblasts which were also found in fibrovascular tissues of primary and recurrent pterygium ***(Dushku and Reid, 1994; Kalluri and Neilson, 2003, Kellermann et al., 2008).***

The basal cell layer of the corneal epithelium was separated from Bowman layer by a wedge of fibroblastic tissue. The basal cells were not greatly altered by the process, but they were elevated and their normal vertical axes had become oblique. Bowman layer and variable amount of the superficial corneal stroma on the other hand were severely affected ***(Cameron, 1983).***

Genetic factors, viral infections and pterygium

UV irradiation leads to DNA damage which follows with gene mutation and genomic instability. Although the relation between pterygium and genetic factors are unclear but a family

history is frequently reported ***(Detorakis et al., 2000; Kau and Tsai, 2004; Rodrigues et al., 2008).***

The Ku70 promoter T-991C polymorphism is also a potential genetic marker for pterygium susceptibility ***(Tsai et al., 2007).***

The VEGF-460C polymorphism in female patients increases the risk of pterygium ***(Tsai et al., 2007).*** Human papilloma virus (HPV) and herpes simplex virus (HSV) were detected in pterygium in several studies ***(Karcioglu and Issa, 1997; Dushku et al., 1999; and Detorakis et al., 2000, 2001).***

Evidence suggests that familial transmission may be a factor in a small proportion of cases. The nature of an underlying genetic basis has not been explored ***(McCarty et al., 2000; Bradley, 2010)*** One family with pterygium affecting eleven members in three generations was reported. The mode of transmission in this particular family was autosomal dominance with low penetrance ***(Zhang, 1987)*** it would appear that it is not the actual lesion which is transmitted but rather the tendency of the eye to react in this way to environmental stimuli ***(Hill and Maske, 1989).***

Regarding HPV presence in human pterygium lesion. pervious studies identified HPV infection in up to 100% of pterygia samples ***(Varinli et al., 1994; Detorakis et al., 2001; Gallagher et al., 2001; Piras et al., 2003; Ateenyi-Agaba et al.,***

2004; Sjo et al., 2007; Rodrigues et al., 2008; Takamura et al., 2008; Piecyk-Sidor et al., 2009; Tsai et al., 2009; Hsiao et al., 2010; Chong et al., 2014). While others were unable to detect it at all ***(McDonnell et al., 1992; Dushku et al., 1999; Chen et al., 2003; Kuo et al., 2006; Schellini et al., 2006; Guthoff et al., 2009; Otlu et al., 2008)***.

Epstein-Barr virus (EBV) was also detected in 10% of primary pterygia patients. Therefore, viral infections may also contribute to the development of pterygium ***(Otlu et al., 2008).***

A multi-step pathogenetic process, with the participation of genetic inheritance, UV radiation and, importantly, oncogenic viral infection has been proposed for the pathogenesis of pterygium ***(Detorakis et al., 2000).***

Clinical Presentation of true Pterygium

Incidence

The majority of pterygium about 95% of cases occur on the nasal side of exposed part of bulbar conjunctiva **(Figure 1)**. The rest 5% occur in the temporal side of blubar conjunctiva **(Figure 2)**. However in uncommon occasion pterygium can occur in both nasal and temporal sides of cornea. Also not uncommonly pterygium can occur bilaterally in the two eyes of the same patient but needless to be semultaneous ***(Manhas et al., 2017).***

Clinically pterygium is presented either in a stationary form or in a progressive form. Stationary appears thin, less vascular, while Progressive: thick and more vascular it may be aggressive or non aggressive, in the aggressive type the eye is irritated with conjunctival reddness, excessive lacrimation, photophopia and may be even ocular pain. The non aggressive is usually present in a quiet eye ***(Othman and Ihab, 2009; and Džunić et al., 2010).***

Progressive pterygium is thick, fleshy and vascular showing the presence of opaque infiltrates ahead of the head of the pterygium. Atrophic pterygium is thin, with little vascularity. The presence of Stocker,s line is suggestive of long standing non-progressive pterygium ***(Othman and Ihab, 2009; and Džunić et al., 2010).***

Stationary pterygium: at some stage the pterygium may still look vascular, but the head of pterygium looks pale and sparsely vascularized and stops growing.losses its vascular appearance and develops Stocker,s line because of tears pooling at its apex and depositing iron into bowman membrane ***(Stocker, 1939).***

Regressive pterygium: a pale, thin, papery, gray, anemic and membranous pterygium appears to be regressing, but in reality the pterygium never gets smaller or disappears. Regressive pterygium has a gray apex resembling a corneal opacity ***(Thomas, 1955).***

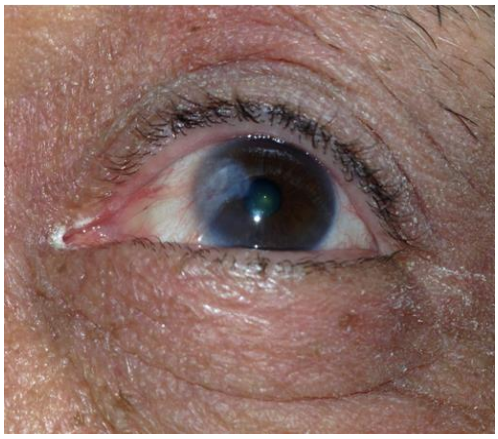

Figure (1): Case gp B No 5 classical pterygium

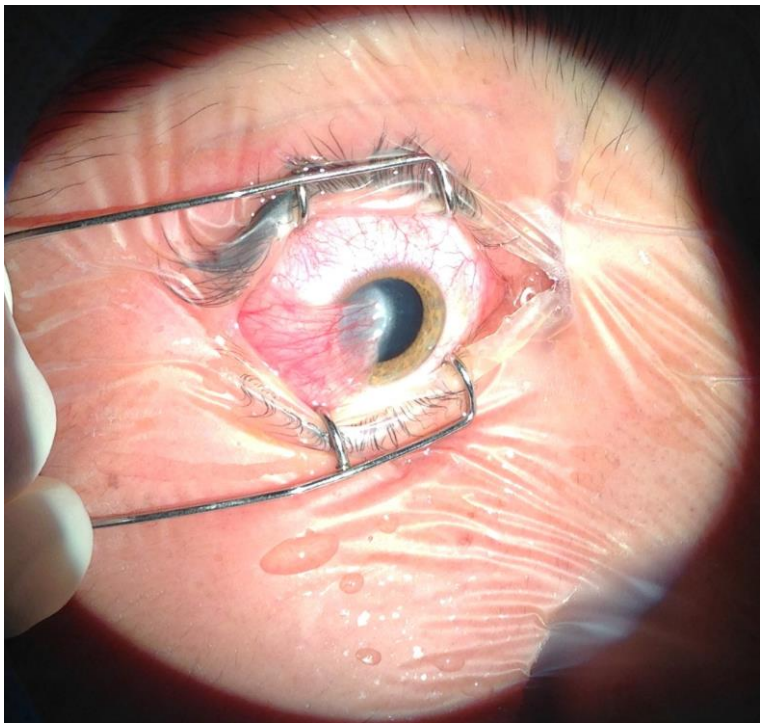

Figure (2): Case gp A No 17 temporal pterygium.

The symptoms of pterygium include persistent redness, foreign body sensation, dry eye symptoms. Pterygium may affect