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Structural Changes in Rabbit Iris Following Excimer Laser Treatment

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Refractive surgery has evolved with the advent of the excimer laser, which is capable of removing an accurate quantity of corneal tissue through photoablation. Many complications of excimer laser treatment including uveitis and relative papillary mydriasis are reported. So we aimed in this study to investigate the possible pathological effects of excimer laser on the iris tissue. This study included thirteen albino rabbits that were used and divided into 3 groups (1, 2, 3). Group 1 used as control group and groups 2, 3 were subjected to photorefractive Keratotomy (PRK) using the excimer laser unit, then decapitated after 24 h and four weeks, respectively. At the end of each experiment histopathological investigations were carried out and the iris tissue specimens were cut into semi-thin sections stained and were examined by both light and transmission electron microscopy. Many findings were demonstrated in groups 2 and 3 including the main layers of the iris which are the anterior stromal border, loose connective tissue blood vessels and the posterior epithelial cells. Mild changes occurred after 24 h which were aggravated after 4 weeks including degenerative changes in iris tissue, rupture of membrane of posterior epithelial cells, reduction of stromal cells and thickening of blood vessels. These changes were confirmed by electron transmission microscope in which the stromal cells were destroyed, fibroblasts were degenerated, disorganized melanocytes with the appearance of phagocytic and apoptotic cells. In conclusion these histopathological findings correlate with the clinical findings affecting the iris and pupil including mydriasis and uveitis

Key words: Excimer laser, iris PRK, complications, inflammation, necrosis

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INTRODUCTION

The public and ophthalmic interest in refractive surgical procedures has been greatly heightened over the past years with the advent and widespread availability of the excimer laser with its ability to precise removing submicron amounts of corneal tissue. Trokel *et al.* (1983) were the first to report the use of excimer laser to ablate corneal tissue and Munnerlyn *et al.* (1988) published an algorithm relating the diameter and depth of ablation of the required refractive correction Since then, the efficacy and safety of excimer laser treatment was the main item of many researchers (Sun *et al.*, 2005). The patient satisfaction and functional outcomes of the excimer laser treating myopic errors of patients was nearly a global interest throughout most studies (Tahzib *et al.*, 2005; Ciolino and Belin, 2006).

The incidence of complications of excimer laser treatment was discussed by many authors (Albietz *et al.*, 2005). Although there have been a number of improvements in excimer laser technology, a large body of conclusive evidence on the chances of long-term complication is not yet in place. A large number of complications affected the iris and the uvea. (Suarez *et al.*, 2002). These complications affecting the iris and uvea may cause many complications as uveitis and relative papillary mydriasis after the procedure (Geerling *et al.*, 2000).

It has been estimated that 10^{-5} of the total excimer laser energy impinging on the cornea is converted to wavelengths between 295 and 320 nm which can penetrate deeper into the eye where phytotoxic, thermal and cataractogenic effects may be generated tissue. (Maldonado *et al.*, 2001). Due to a large number of complications of excimer laser appeared to effect the uvea and iris tissue changing its normal physiological response and may produce many pathological effect on the iris tissue it self, our aim in this study was to give an evidence that excimer laser treatment may have an effect on the iris tissue itself. So we aimed in this study to report the histopathological findings of the effect of excimer laser on the iris tissue.

From the clinical point of view, it is difficult to have an iris specimen from a human case that have undergone excimer laser surgery, so our approach in this study was experimental, as we chosen to undergo our experiment on rabbit eyes, were we can easily obtain iris specimens following excimer laser treatment and correlate the evidence of our clinical work by this experimental approach. So we subjected the iris specimens of rabbits undergoing excimer laser surgery to both light and transmission electron microscopy to give a full picture of the possible effects of photorefractive keratotomy (PRK) excimer laser corneal surgery on this tissue.

MATERIALS AND METHODS

Thirteen albino rabbits weighing 2-2.5 kg were used in the study and classified into three group as follows:

Group 1: Consists of 3 rabbits and used as control group.
Group 2: Consists of 5 rabbits submitted to PRK excimer laser and decapitated after 24 h.
Group 3: Consists of 5 rabbits submitted to PRK excimer laser and decapitated after 4 weeks.

Excimer laser treatment: Animals were anesthetized, then the corneal epithelium of the rabbit eye was mechanically removed with a blade, the stroma of all treated rabbits were exposed to the same excimer laser energy (96 mj). The photoablative treatment were preformed using (NIDEKEC 5000 excimer laser machine). The laser pulse rate was fixed at 40 Hz, the optical zone diameter was (5.5 mm), the scan number was (162 scan) and the operative time was (40 sec).

After the demonstrated periods rabbits were decapitated, then the eyes were enucleated and immediately fixed in phosphate buffered glutaraldehyde. Iris slices (1×1 mm^2) were then, washed in phosphate buffer and post fixed in 1.33% osmium tetroxide, dehydrated through graded alcohols and embedded in araldite Cy_{212}. Semi-thin, 1 μm thick, sections were cut and stained with toluidine blue for light microscopy examination.

Ultra-thin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy.

RESULTS

Histopathological examination of section prepared from the iris tissue of the control group (Gr. 1) revealed the normal appearance of the iris layers (Fig. 1).

- Anterior border stromal layer (condensation of fibroblasts and melanocytes)
- The stroma was formed of loose connective tissue and blood vessels
- Posteriorly the iris was lined by epithelial cells

Light microscopic examination of the sections prepared from iris of group 2 (after 24 h of excimer laser exposure) demonstrated mild changes in the stroma where scattered fibroblasts and dilated blood vessels with mild congestion as well as focal loss of epithelial cells nuclei were seen in (Fig. 2)

Four weeks later (Gr. 3) marked degeneration changes were detected in the iris tissue, generally edema was observed in the group. The anterior border layer exhibited

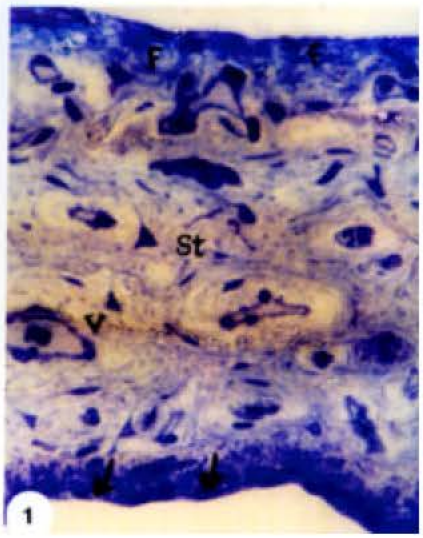

Fig. 1:Length micrograph of iris obtained from the control group showing the normal appearance of the iris (a) border stromal layer condensation of fidroblast (f), (b) loose connective tissue stroma (st) containing blood vascular channels (v), (c) posterior epithelial cells (arrows) (x 500)

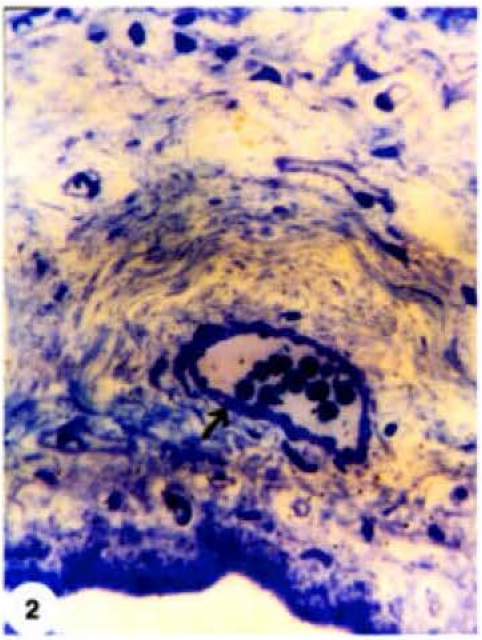

Fig. 2: Light micrograp of iris obtained from groups 2 (after 24 h of treatments) howing delatation and mild congestion of blood vessels (arrow) as well as focal loss of nuclei in the epethelial cell layer (x 500)

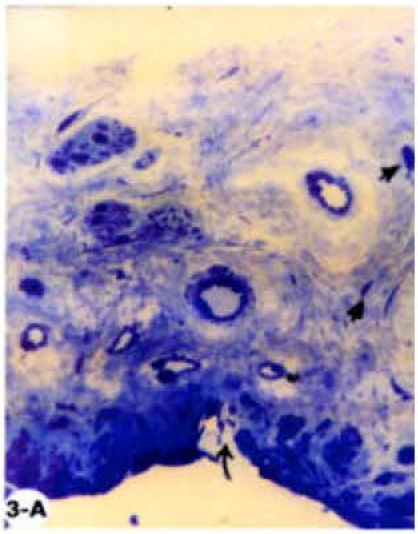

Fig. 3A: Revialing loss of anterior stromal fibroblast, stroma contains few scattered fibroblast (arrow heads). Note, ruptureof epithelium cell membrance at some areas (arrow) (x 250)

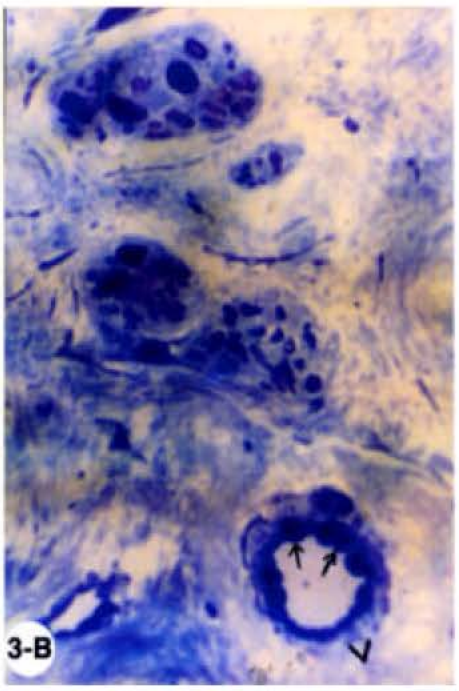

Fig. 3B: Higher magnification of the same field showing markedlythickened wall vascular channels (v) and swelling of endothelial cells (arrows). Note reduction of pericytes around the vascular channels (x 5000)

marked degenerative changes manifested by loss of anterior border (fibroblasts and melanocytes) except in few areas. Rupture of membrane of posterior epithelial cells was also observed in few areas. In addition the reduction of the stromal cells was observed (very few scattered fibroblasts). The stroma showed disintegration in many areas, marked thickening of the blood vessels walls with bulging of the endothelial cells and swelling of some pericytes around iris vessels with reduction in its number. Besides, macrophages engulfing many dense granules (variable in size) were observed. The epithelial layer appeared highly vacuolated with focal loss of their nuclei (Fig. 3a, b and 4)

By electron microscopic examination, dissociation and disintegration of collagen fiber were observed in many sections. The stromal cells were destroyed with no detailed structure except few cells, which appeared irregular in shape and contained undistinguishable organelles as well as electron dense bodies varied in size. Some fibroblasts showed signs of degeneration in the form of vacuolated cytoplasm, swollen mitochondria with loss of their cristae, dilated endoplasmic reticulum, lamellar body and homogenous chromatin. Also disorganized melanocytes and necrotic cells (broken open with disorganized release of cellular content) were observed in addition, macrophages (phagocytic cells) were showed engulfing dead cells (apoptotic cells) The blood capillaries were dilated, the lining endothelial cells and the pericytes appeared swollen with no detailed structure. The basement membrane showed rupture at one side (Fig. 5a-d and 6).

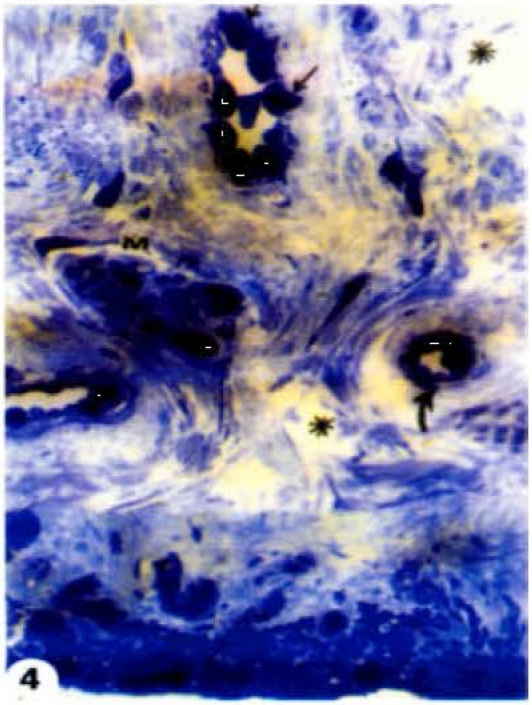

Fig. 4: Light micrograph of iris from groups 3 (another field) exhibiting vacuolization of the epithelium with focal loss of its nuclei. Disintegration of stromal collagen fiber in some parts (*), swollen of endothelial cells lining the blood vessels (arrows) as well as markedly thickened wall (curved arrow) and note also a macrophage (M) (x 500)

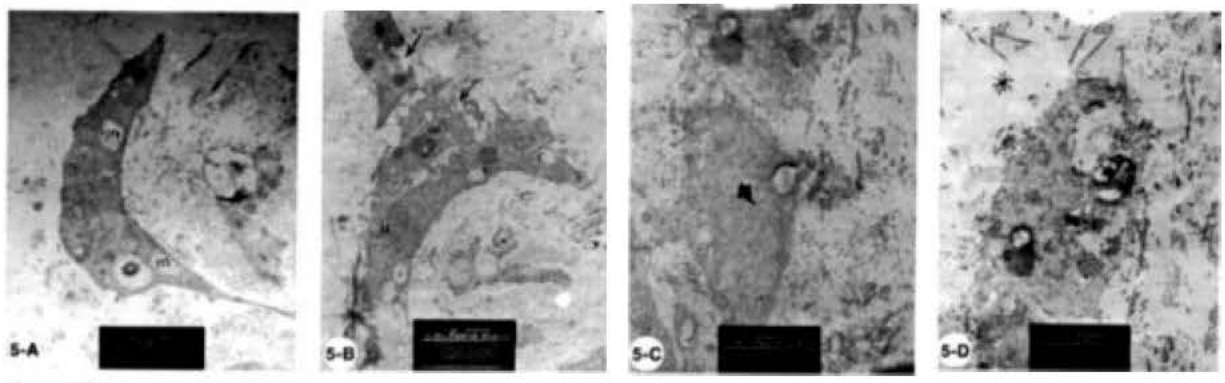

Fig. 5: Electron micrograph of iris stroma from the group 3 showing: (A) Fibroblast contain swollen degenerated mitocondoria (m) and vacuoles containing lamerllar bodies. Note, dissociation of stromal fibers into subfibil (x 13000) (B), Another fibroblast revealing vacuolated cytoplasm (C) and irregularity of nuclear membrance (a) Note, disorganized melancytes (arrow) (x 6300), (C) Swollen cell with homogenous nuclei (n), degenerated mitochondria, dilated endoplasmic reticulum and rupture of cell membrane (x 10000), (D) Disintegrated stromal collagen fibers (*), macrophages engulfing dead cells (membrance bound apoptotic bodies) Note, chromatin condensation (x 10000)

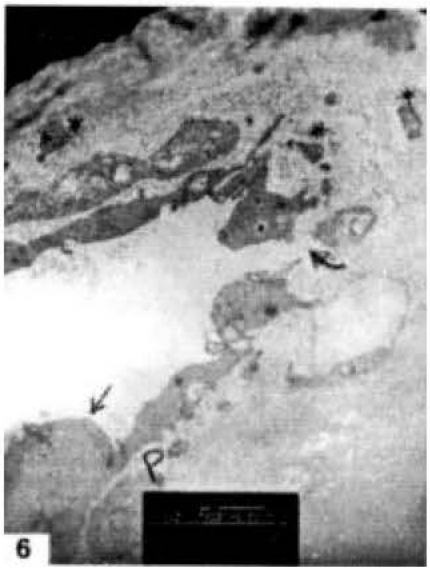

Fig. 6: Electron micrograph of the blood vessel of treated iris revealing dilation with swelling of endothelial cells (arrow), rupture of blood vessel wall (curved arrow), pyknoticpericyte (P) and electron dense microbodies (*) (x 8000)

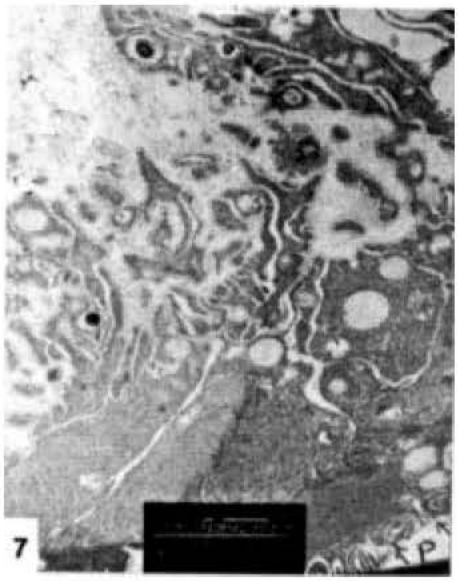

Fig. 7: Electron micrograph of the iris posterior epithelium obtained from group 3 exhibiting: Homogenous nucleus, dilation of nuclear envelop in some cells, vacuoles at different size, degenerated mitochondria, dilation of intercellular space. Note also, the posterior epithelium displays cells with numerous basal infoldings (arrow) P: Posterior chamber of the eye (x 2300)

In the posterior epithelial cells, the staining character were not the same, some cells were electron pale while others were electron dense. In the dark stained cells nucleus was crenated and its chromatin was homogenous. Moreover, many changes were observed in this layer in the form of slight widening of nuclear envelope, swollen degenerated mitochondria, as well as intracytoplasmic vacuoles of different size. Generally, the cells were separated from each other by wide space (Fig. 7).

DISCUSSION

The argon floride (Arf) excimer laser causes high powered pulses of ultraviolet light (193 nm). Part of the energy delivered is reflected, part is consumed by the corneal surgery itself and part is transmitted into the eye as secondary radiation (Wachtlin *et al*., 2000)

This successful tool has been responsible from treatment of millions world wide suffering from refractive errors.

The incidence of refractive surgery patients having unresolved complications six months after surgery has been estimated to range between 3 to 6% (Albietz *et al*., 2005). These complications included all structures of the eye as cornea, iris, uvea, lens vitreous and the retina. (Majmudar, 2004; Mirshahi *et al*., 2006; Arevalo *et al*., 2005; Netto *et al*., 2006). Many complications included the iris structure and the pupil as in night vision disturbances (Fan-Paul *et al*., 2002), relative papillary mydriasis after photorefractive keratotomy (PRK) and glare which is a commonly reported complication (Tahzib *et al*., 2005, Greerling *et al*., 2000, Nagy *et al*., 2002). Other complications of excimer lase treatment on the iris tissue included iritis and uveitis with its complications. (Suarez *et al*., 2002; Ruiz-Moreno and Alio, 2003) These studies interpretated the effect of excimer laser on the iris tissue only from the clinical point of view. As the iris is the most sensitive tissue for many types of lasers (argon, ruby and Yag) (Dannhein and Rassow, 1978). We focused in this study to correlate the clinical interpretation of several studies with the histopathological findings in the iris tissue of an animal model following its treatment by excimer laser. To our knowledge there are no previous reports about studying the possible histological effects of excimer laser on the iris tissue apart from the studies on the effect of another lasers on this tissue (Chen and Zhang, 1994; Chew *et al*., 2000). Hence this work designed to study the effect of excimer laser on the iris In the current study, the histopathological specimens of rabbit iris showed all changes that may be responsible for iris complications following excimer laser treatment. These changes included signs of inflammation and degeneration

that included the iris tissue. The degenerative inflammatory changes in the anterior border layer were in the form of cell loss (fibroblasts and melanocytes) and reduction in number of stromal cells. The electron microscopic study confirmed these changes of inflammation, degeneration and necrosis in both nucleus and cytoplasm of stromal cells were the nuclear chromatin was clumped into masses (karyorrhexis) in few cells. We have correlated our results by similar results that used excimert laser but on other tissues. This was observed in the work done by Sbimmura *et al.* (1999) on the cornea who claimed that reactive oxygen species formation (by excimer laser), such as hydroxyl radical may be responsible for the initial death of keratocytes and may also play a role by compromising cell membrane integrity or acting synergistically with other proapoptotic agents. Furthermore, keratocyte loss after excimer laser photoablation has been reported to be a result of cell destruction and cell migration (Erie *et al.*, 2005). Also changes in nuclear chromatin seen in this study by electron transmission microscopy was also seen in other studies in cases of necrosis resulting from various noxious agents as, Xrays, where Robbin and Kumar (1987) claimed that the process of necrosis is characteristic of hypoxia and death of cells. Swelling of the mitochondria and dilation of the endoplasmic retculum which was observed in this study constitute the change called cloudy Swelling due to entry of water and solutes into the organelles, that can be engendered by numerous agents which produce cell damage. More over similar results were seen by Ghadially (1982) in many conditions and he suggested that the primary cause of cell death is the intercellular water accumulation in cells subjected to toxic stress.

Ultraviolet radiation and other chemical or physical agents can cause different stresses to a tissue and can finally induce cell death by killing or indirectly by DNA, cell membrane and cell organelles damage (Micbael *et al.*, 1998). This confirmed by our results where damage of cell membranes and cell organelles were seen by electorn microscopic examination in many cells. We compared our work here by different studies that used other types of lasers on the iris tissue. A study by Huber *et al.* (1979) analysed the effect of argon laser upon iris tissue and found that the lesion is characterized by invasion of macrophages into the damaged area 24 h after irradiation.

Dixon (1987) also concluded that the clearing of damaged area by macrophages, called clump cells is the first step in the reparative process. In this study, autophagic vacuoles and phagocytosis is characterized by the engulfment of dead cells (or membrane-bound apoptotic bodies) by neighboring were demonstrated. Dissociation of stromal collagen fiber in some areas and disintegration in another one were showed in this study. Tawara and Inomata (1987) studied the effect of argon laser on iris and ciliary body and they noticed tissue defects in the form of degenerated cells and destroyed extracellular substance as well as degeneration in the cell construction of the vessels wall. The disintegration which was produced in the stroma by excimer laser may be attributed to free radical formation where many authors reported that argon fluoride excimer laser irradiation can generate species of free radicals (Brancato *et al.*, 2000). Similar results were reported by Jain *et al.* (1995) who studied the effect of free radicals on the corneal stroma and concluded that reactive Oxygen species (O_2^-, OH^-) degrade stromal macromolecules (proteoglycan and collagen) directly by scission of covalent bond and indirectly by enhancing susceptibility of hydrolytic enzymes and alter collagen-fibroblast interaction. Disintegration of collagen fiber may be also attributed to fibroblasts damage where the fibroblasts are responsible for collagen fiber formation (Netto *et al.*, 2006).

In this study electron microscope revealed swollen of endothelial cells lining blood vessels with the reduction of pericytes. As the capillary-associated pericytes serve as the chief progenitor cells for the fibroblasts, this may explain the reduction of the fibroblasts.

Also the degenerative changes in the epithelial layer have taken place in other studies that used a different type of lasers (Yag) and argon in treating animal irides (Dueker *et al.*, 1990).

Our main aim in this study is to correlate the complications of excimer laser treatment mainly on the iris tissue including glares, papillary mydriasis iritis and uveitis with the histopathological findings. Since our study is the only study available uptill now to examine the iris tissue histologically after corneal treatment by excimer laser on we can clearly correlate these histopatholgocial finding to the complications of excimer laser treatment on the iris tissue in humans.

Excimer laser in the present study, resulted in degenerative changes in the epithelial layer in the form of focal loss of nuclei and cytoplasmic vacuolar degeneration. Despite the initial success about uses of excimer laser in ophthalmology, the study of its effect on the iris has aroused cautiousness and concern about the possibility of accidental damage to the iris, which may occur inadvertently during excimer laser application to cornea.

Many studies proved the injurious effect of excimer laser on eye including the cornea (Netto *et al.*, 2006), the lens with potential risk of cataractogenesis (Wachtlin-Jetal, 2000), the choroids (Ruiz-Moreno *et al.*, 2000), retina and retinal nerve fibre layer (Roberts *et al.*, 2002; Loewenstein *et al.*, 2002).

This study clearly signifies the injurious effects of excimer laser on the iris tissue that is mainly responsible for all complication affecting the patient's iris and pupil in the form of papillary mydriasis, glare, iritis and uveitis. Excimer laser is a wonderful tool for treating refractive errors, but the potential risks of any tool must be clearly signified to start early treatment of any complication.

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