Comparing the efficiency of two prophylactic approaches in patients at risk of developing Intraoperative Floppy Iris Syndrome

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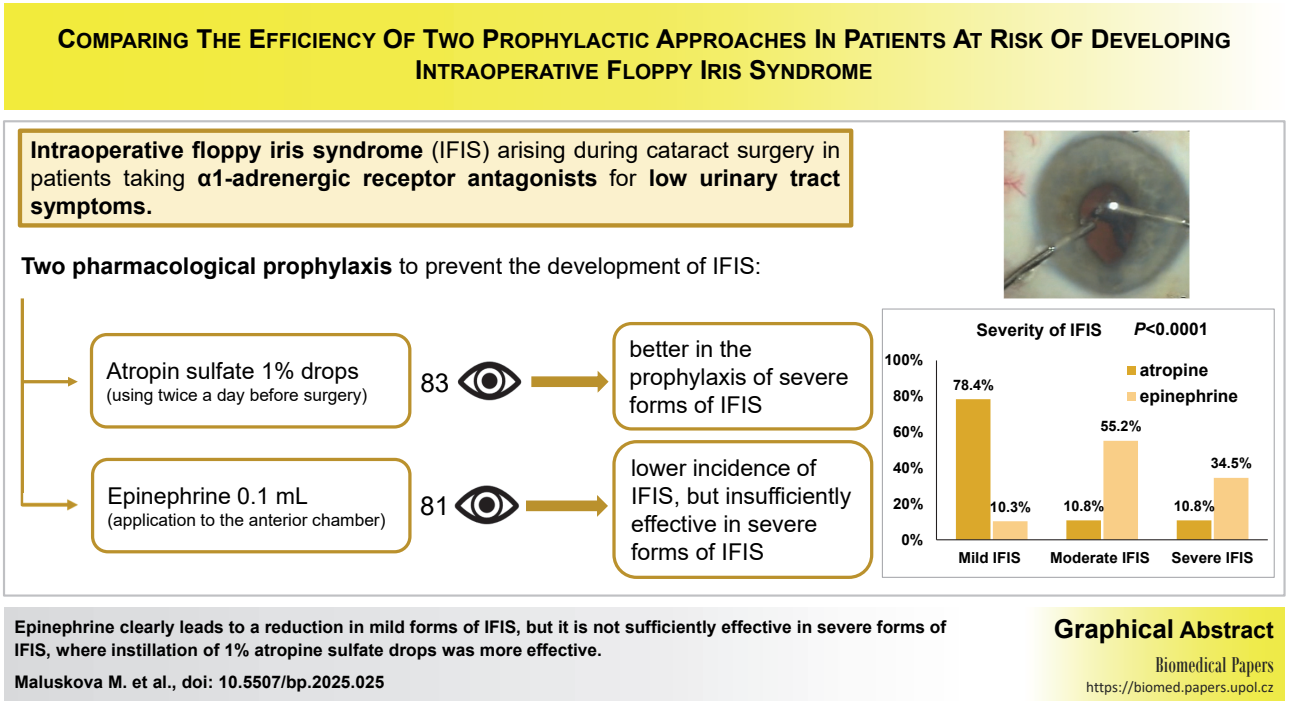
Background and Aims. Intraoperative Floppy Iris Syndrome (IFIS) is a condition that can lead to a number of complications during cataract surgery that threaten final vision of patients using alpha1-adrenergic receptor antagonists (α1-ARAs). There are two recommended pharmacological approaches to preventing IFIS development: intraoperative intracameral epinephrine and application of atropine drops twice a day for one week before surgery. The primary aim of this study was to identify the incidence and grade of IFIS using these two prophylactic approaches in patients at risk of IFIS development and to determine which approach is more effective for mild and severe IFIS. The second aim was to evaluate complication rates between the two.

Methods. The study consisted of 164 eyes of 164 men using α1-ARAs. 83 eyes were treated with atropine sulphate 1% drops (Group 1) while 81 eyes received an injection of epinephrine to the anterior chamber during cataract surgery (Group 2).

Results. Even though the incidence of IFIS was lower in the epinephrine group this was not statistically significant at the 5% level (P=0.269). Apropos severity, there was a statistically significantly higher incidence of moderate and severe forms of IFIS in Group 2 (P<0.0001). There was no significant difference between groups in the development of surgical complications (P=0.165).

Conclusions. The instillation of epinephrine was more effective in preventing the overall incidence of IFIS. Nevertheless, the occurrence of IFIS in its moderate and severe forms was statistically significantly higher in this group.

Trial registration. The study protocol was retrospectively registered in the Clinical Trial registry clinicaltrials.gov under ID number NCT06266962



Key words: IFIS, epinephrine, atropine, α1-ARAs

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INTRODUCTION

Intraoperative Floppy Iris Syndrome (IFIS) is a condition that may develop during cataract surgery. IFIS is characterised by an unstable iris whose increased elasticity may lead to a number of complications during cataract extraction with a negative impact on vision outcomes. IFIS was first described in 2005 by cataract surgeons Chang and Campbell in patients using tamsulosin. Basic features of IFIS are a "floppy" iris that "ripples" in irrigation, insufficiently inducible mydriasis with progressive intraoperative miosis (despite repeated application of mydriatics) and the tendency of the iris to prolapse toward the phacoemulsification probe and through corneal incisions[1-4]. Chang and Campbell used the presence of individual signs to distinguish three degrees of IFIS – mild, moderate and severe[5].

IFIS is most commonly associated with the chronic use of tamsulosin and other α1-ARAs prescribed in patients with low urinary tract symptoms (LUTS) (ref.[1]). However, an association with the development of IFIS has also been reported in other drugs (mainly described finasteride from the group of 5α-reductase inhibitors[3,6,7]; some antipsychotics such as chlorpromazine, zuclopenthixol or quetiapine[8-10]; some anxiolytic agents such as benzodiazepines[11]; duloxetine, mianserin and imipramine from the group of antidepressants[12-14]; some cognitive agents such as donepezil and rivastigmine[4], antihypertensive drugs such as losartan and labetalol; ropinirole as the antiparkinsonian agent[15-17]), albeit in a milder extent[3,18]. An interesting study is Tzamalis et al. from 2019, which describes angiotensin receptor antagonists as a significant risk factor for the development of IFIS in women undergoing cataract surgery. Of the total number of 1678 women included in the study, 25 of them developed IFIS (1.29%). None of these women were taking α1-ARAs. When determining the pharmacological history, all women were taking angiotensin receptor inhibitors for arterial hypertension, atrial fibrillation, heart or renal failure at the time of cataract surgery[19].

IFIS is a condition that makes surgery more difficult for the eye surgeon. There is a risk of intraoperative complications such as rupture of the posterior capsule with lens masses luxation into the vitreous body, damage to the iris by surgical instruments, damage to the endothelium with washout of endothelial cells, hyphaema, or prolapse of the vitreous body into the anterior chamber[4,20-23]. After surgery, IFIS patients have an increased risk of intraocular inflammation and cystoid macular oedema. Damage to the iris may subsequently lead to permanent deformation of the pupil and the development of photophobia[1,3,24].

It is essential to identify high-risk patients for intraoperative development of IFIS. There are several surgical approaches to prevent the development of IFIS and facilitate easier management of the entire cataract extraction in unstable iris[4,25]. One of the possible pharmacological prophylaxis is the application of atropine sulfate 1% drops twice a day for one week before a cataract surgery[26]. A more elegant method is the administration of epinephrine into the anterior chamber at the beginning of the cataract surgery. Epinephrine leads to the development of mydriasis and stabilises the iris[27]. Furthermore, mydriasis can be supported by viscoelastic material administered into the anterior chamber, iris hooks or pupil expanders[21,24,28,29].

The overall prevalence of IFIS is on the rise. An Austrian study by Wahl et al. reports 12.6%. The increasing prevalence is probably caused by population ageing and chronically used drugs. Another reason may be the higher awareness of eye surgeons about the existing syndrome and their ability to identify even milder forms of IFIS (ref.[18,30]).

The aim of this study is to evaluate the efficacy of two mydriatic agents – the administration of atropine 1% drops and the instillation of epinephrine into the anterior chamber. We ultimately compared the effectiveness of the two pharmacological approaches in preventing IFIS. Primary we identified the incidence and severity of IFIS, secondary we evaluated complication rates between the two prophylactic approaches. The aim was to determine whether any method was more effective in mild and severe forms of IFIS.

MATERIAL AND METHODS

We designed a prospective study that included men using α1-ARAs therapy for LUTS with diagnosed benign prostatic hyperplasia (BHP) and were thus at risk for intraoperative development of IFIS during cataract surgery. Between July 2020 and September 2022, we enrolled a total of 164 men aged 55 to 92 during biometric examination before cataract surgery at the Department of Ophthalmology of the University Hospital and the Faculty of Medicine in Olomouc. We divided patients into two groups based on the two prophylactic approaches. In the first half of the follow-up period we recommended to administer atropine sulphate 1% drops twice daily for one week before the surgery to 83 men at high risk of IFIS development (a 1st group). In the second half of the follow-up period, we formed a 2nd group with 81 men on α1-ARAs therapy. Patients in the 2nd group underwent the instillation of epinephrine into the anterior chamber at the beginning of cataract surgery. No preoperative preparation with atropine sulphate 1% drops was utilised in the 2nd group of patients.

We based our sample size estimation on the study by Nuzzi et al. who addressed the same issue[20]. Based on sample size estimates (alpha=0.05, beta=0.9), it was necessary to collect 62 patients, which we achieved in our study.

The monitored data included age, type of α1-ARAs used, development and severity of IFIS and incidence of intraoperative complications. We always assessed the first operated eye in each patient. Examination before cataract surgery included the measurement of refraction, assessment of best-corrected distance and near visual acuity, measurement of intraocular pressure, slit lamp examination of the anterior and posterior segment of the eye in mydriasis, and biometric measurement of the intraocular lens. Personal and pharmacological history with the focus

on diagnosed BHP and current or previous use of α1-ARAs were collected in all the patients.

The majority of patients used tamsulosin therapy (60 patients in the 1st group, 56 patients in the 2nd group). Doxazosin was used by 13 patients in the 1st group and 5 patients in the 2nd group. Terazosin was used by 2 patients from the 1st group. Silodosin was solely used by one patient in the 2nd group. Combined tamsulosin and 5α-reductase inhibitor therapy was used in 8 patients in the 1st group and 19 patients in the 2nd group.

All patients provided informed consent for participation in the study, the course of cataract surgery and the preoperative preparation.

Tropicamide 1% and phenylephrine hydrochloride 10% drops was administered three times before the cataract surgery to induce artificial mydriasis in all patients. An infusion solution of 500 ml containing BSS (balanced salt solution) and 20 mg of Gentamicin was used during the surgery. Viscoelastic material was administered into the anterior chamber in all patients to protect the endothelial cells. In the 2nd group of patients, it was standardly administered 0.1 mL (0.2 mg/mL) of epinephrine in the initial phase of the surgery.

In the study we included 4 cataract surgeons with more than 10 years of experience at a university hospital. We informed them about the high-risk patients in advance. All surgeons used the same surgical technique – phacoemulsification from the temporal or superior corneal approach and worked with Alcon Centurion Vision System and Alcon Constellation Vision System instrumentation. All were familiarized with all grades of IFIS to standardize their evaluation. We based our analysis on the IFIS classification according to Chang and Campbell from 2007. The mild form of IFIS is characterized by an unstable fluttering iris. In addition, the presence of a significant miosis or small iris prolapse into the corneal incisions is typical for the moderate form of IFIS. The severe grade of IFIS is characterized by previous signs with strong tendency for iris prolapse into the corneal incisions[8]. The IFIS classification is shown in Table 1.

The exclusion criterion for the study was the presence of any pupil deformity due to, e.g., post-traumatic condition, an iris defect of any aetiology, including status post anterior uveitis.

IBM SPSS Statistics version 23 (Armonk, NY: IBM Corp.) was used for data analysis. The Mann-Whitney U test was used to compare the epinephrine and atropine group for quantitative parameters. Chi-square or Fisher's exact tests were used to compare qualitative parameters. Data normality was evaluated using the Shapiro-Wilk test. All tests were performed at the significance level of 0.05.

RESULTS

There was no significant difference in the average age of the patients in the two groups at the time of surgery. The average age during cataract surgery was 75.0±7.5 in the 1st group and 76.3±6.7 years in the 2nd group (P=0.26). Of the total number of treated eyes, IFIS developed in 66 eyes. In the 1st group of patients, the incidence of IFIS was numerically higher compared to the 2nd group of patients (37 versus 29 eyes), but the difference was not statistically significant (P=0.27). A mild form of IFIS was more common in the 1st group (29 eyes) when compared to the 2nd group (3 eyes). On the other hand, moderate and severe forms of IFIS were more common in the 2nd group (26 eyes) when compared to the 1st group (8 eyes). A statistically significantly higher incidence of moderate and severe IFIS was demonstrated in the 2nd group of patients with epinephrine application (P=<0.0001). The data are provided in Table 2.

In 28 eyes in the 1st group of patients (75.7%) with sudden appearance of significantly fluttering iris during cataract surgery, surgeons administered 0.1 mL (0.2 mg/mL) of epinephrine into the anterior chamber to stop the progression of IFIS to a more severe form and to avoid damaging the iris with the phacoemulsification probe. In 23 of these eyes, IFIS persisted in a mild form, in 3 eyes IFIS progressed to a moderate form, and in 2 eyes there

Table 1. Classification of Intraoperative Floppy Iris Syndrome.

Grade of Intraoperative Floppy Iris Syndrome	Signs
Mild	Unstable fluttering iris
Moderate	Unstable fluttering iris + significant miosis/small iris prolapse into the corneal incisions
Severe	The presence of all signs with strong iris prolapse into the corneal incisions

Table 2. Comparison of incidence and severity of Intraoperative Floppy Iris Syndrome in both surgical groups.

		1st group (83 eyes)		2nd group (81 eyes)		P
		Number of eyes	%	Number of eyes	%	
Occurrence of IFIS[1]	Without IFIS[1]	46	55.4	52	64.2	0.269
	With IFIS[1]	37	44.6	29	35.8	
Grade of IFIS[1]	Mild	29	78.4	3	10.3	**< 0.0001**
	Medium	4	10.8	16	55.2	
	Severe	4	10.8	10	34.5	

[1]IFIS, Intraoperative Floppy Iris Syndrome.

was severe IFIS. The severity of the syndrome did not worsen in the majority of eyes after the extension of the prophylactic therapy. This difference was not statistically significant (*P*=0.70).

Intraoperative complications developed in a total of 8 patients (in 2 eyes in the 1st group and in 6 eyes in the 2nd group), the difference was not statistically significant (*P*=0.17). The data are provided in Table 3. The most common complication was rupture of the posterior capsule of the lens. This complication developed in 1 eye in the 1st group. We implanted an artificial lens into the sulcus ciliaris. In the 2nd group, the posterior capsule ruptured in 4 eyes – an artificial lens was implanted into the sulcus ciliaris in 2 eyes, in 1 eye it was implanted into the lens capsule, and in 1 case the eye was left aphakic. In the 1st group, there was substantial intraoperative hypotonia in 1 eye as a consequence of the increased outflow rate of the ventricular fluid through the corneal incisions; furthermore, there was a tendency of iris incarceration into corneal incisions. As for other complications, we noted an iris defect in 2 eyes in the 2nd group of patients. In the 1st group, both patients with complications developed a severe form of IFIS, one of them used tamsulosin, the other patient doxazosin. Regarding complications in the 2nd group, 2 patients with iris damage developed a severe form of IFIS and both were on tamsulosin therapy. A severe form of IFIS was seen in one patient with posterior capsule rupture on doxazosin therapy. The last 3 patients with the same complication were on tamsulosin therapy, one of them developed a mild form of IFIS and the other 2 patients did not develop any signs of IFIS.

DISCUSSION

Cataract extraction is one of the most common surgical procedures in ophthalmology. Cataract incidence increases with age; it affects 60–70% of the population over 70 years of age[3]. LUTS increases with the upper age limit of the ageing population. Currently, patients on α1-ARAs therapy are increasingly common in our clinical practice. Epidemiological data shows that a large pro portion of patients with BHP are indicated for cataract extraction even several years after they are started on α1-ARAs therapy[20]. It is not recommended to discontinue α1-ARAs before cataract surgery due to the risk of acute urinary retention, especially with simultaneous administration of atropine drops[21]. Preoperative discontinuation of this medication will not prevent the development of IFIS because of irreversible iris atrophy[21,22].

The examination before cataract surgery therefore requires a careful evaluation of medication, not only in men with a urological diagnosis but also in patients using antipsychotics, antidepressants, etc. (ref.[3,18,20]).

The average age of cataract extraction in our cohort corresponds with the epidemiological data on the occurrence of cataracts. Tamsulosin is one of the most commonly used α1-ARAs and is often the drug of first choice in LUTS patients[27]. Most patients in our cohort were treated with tamsulosin (72.3% in the 1st group, 69.1% in the 2nd group). Silodosin, like tamsulosin, is a highly selective α1-ARA. This drug was introduced in 2008 (ref.[31]). In our study, only 1 patient in the 2nd group used silodosin. Given its high uroselectivity with minimal systemic side effects, it was assumed that silodosin would lead to a lower incidence of IFIS. However, Christou et al. confirmed that silodosin must also be considered a significant factor for the possible intraoperative occurrence of IFIS. They verified their claim by the development of IFIS in 37.2% of patients who used silodosin at the time of cataract surgery[32]. This fact is based on the high binding affinity of silodosin to the α_{1a}-adrenergic receptor, which is 56 times greater than the α_{1b} and α_{1d} subtypes[33] (α_{1a}-adrenergic receptor is dominant in hyperplastic prostate tissue, but it is also found in the dilator pupillae muscle) (ref.[34-36]).

Our study evaluated the efficacy of two pharmacologic prophylactic approaches to prevent the development of IFIS by inducing sufficient mydriasis and restoring iris rigidity; we also assessed the rate of perioperative complications.

We initially focused on the effectiveness of atropine sulphate 1%. Its parasympatholytic effect leads to inhibition of muscarinic acetylcholinergic receptors (M3) of the sphincter pupillae muscle. In addition, it induces ciliary body relaxation, thereby moving the iris-lens diaphragm backward and reducing the pressure gradient between the anterior and posterior chambers. This results in a greater distance between the iris and the corneal incisions[37]. We subsequently assessed the effectiveness of epinephrine – it is a sympathomimetic agent stimulating α1-adrenergic receptors of the dilator pupillae muscle[35]. Nuzzi et al. describe two mechanisms of action in the development of IFIS: drug-induced receptor antagonism and anatomical modification at the level of the dilator pupillae muscle. The study describes a statistically significantly lower incidence of IFIS in patients with intracameral administration of epinephrine compared to a group with preoperative administration of 1% atropine drops (60.53% versus 86.05%, *P*=0.0115). A mild form of IFIS was less common in the epinephrine group (15.79%) compared to the atropine group (34.8%). The authors hypothesised that the mild form of IFIS is caused by the antagonistic effect of α1-ARAs on α1-adrenergic receptors of the dilator pupillae muscle. Epinephrine has a stronger affinity for

Table 3. Evaluation of the occurrence of intraoperative complications.

		1st group		2nd group		*P*
		Number of eyes	%	Number of eyes	%	
Intraoperative incidence of complications	Yes	2	2.4	6	7.4	0.17
	No	81	97.6	75	92.6	

α1-adrenergic receptors. This leads to the release of tamsulosin from the bond and an increase in the muscle tone of the pupil dilator. Atropine competitively inhibits muscarinic receptors of the iris sphincter and ciliary muscle. Its indirect effect in mydriasis induction is hypothesised to be the cause of its lower effectiveness in the prevention of mild forms of IFIS. The difference in the incidence of moderate and severe IFIS was not statistically significant in their patient cohorts. The authors consider the cause to be the atrophy at the level of the pupil dilator, where pharmacological prophylaxis does not prevent the development of IFIS[38]. Our results partially correspond to Nuzzi et al. IFIS was less common in the epinephrine group in our cohort (especially mild forms of the syndrome). However, the extent to which the advanced anatomical changes at the level of the pupil dilator muscle, limit the effect of the two mydriatic agents is not clear.

In the comparison study, patients applied atropine sulphate 1% at 40 and 20 min before cataract surgery. In terms of pharmacodynamics, the mydriatic effect of atropine sulphate 1% starts after about 40 min. For our study, we followed the recommendation of American Academy of Ophthalmology for preoperative preparation with atropine sulphate 1% twice daily for a week before surgery.

Unlike the aforementioned study, we confirmed a statistically significant difference between the two groups in favour of atropine in moderate and severe forms of IFIS. However, it must be noted that we injected epinephrine into the anterior chamber in 28 eyes of the atropine group with the aim to further support the stability of the iris and prevent the development of possible intraoperative complications. IFIS persisted in a mild form and the severity of the syndrome did not progress in the majority of eyes where combined prophylactic therapy was used.

The use of combined IFIS prophylaxis is currently a matter of discussion in the recent world literature. The first protocol of preventive combined therapy with 1% atropine administered 3 times a day 2 days before surgery followed by intracameral instillation of epinephrine was published by Masket et al. They tested 20 patients using α1-ARAs therapy. IFIS only developed in 1 case[39].

Sharon et al. also published results of much interest, comparing the effectiveness of combined prophylactic local therapy in the form of 1% atropine drops and NSAIDs (nepafenac, diclofenac) administered 3 days before the cataract surgery in a high-risk group of patients. The control group consisted of high-risk patients without the preoperative described local treatment. These authors report a statistically significantly lower incidence of IFIS in patients using combined prophylaxis compared to patients without preventive therapy (12% versus 30.4%, P=0.001) (ref.[40]). NSAIDs inhibit the action of cyclooxygenase – this enzyme is necessary for prostaglandin formation. Zanetti et al. published a randomised study on local administration of NSAIDs effectively reducing intraoperative pupil narrowing[41]. Significantly more effective prevention of progressive miosis and iris incarceration in corneal incisions was confirmed by Silverstein SM et al. The study protocol included the injection of a combination of NSAIDs (ketorolac) and epinephrine into the anterior chamber at the beginning of the surgery[42].

A main limitation of our study is the use of intracameral epinephrine in Group 1, which biases the interpretation of the results. As mentioned above, in the 28 patients who developed sudden iris flutter with a tendency to prolapse into the phacoemulsification probe, the surgeon decided to use intracameral epinephrine to avoid iris damage. Our goal was to ensure the best possible postoperative result at all costs and thus eliminate permanent photophobia, which can occur with iris damage. Aware of the expansion of prophylactic therapy in Group 1, we did not make a new division of patients into 3 groups (i.e. the atropine group, the epinephrine group and the combined prophylaxis group), because we wanted to keep the original idea of randomization.

Another limitation to this study is the range of exclusion criteria – patients with glaucoma or pseudoexfoliation syndrome and general diseases such as diabetes mellitus, hypertension and other conditions were not excluded from this study. The available literature does not allow us to evaluate the possible influence of these diagnoses on the intraoperative behaviour of the iris. Published opinions regarding the effect of hypertension on the development of IFIS are inconsistent[11,43]. No association with the development of IFIS has been statistically confirmed in other mentioned conditions[36,44]. Therefore, the presence of aforementioned diagnoses does not necessarily have to be considered an exclusion criterion. Another relative limitation of the study is the use of two surgical machines whose settings may differ slightly.

The other limitation of our study is that we did not confirm a statistically significant difference in the occurrence of IFIS between the 1st and 2nd group. As we describe above, initial estimates of the sample size were based on the study by Nuzzi et al. For alpha=0.05 and beta=0.9, the sample size estimate was 62 patients. For alpha=0.05 and beta=0.8, the sample size estimate was 46 patients. Our study met the required number of patients for both estimates. The difference in our study did not turn out to be statistically significant as the study by Nuzzi et al. had a higher incidence of IFIS. If we wanted to obtain a statistically significant difference in our study, then the ideal sample size for alpha=0.05 and beta=0.9 is 245 patients and for alpha=0.05 and beta=0.8 is 184 patients.

Four experienced cataract surgeons performed the procedures in the presented cohort, which may also be considered a weakness of our study. The subjective evaluation of syndrome severity may differ among individual operators despite the existing criteria for individual degrees of IFIS severity established by Chang and Campbell[21]. On the other hand, we only included surgeons with several years of experience in our study. We supported the generally established surgical recommendations for IFIS risk and achieved minimal rate intraoperative complications at the same time.

In our study 8 patients developed perioperative complications, 6 of these patients were on tamsulosin therapy, 2 of them were taking doxazosin. The rate of perioperative

complications in patients with IFIS on doxazosin (109 patients) and tamsulosin (52 patients) therapy is described in the study by Haridas et al.[45]. The study resulted in a significantly higher rate of perioperative complications in patients receiving tamsulosin compared with doxazosin (13.5% versus 1.9%, *P*=0.0062). Doxazosin, like other non-selective α1-ARAs, has a lower binding affinity to the α_{1A}-adrenergic receptor compared to tamsulosin, which may explain the lower incidence of IFIS and perioperative complications compared to selective α1-ARAs (ref.[5,45-47]).

In addition to pharmacological prophylactic approaches, the use of a viscoelastic material with a high concentration sodium hyaluronate is recommended in patients at high risk of developing IFIS, which acts as protection against iris incarceration in the corneal incisions and the endothelium from damage[28,29]. In terms of surgical procedures, it is recommended to create appropriate incision sizes and to entry anterior to the iris root[48]. It is also recommended to reduce the irrigation/aspiration flow with keeping the irrigation probe above the iris plane and away from the pupillary margin[40]. When implanting an intraocular lens, the cartridge should be turned with the bevelled end upwards to prevent prolapse of iris into it[49]. To create a larger pupil, we have iris hooks that dilate the pupil into a square shape. They have the disadvantage of additional separate incisions, increasing the risk of postoperative inflammation[23,24]. Pupil expansion ring is another option that stabilizes the iris while minimizing deformation. No additional incisions are needed in case of pupil expansion ring. Pupil enlargement devices are recommended for severe forms of IFIS with significant miosis[23]. The disadvantage of mechanical expanders over pharmacological approaches is their higher cost.

In our department, we mainly prefer the administration of epinephrine, mainly because of its elegant instillation and lower burden for the patient when compared to the preoperative drops.

However, our study shows that epinephrine is not sufficiently effective in severe forms of IFIS. The higher prevalence of severe forms of IFIS despite the application of epinephrine in our study is consistent with the study of Keklikci et al. Authors of the study describe that the use of epinephrine is appropriate and can be easily applied during surgery. However, it is only effective in approximately 50% of patients with IFIS and is ineffective in most of the eyes with severe forms of the syndrome[25].

The results of our study suggest that if a patient develops severe IFIS in one operated eye, it is advisable to administer atropine sulfate 1% twice daily for a week before the planned surgery of the other eye to rule out the possibility of IFIS development. However, this hypothesis needs to be supported by further studies.

CONCLUSION

There is no current clear recommendation for preventive pharmacological therapy for patients at high risk of IFIS. In the presented study, intracameral application of epinephrine was more effective in preventing mild IFIS forms. However, severe forms of IFIS were significantly more common in this group than in the atropine group.

Further education of patients and physicians about the adverse effects of α1-ARAs on the iris dilator muscle is of utmost importance. Adequate awareness may prevent possible intraoperative complications.

ABBREVIATIONS

IFIS, Intraoperative Floppy Iris Syndrome; α1-ARAs, alpha1-adrenergic receptor antagonists; LUTS, low urinary tract symptoms; BHP, benign prostatic hyperplasia; BSS, balanced salt solution; M3, muscarinic acetylcholinergic receptors; NSAIDs, nonsteroidal anti-inflammatory drugs.

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Ethic approval and informed consent: Study protocol was approved by the local Ethics Committee of University Hospital Olomouc and Faculty of Medicine, Palacky University Olomouc (Date 15 January 2024/ No.3/24). The study was performed in accordance with the tenets of the Declaration of Helsinki. The study protocol was registered in the Clinical Trial registry clinicaltrials.gov under ID number NCT06266962, https://clinicaltrials.gov/study/NCT06266962. Informed consent was obtained from all individual participants included in the study.

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