

Antimicrobial Assay of Chlorhexidine-Wetted Textile Napkins for Surgical Site Disinfection in Ocular Surgery

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ABSTRACT

Background: As a new intraoperative disinfection method, chlorhexidine-wetted textile napkins have been employed in order to cover the upper and lower eyelid edges, eyelid skin, eyelashes, lid margins and palpebral conjunctiva during phacoemulsification cataract extraction. This study was conducted to compare the antimicrobial activity of textile napkins before and after their use. **Methods:** This study evaluated 80 textile napkins wetted with 0.02% aqueous solution of chlorhexidine. All textile napkins were divided into groups. The study group consisted of 60 used textile napkins which were collected from 29 patients (30 eyes) at the end of phacoemulsification, and the control group included 20 unused sterile textile napkins. Antimicrobial assay was performed by means of measuring the growth inhibition zones of the standard or clinical isolate strains under the textile napkins on the surface of agar media. **Results:** The number of textile napkins and the diameter of the growth inhibition zones (mm) in the study group and in the control group relating to gram-positive, gram-negative, and fungi were: 24/31 vs. 8/31, 32/30 vs. 8/30, and 4/30 vs. 4/30. The diameter of the growth inhibition zones of gram-positive bacteria was more than other investigated microorganisms. In the growth inhibition zones, exogenous microorganism colonies were not found. **Conclusion:** Antimicrobial activity of textile napkins wetted with 0.02% aqueous solution of chlorhexidine against gram-positive bacteria is more than gram-negative bacteria and fungi, and is preserved to the end of the phacoemulsification.

Keywords: Chlorhexidine; Endophthalmitis; Levofloxacin; Phacoemulsification; Surgical Site Disinfection

1. Introduction

Post-operative endophthalmitis after cataract surgery is a rare but serious and potentially blinding complication [1,2]. The sources of ocular bacterial contamination are commonly the conjunctival sac and the eyelids [3,4]. Different methods of antimicrobial prophylaxis are used to prevent the post-operative infectious complications. The main idea of these methods is to eliminate the transient organism and reduce the resident flora to as low level as possible [5].

Since 2005, a new disinfection method in order to prevent microbial contamination of surgical wound during intraocular surgery has been employed in Ukraine. The components of the method are sterile textile napkins, and an antiseptic for instance, 0.02% aqueous solution of chlorhexidine (CHG) [6].

The purpose of this work is to compare the antimicrobial activity of textile napkins wetted with 0.02% CHG

before and after their use during phacoemulsification cataract extraction.

2. Materials and Methods

2.1. Patients

Thirty eyes of 29 patients with cataract were included in the study and operated by phacoemulsification cataract extraction and intraocular lens implantation. Patients underwent day surgery at the Central Kiev Ophthalmic Hospital "Eye Microsurgery Centre" by one surgeon. The exclusion criteria included systemic or local infection, conjunctivitis, blepharitis, dacryocystitis, meibomian gland dysfunction and diabetes mellitus. The eye scheduled for surgery received one drop of 0.5% levofloxacin ophthalmic solution and, 0.1% dexamethasone ophthalmic solution five times per day for four days (the last drop for an hour before surgery were administered by nurses). One hour before the surgery, the patients received the

standard eye drops to dilate the pupil. Retrobulbar anesthesia was performed in addition to the preoperative application of ocular compression.

2.2. Surgical Site Disinfection

During the preoperative procedure used chlorhexidine formulations were two-fold as follows. Firstly, 0.05% alcohol-based chlorhexidine solution which was prepared by 1:400 dilution of 20% CHG (Chlorhexidine, 1,6-di(4'-chlorophenyl-diguanido)hexane) using 70% ethanol for disinfection. Secondly, 0.02% CHG which was prepared by 1:1000 dilution of 20% CHG using sterile purified water. The solutions were prepared in the hospital laboratory with the potential concerns about quality control and safety for these solutions as their sterility, stability of pH, and shelf life. Antiseptic eye skin surface treatment was carried out twice with two fresh sterile cotton swabs soaked with 0.05% alcohol-based chlorhexidine in 2 min interval by circular movements outwards (for the left eye—clockwise, for the right—on the contrary). The brow, upper and lower eyelids, eyelashes, and adjacent forehead, nose, cheek and temporal orbital area were scrubbed for 5 min before surgery and meticulous draping was used to isolate the effected eye. Prior to beginning of surgery, the upper and lower eyelid edges, eyelid skin, eyelashes, lid margins and palpebral conjunctiva were covered with two textile napkins (Calico fabric), prepared by a punch press and sterilized by autoclave in the hospital laboratory by flushing them with 1 ml of 0.02% CHG. After two min the ocular surface was vigorously rinsed with 5 ml balanced salt solution of Sodium Chloride (BSS).

Preventing CHG toxicity carried out according to the following approaches: firstly, choosing Calico fabric which CHG has ability to connect with [5], secondly, preparing the textile napkin diameter 30 mm, thickness 1 mm that totally absorbs 6 drops of 0.02% CHG, thirdly, meticulous batting, showering the ocular surface and the textile napkins just before beginning the surgery with 5 ml BSS and lastly, using the constant flow of BSS from an anterior chamber maintainer during phacoemulsification. Thereby the expected residue of 0.02% CHG in the operative area is much lower than toxicological dose of CHG [7,8] likewise, the emergence of entry residual CHG into the anterior chamber is avoided.

2.3. Surgical Technique

Phacoemulsification cataract extraction was performed through a superior clear corneal incision with implantation intraocular lens using an injector system. During the operations additional antimicrobial treatment was not given. On completion of the surgery after sealing corneal incisions, two textile napkins were withdrawn from each

eye by sterile forceps and placed in separate sterile Petri dish with slightly opened lid. Then the lid was secured with scotch tape and immediately sent to the Microbiology Laboratory. After a subconjunctival injection of 0.5 ml dexamethasone and ceftriaxone, the conjunctival cavity was washed with 2 ml BSS, this was followed by instilling one drop of 0.5% levofloxacin ophthalmic solution into the inferior culs-de-sac and the eye was closed with aseptic eye gauze pad. The duration of operation ranged from 20 ± 5 min. Operations were completed without surgery-related complications.

2.4. Study Sample

60 used textile napkins collected at the end of the surgery were employed as study group. 20 unused sterile textile napkins wetted with 6 drops of 0.02% CHG and employed as control group. Microorganism strains were used to compare antimicrobial activity of unused textile napkins with used ones wetted with 0.02% CHG. The source of the microorganism strains for this work was formed by pure clinical patterns isolated from ocular post-operative infection, laboratory strains and standard culture collection types of microorganisms. The microorganism strains were employed for the successful accomplishment of the study viz. gram-positive bacteria, gram-negative bacteria and fungal species.

2.5. Antimicrobial Activity Assay

Antimicrobial activity assay was performed particularly on the principle of Bauer-Kirby disc-diffusion sensitivity method [9]. Relevant nutrient agars were used for microbial cultures: Muller-Hinton agar media, some of them were supplemented by 10% fetal calf serum and Sabouraud's dextrose agar. More than one textile napkin was examined for each microorganism strain. Determination of antibacterial activity was carried out using standard microbial suspension containing 10^6 CFU/ml by optical turbidity standard. 1 ml of obtained standard microbial suspension of each microbial strain was deposited on the surface of the relevant microorganism nutrient agar in Petri dishes, spread with a sterile spatula and covered all surface of medium evenly to achieve uniform spread. This was followed by keeping the Petri dishes within 15 - 20 min at ambient temperature. Then the two textile napkins from each eye (study group) or one textile napkin (control group) were superimposed with flame sterilized forceps gently onto the surface of the separate solid nutrient media and pressed them onto the surface of the medium. After placing of the textile napkins, the study and control Petri dishes with bacteria species were incubated in thermostat at 37°C during 24 h, Petri dishes with *Candida* species for 48 h at 35°C . The antimicrobial activity was evaluated by measuring the diameter of the

growth inhibition zones of corresponding microorganism under the textile napkins and around them in mm. A further step was the identification of possible appearance of microbial colonies in the growth inhibition zones of the microorganisms. For this purpose the textile napkins from the surface of nutrient media were removed, and each Petri dish was re-incubated in thermostat at 37°C during 24 h (Petri dishes with *Candida* species for 48 h at 35°C).

2.6. Ethics Approval

Approval for accessing the patient health records was obtained from the local research ethics committee. Informed consent was obtained from each patient. The study protocol and the safety and efficacy of the interventions were explained to all of the participants prior to their enrolment.

2.7. Statistical Analysis

The Mann-Whitney U-test (for small samples) and the Z-test were used to compare the studied variables. A *P* value less than 0.05 was considered statistically significant.

3. Results

The results of this study are summarised in **Table 1**.

The diameter of the growth inhibition zones of microorganism strains under the textile napkin and around them varies for different microorganisms after incubation. When removing the textile napkins from the surface of nutrient agar media, no colony was seen on their inner side. As well as after re-incubation of these media in the field of the growth inhibition zones of the microorganism strains identified at the first stage, no colonies of microorganism strain and exogenous microbial contamination were formed. No ocular toxicity associated with CHG was noted.

4. Discussion

Local preoperative antibiotic prophylaxis, sterile preparation of the skin surrounding the surgical eye with Povidone-Iodine 10%, meticulous draping of the lids and eyelashes [10], and instillation of Povidone-Iodine 5% onto the ocular surface at least 3 - 5 min prior to surgery are widely used in many countries. Many authors believe these measures have the most long-standing and highest quality which is the evidence of their efficacy [11-14]. With this approach, bacteria were isolated in the conjunctival sac or in the anterior chamber at the beginning of cataract extraction and on its completion [15,16]. Thus the duration of action of these preoperative regimens is disputed. Numerous studies have reported that after ap-

Table 1. Comparison of antimicrobial activity of textile napkins wetted with 0.02% aqueous chlorhexidine.

Microbial strains	Number of textile napkins/ The inhibition zone (mm)	
	Study group (n = 60) ^{*1}	Control group (n = 20) ^{*2}
<i>Staphylococcus aureus</i> 209p	8/31 ^{*3}	2/31
<i>Staphylococcus epidermidis</i> 1534	8/31 ^{*3}	2/31
<i>Corynebacterium diphtheriae mitis</i>	4/31 ^{*3}	2/31
<i>Corynebacterium xerosis</i>	4/31 ^{*3}	2/31
Total Gram-positive strains	24/31 ^{*4}	8/31 ^{*5}
<i>Salmonella typhimorium</i> 79	8/30 ^{*3}	2/30
<i>Klebsiella pneumoniae</i> 5758	8/30 ^{*3}	2/30
<i>Escherichia coli</i> O111	8/30 ^{*3}	2/30
<i>Pseudomonas aeruginosa</i>	8/30 ^{*3}	2/30
Total Gram-negative strains	32/30	8/30
<i>Candida albicans</i>	2/30 ^{*3}	2/30
<i>Candida tropicalis</i>	2/30 ^{*3}	2/30
Total fungal strains	4/30	4/30

^{*1}Used textile napkins, ^{*2}Unused textile napkins, ^{*3}*P* = 1 compared with the control group, ^{*4}*P* = 0 compared with the total Gram-negative strains, ^{*5}*P* = 0 compared with the total fungal strains. ^{*3}*P* = 0 compared with the total Gram-negative strains; ^{*3}*P* = 0.001 compared with the total fungal strains.

plying the various invasive techniques, including the addition of antibiotics in the balanced salt solution, or their infusion into the anterior chamber, the material taken from anterior chamber at the conclusion of operations or postoperative endophthalmitis cases may release bacteria sensitive to these antibiotics [17]. A disadvantage of the intracameral antibiotics has potential risks, such as toxic anterior segment syndrome secondary to dilution errors [18].

In this study, antiseptic was presented in operation area by noninvasive method to ensure sterile preparation during ophthalmic surgery and its antimicrobial activity was investigated directly by providing a comparative analysis of activity of textile napkins wetted with 0.02% CHG before and after their use. Main reasons for choosing 0.02% CHG are as follows. Firstly, 0.02% CHG is the most stable within the pH range 5 to 8 [5], in the pH range of 0.5% levofloxacin ophthalmic solution [19]. Secondly, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Micrococcus* sp., *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa* are sensitive to it [20] and lastly, methicillin-resistant *Staphylococcus aureus* exhibits low-level resistance to chlorhexidine [21]. Additionally, as conducted research reveal, CHG as a cationic molecule [5] binds to negatively charged cells of the oral

cavity. Thus, for a certain period of time, the oral cavity becomes a CHG reservoir, which prolongs its chemical activity in preparations [22]. The ocular surface (cornea and conjunctiva) is also negatively charged [23,24], and the paracellular space is more permeable to cations than to anions at physiological pH [25,26] consistently the ocular surface may also become a CHG reservoir, which prolongs its chemical activity in preparations. This phenomenon depathogenizes the ocular surface flora intraoperatively and postoperatively but bacteriological culture from the ocular surface may continue to be positive.

Results showed that the use of BSS during cataract surgery did not totally decrease the antimicrobial activity of the textile napkins due to CHG absorbed in the fibers of certain textile, particularly cotton, and consistently resisted removal by washing [5]. Thereby, the textile napkin virtually serves as a sustained release reservoir of CHG during phacoemulsification. Moreover, equal diameter of the growth inhibition zones before and after phacoemulsification cataract extraction also indicates that 0.5% levofloxacin ophthalmic solution instilled preoperatively is not an antagonist of 0.02% CHG. Additionally, antimicrobial activity of both unused textile napkins and used textile napkins, wetted with 0.02% CHG against gram-positive bacteria is more than gram-negative (**Table 1**), that is comparable to certain reports [5, 20]. Since microbial flora under the textile napkins mixed with flora of the conjunctival sac and the lid margin, the result of microbial culture from the conjunctival sac after withdrawing the textile napkins on completion of the surgery is disputed. Test strains colonies were not seen after incubation of Petri dishes on the surface and inner side of used textile napkins. Likewise in the growth inhibition zones after re-incubation, exogenous microorganism colonies that could contaminate used textile napkins during surgery were not seen.

5. Conclusion

The antimicrobial activity assay of CHG-wetted textile napkins indicates a persistent antimicrobial effect of a residue of CHG in the textile napkins during phacoemulsification cataract extraction. Intraoperatively, the isolation of the lid edges with 0.02% CHG-wetted textile napkins in combination with a preoperative antibacterial prophylaxis for instance 0.5% levofloxacin ophthalmic solution reliably prevents microbial surface contamination during ophthalmic surgery.

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