• Clinical Research •

# Effectiveness of oral probiotics supplementation in the treatment of adult small chalazion

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Received: 2021-02-21 Accepted: 2021-09-03

## Abstract

● **AIM:** To define the possible beneficial impact of probiotics oral supplementation on patients affected by chalazion.

● **METHODS:** Prospective comparative pilot study on 20 adults suffering from chalazion randomly divided into two groups. The first group (*n*=10) received conservative treatment with lid hygiene, warm compression, and dexamethasone/tobramycin ointment for at least 20d. The second group (*n*=10), in addition to the conservative treatment, received a mixture of probiotic microorganisms of *Streptococcus thermophilus* ST10 (DSM 25246), *Lactococcus lactis* LLC02 (DSM 29536) and *Lactobacillus delbrueckii* (DSM 16606) once a day up to 3mo. Chalazia were classified according to their size into three groups: small (<2 mm), medium (≥2 to <4 mm), or large (≥4 mm). When conservative treatment with and without probiotics supplementation failed to resolve the lesion, invasive methods were used, intralesional steroid injection in medium size chalazion and surgical incision and curettage for the largest ones.

● **RESULTS:** Medical treatment with or without probiotics supplementation was effective only on the small size chalazia. There was a significant difference in the time taken for complete resolution of small size chalazia between the two groups in favor of the patients receiving probiotics (38.50±9.04d *vs* 21.00±7.00d, *P*=0.039). Medium and large size chalazia did not respond to medical treatment with or without probiotics supplementation over the follow-up period (3mo). The treatment did not induce any complications in both groups and no recurrence of chalaziosis was recorded in both groups.

● **CONCLUSION:** The considerable difference in time taken for complete resolution of small chalazia between the two groups in favor of the experimental one confirms the presence of a gut-eye axis.

● **KEYWORDS:** microbiome; probiotics; chalaziosis; adults
**DOI:10.18240/ijo.2022.01.06**

**Citation:** Filippelli M, dell'Omo R, Amoruso A, Paiano I, Pane M, Napolitano P, Campagna G, Bartollino S, Costagliola C. Effectiveness of oral probiotics supplementation in the treatment of adult small chalazion. *Int J Ophthalmol* 2022;15(1):40-44

## INTRODUCTION

Chalazion is one of the most common inflammatory eyelid lesions diagnosed in ophthalmology, due to a block in the efflux from an oil gland[1]. It appears as a small lipogranulomatous tender swelling[2]. Although the chalazion size is generally less than 1 cm, its dimension will often vary over time; moreover, a small percentage of the chalazia can remain for weeks or months in the lid, as a non-tender lump[3]. Multiple factors are claimed in the pathogenesis of chalazion and, among these, constitutional atopic and seborrheic, hormonal, immunological, presence of irritable bowel disease, iatrogenic[4-5], infectious, mainly related to *Staphylococcus aureus* and *Cutibacterium acnes*, demodicosis (demodex mite infestation)[6], dysmetabolic factors such as vitamin A deficiency and diabetes[7-9]. The treatment options for chalazia vary from conservative methods (eyelid hygiene, warm compresses, topical medications, *i.e.*, antibiotic, corticosteroid or antibiotic corticosteroid combinations) to invasive methods (intra-lesion steroid injection and surgical incision and curettage). When the initial conservative methods fail, intralesional corticosteroid injections or incision and curettage to excise the lesion are performed. Conservative methods offer the lowest complication rate accompanied by a small success. Contrarily, invasive methods are the more effective, even if they exhibit the highest possibility of complications. In fact, the intralesional injection may be complicated by ocular

penetration of steroids, subcutaneous fat atrophy and topical depigmentation, retinal and choroidal vascular occlusion (rarely), anterior segment ischemia, intraocular pressure (IOP) elevation, visual loss[10], whereas surgical incision and curettage risks include pain, bleeding, and scarring[11].

Probiotics supplementation has proved encouraging effects against numerous enteric pathogens by triggering the activation of specific genes inside and outside the host intestinal tract[12]. Some probiotic bacteria have also shown promise in reducing the production of pro-inflammatory cytokines[13]. Specific populations of gut microbiota are specialized in the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate as the product of the fermentation of fiber-rich prebiotics. SCFAs act as key mediators of cell function in a range of local, intermediary and peripheral tissues, confirming their role as signaling molecules. In fact, SCFAs have been proven to be relevant in modulating the immune system, cell death and proliferation, and inflammatory status[14]. Significant data support a possible relationship between intestinal dysbiosis and ophthalmic diseases, such as uveitis[15], vernal keratoconjunctivitis[16], keratitis, Sjögren's syndrome, dry eye[17], open angle glaucoma, age-related macular degeneration and diabetic retinopathy[18-21]. Recently, a study has been conducted on the use of probiotics in a pediatric population affected by chalaziosis; this study showed that probiotic supplementation can boost the effectiveness of traditional therapies by promoting the full resolution of chalaziosis faster, easily and feasibly[22]. Therefore, notably any eating disorder, such as excessive intake of saturated fats, can lead to a change in the lipid composition secreted by the meibomian glands, decreasing its fluidity, making it difficult to spill the glandular secretion ending in an inflammation and chalazion formation.

The aim of this study was to verify whether chancing the intestinal microbiome with probiotics oral supplementation can increase the effectiveness of traditional conservative therapies in both obtaining a complete resolution of chalazion and reducing its recurrences.

## SUBJECTS AND METHODS

**Ethical Approval** In accordance with the Declaration of Helsinki a written informed consent was taken from the all the enrolled patients, and the ethical approval was obtained from the CTS of the Department of Medicine and Health Sciences "V. Tiberio" of Molise University, Campobasso, Italy. The trial was retrospectively registered (08/04/2020) at Clinical Trials.com as NCT04342507.

The study was conducted from February 2019 to February 2020 at the Department of Medicine and Health Sciences "V. Tiberio" of University of Molise, Campobasso (Italy). All enrolled patients underwent a complete ophthalmologic examination, including the assessment of size, duration and location of the chalazion before the recruitment. Inclusion criteria were: 1) history of sudden onset of painful inflamed mass with an unchanged size for more than 2mo; 2) location and clinical aspect of the lesion. Exclusion criteria were: 1) presence of an infection of eyelids; 2) nonpalpable chalazion; 3) chalazion duration <1mo; 4) pregnancy; 5) suspicion of malignancy; 6) comorbidities such as systemic hypertension, diabetes, chronic intestinal diseases and hormonal or cutaneous imbalances infections mostly due to demodex mite infestation, *S. aureus* and *Cutibacterium acnes*, vitamin A deficiency and personal habits (smoking, eating disorders, *etc*.).

This prospective comparative pilot study was performed on 20 patients (7 males and 13 females, age range 39-54y, mean age 48.25±4.54y) randomly divided into two groups (group A and group B). The group A consisted of 10 patients (4 males and 6 females) who received conservative treatment with tobramycin/dexamethasone ointment for at least 20d combined with the use of warm compression and lid hygiene. The group B comprised 10 patients (3 males and 7 females). In addition to the conservative treatment, group B patients, received a mixture of probiotic microorganisms once a day for up to 3mo. According to their size chalazia were classified in large (≥4 mm), medium (≥2 to <4 mm) and small (<2 mm).

When conservative treatment (with and without probiotics supplementation) failed to resolve the lesion, invasive methods were used, and specifically intralesional steroid injection in medium size chalazion and surgical incision and curettage for the largest, according to the technique described by Nabie *et al*[10].

A single dose of the active probiotic product was packaged in a sachet and consisted of a powder of $\geq 1\times10^9$ live bacteria of *Lactococcus lactis* LLC02 (DSM 29536), $\geq 1\times10^9$ live bacteria of *Lactobacillus delbrueckii* subsp. *bulgaricus* (DSM 16606) and $\geq 1\times10^9$ live bacteria of *Streptococcus thermophilus* ST10 (DSM 25246), and the bulking agent was maltodextrin (Probiotical S.p.A., Novara, Italy). Participants were advised to dissolve the powder in water or milk and to drink it once a day. The active probiotic mixture consisted of probiotic strains used in food supplement formulations which are commercially available. Flow-cytometry (ISO 19344:2015 IDF 232:2015, results $>3\times10^9$ AFU) was used to study materials (Biolab Research S.r.l., Novara, Italy) and plate count method was used to confirm target cell count (Biolab Research Method 014-06, results $>3\times10^9$ CFU). In order to ensure that minimum cell counts were maintained shelf-life was monitored. Moreover, unused sachets restored from the study were tested for their viability using AFU/CFU methods. With rare exceptions, the viability went beyond the minimum dosage essential across the study. Patients of group B were informed to keep the probiotic mixture in a refrigerator at 2℃-8℃.

**Table 1 Differences in baseline and outcome between the two groups** *n* (%)

| Characteristics | Group A | Group B | *P* |
|---|---|---|---|
| Sex (F/M) | 6 (46.15)/4 (57.14) | 7 (53.85)/3 (42.86) | 1.00 |
| Age, mean±SD (95%CI), y | 47.90±3.48 (45.41 to 50.39) | 48.60±5.58 (44.61 to 52.59) | 0.74 |
| Laterality (uinilateral/bilateral) | 5 (55.56)/5 (45.45) | 4 (44.44)/6 (54.55) | 1.00 |
| Chalazion size (mm) | | | 1.00 |
| Small (<2) | 4 (40.00) | 3 (30.00) | |
| Medium (≥2 to <4) | 4 (40.00) | 5 (50.00) | |
| Large (≥4) | 2 (20.00) | 2 (20.00) | |
| Recurrence | 0 | 0 | - |

**Statistical Analysis** Continuous variables were shown as mean±standard deviation (SD) and 95% confidence interval (95%CI). Absolute frequencies and percentages [*n* (%)] were used to present categorical variables. Associations between categorical variables and groups (group A *vs* group B) were performed by the $\chi^2$ test or Fisher's exact test when appropriate. The Shapiro-Wilk test was used to verify the normality of the distribution of continuous variables. Comparisons between group A *vs* group B and continuous variables (age and time of resolution) were analyzed by Student's *t*-test. The Cochran-Armitage exact test for trend was using to verify the association between chalazion size and groups. A $P<0.05$ was considered statistically detectable. Statistical analysis was performed using SAS version 9.4 and JMP PRO version 15.1 (SAS Institute, Cary, NC, USA).

## RESULTS

The trial was accomplished by all the participants. The mean age was matched in the two considered groups ($P=0.74$). Both groups had comparable baseline characteristics in terms of location, size and duration of the chalazion, with the exception of the sex; in fact, there was a higher prevalence in the female (Table 1).

The medical treatment with or without probiotics supplementation was effective only on the small size chalazia. However, in this subgroup a significant difference in the time required for complete resolution between group A (38.50±9.04d; 95%CI: 24.12 to 52.88d) and group B (21.00±7.00d; 95%CI: 3.61 to 38.39d) was recorded, being shorter in the second ones ($P=0.039$).

Medium and large size chalazia did not respond to medical treatment with or without probiotics supplementation over the follow-up period (3mo). In these patients, invasive methods were used, more specifically intralesional steroid injection in medium size chalazion and surgical incision, and curettage for the largest.

The treatment did not induce complications in any of the groups (Table 2). No recurrence of chalaziosis was registered in the two groups.

**Table 2 Absolute frequencies of adverse events between the two groups** *n*

| Adverse effects | Group A | Group B |
|---|---|---|
| Diarrhea | 0 | 1 |
| Constipation | 1 | 0 |
| Appetite loss | 1 | 2 |
| Increased appetite | 2 | 1 |
| Skin rash | 1 | 0 |
| Ocular discomfort | 4 | 3 |

## DISCUSSION

In our study, the conservative treatment is effective only on small chalazia. After the failure of the conservative approach on medium and large chalazia, intralesional injection of triamcinolone acetonide and incision and curettage, respectively, were performed. These invasive procedures are those with the highest success rate for each considered size subgroup[10-11].

The International Workshop on Meibomian Gland Dysfunction defines chalazion as a condition with localized meibomian gland dysfunction[23]. Histopathologically, chalazion is a lipogranulomatous reaction caused by liberated lipid. A connective tissue impermeable pseudocapsule is often present around the lesion, especially in those largest and oldest, where fibrosis easily occurs[24]. Any eating disorder, such as an over-intake of saturated fats, can make the secretion of meibomian glands less fluid. So, it becomes hard to spill the glandular secretion leading to chalazia formation. In small size lesions meibomian gland ducts, although with a reduction in flow, still work, allowing resolution through conservative treatment (antibiotic ointment containing steroid and warm compresses). Several pieces of evidence indicate that the microbiome of the ocular surface has potent immune-regulatory functions, and it is relevant in the physiologic preservation of healthy eyes and in the pathogenesis of ocular diseases[25].

Different microbial communities colonize the human ocular surface, which composition can vary with age, sex[26], as well as with other factors like alcoholism, auto-immune diseases,

hyperlipidemia[27], including dry eye syndrome, contact lens wear, antibiotics, and infection. Furthermore, alteration of the normal ocular surface microbiome may lead to ophthalmic disorders[28].

Currently several studies shown that the microbiome of different areas of the body are implicated in the pathophysiology of specific ophthalmic diseases, such as the oral microbiome and glaucoma, together with the intestinal microbiome and uveitis[29]. The different confined microbiomes are indeed linked among them through noncoding small RNAs (miRNAs) signaling activity. MicroRNAs are crucial epigenetic regulators implicated in pathologic signaling and have been found extracellularly in different body fluids. They act in a post-transcriptional fashion, playing a critical role in several biological events. Recently, Rizk and Tüzmen[30] claimed a possible cross-talk between miRNAs and the microbiota. Supporting evidence of miRNAs has already been underlined in some latest studies[31]. Interestingly, altered serum levels of miRNA-223 have been linked to microbiota dysbiosis and an upregulation of miRNA-223 was detected in the autoimmune uveoretinitis rat model[32]. Therefore, it is plausible to hypothesize a role for miRNAs in several ocular diseases. Concerning chalaziosis, it is persuasive to suppose a connection between probiotics, miRNAs and changes in the composition of the secreted fat from the Meibomian glands, making it more or less fluid. Gut microbiome dysbiosis has been associated with immune-mediated inflammatory diseases, both enteric (*e.g.*, inflammatory bowel disease) and extra-enteric (*e.g.*, multiple sclerosis, rheumatoid arthritis, psoriasis, ankylosing spondylitis, systemic lupus erythematosus)[33]. In this prospective comparative pilot study, we demonstrate that probiotics supplementation can increase the effectiveness of traditional therapies by promoting a complete resolution of the small size chalazia. The considerable difference in the time taken for complete resolution of the chalazion between the two groups in favor of the experimental one confirms the presence of a “gut-eye” axis. SCFAs, mainly butyric acid but also propionic and acetic acid produced by fermentation of dietary starches by commensal taxa, have been found to have great immunomodulatory activity[34]. Furthermore, as already described by Kugadas *et al*[35], exposure of the host to gut commensal species may serve as a priming signal to generate B-cell repertoires at sites different from the gut, such as eye-associated lymphoid tissues.

The supplementation of probiotics was composed of a mixture of live cells of *Lactococcus lactis*, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which are the most known microorganisms used. They are Gram-positive, anaerobic bacteria and several types produce not only lactic acid but also other antimicrobial substances, such as hydrogen peroxide and bacteriocins (ribosomal synthesized antimicrobial peptides with bactericidal effects)[36]. Lastly, the probiotics supplementation has proved to be safe and effective, in fact, there were no side effects related to their use.

The lack of efficacy on medium and large size lesions could be due to their histopathologic characteristics, namely to the presence of the connective tissue impermeable pseudocapsule[24], which isolates the lesion from the systemic context and therefore makes it only treatable with an invasive approach. Probably, the study conducted on chalaziosis in children gave more consistent results both for the type and size of the lesion and for a greater ease of action on the modulation of the microbiome[22].

In conclusion, oral probiotics supplementation is able to have a favorable impact on the clinical course of one of the commonest eyelid disorders, at least for the small size lesions, without inducing remarkable complications. Further studies are needed to confirm this first pilot trial.

**ACKNOWLEDGEMENTS**

The authors wish to thank the patients who participated in the study. We also gratefully acknowledge the support of Probiotical S.p.A for the assistance and for providing probiotics. Nina Vinot contributed to the English proofreading of the manuscript.

**Conflicts of Interest: Filippelli M,** None; **dell’Omo R,** None; **Amoruso A,** None; **Paiano I,** None; **Pane M,** None; **Napolitano P,** None; **Campagna G,** None; **Bartollino S,** None; **Costagliola C,** None.

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