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Research Article

CLINICAL COMPARISON OF TYMPANIC MEMBRANE PERFORATION CLOSURE WITH TRICHLOROACETIC ACID AND *APAMARGA KSHARA* (*ACHYRANTHES ASPERA* LINN. ALKALINE EXTRACT)

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ABSTRACT

Tympanic membrane perforations are broadly pathological and traumatic in origin. The fibrosed rim of perforation and medially migrated epithelial layer of the perforated membrane offers a cauterization opportunity which conspicuously breaks the epithelial barrier, dissolves necrosed tissue over growth and enhances neo membrane formation. Trichloroacetic acid chemical cautery and patching is used from decades in order to reconstitute the tympanic membrane integrity with a successful rate hovering around 85-92% in various clinical studies. Nowhere in the classical Ayurveda texts or in the modern literature *Apamarga Kshara* (*Achyranthes aspera* Linn. alkaline extract) paste cauterization is used for re-epithelialization of the tympanic membrane although its tissue generative properties and antimicrobial traits are quite well documented. 34 tympanic membrane perforation patients after ramifying them into two groups were selected for the present clinical study which intends to compare the effectiveness of tympanic membrane perforation closure by application of trichloroacetic acid vis-a-vis application of *Achyranthes aspera* alkaline extract on the margins of the perforation. *Achyranthes aspera* alkaline extract for the current clinical trial as a trial drug has its proven tissue regeneration and vasoproliferative properties which is an indispensible prerequisite in any attempt to restore the integrity of a perforated tympanic membrane, also its antimicrobial and vasoproliferative capacity also compliments the selection of *Achyranthes aspera* alkaline extract. This cauterization with *Apamarga Kshara* was found to be quite effective as the results were encouraging and can successfully be advocated as an Ayurvedic cauterization substitute for the resurfacing of the ruptured tympanic membrane.

KEYWORDS: Tympanic membrane, Trichloroacetic acid, *Apamarga Kshara*, *Achyranthes aspera*, Cauterization, Pure tone audiometery (PTA).

INTRODUCTION

Tympanic membrane perforation (TMP) is a condition as old as the human species. TMPs can result from disease (particularly infection) and trauma. Perforations can be temporary or persistent. Effect varies with size, location on the drum surface, and associated pathologic condition. Infection is the principal cause of tympanic membrane perforation (TMP) is infection. Acute infection of the middle ear may cause a relative ischemia in the drum concurrent with increased pressure in the middle ear space. This leads to a rupture of the tympanic membrane that is usually preceded by severe pain. If the perforation does not heal, it leaves a residual tympanic membrane perforation (TMP).

Traumatic perforations occur from blows to the ear, severe atmospheric overpressure, exposure to excessive water pressure (e.g. in scuba divers), and improper attempts at wax removal or ear cleaning. Traumatic perforations usually heal spontaneously, and it is preferable to wait for at least 3 weeks prior to any intervention.

Perforations result from acute otitis media and trauma heal spontaneously in the majority of cases. But if there is repeated infections or if the infection is persistent, there is less possibility of spontaneous healing of these perforations. Long-standing tympanic membrane perforations may cause conductive hearing loss and middle ear infection even if they are small. Although 88% of tympanic membrane perforations of any size heal without any interventions, the rest become chronic & require surgery. These non-healing perforations typically require myringoplasty and tympanoplasty for closure. Medical costs associated with tympanoplasty have recently compelled investigators to search for less expensive, simple non-surgical methods.

The purpose of closure of chronic dry tympanic membrane perforations is to restore the continuity of the tympanic membrane in order to improve hearing and decrease the incidence of middle ear infections. Closure isolates the middle ear from external environment and prevents contamination by exposure to pathogens and restores the vibratory area of the membrane and affords round window protection.

Closure of these perforations is gratifying to both the patient and the surgeon. The patient stands to gain as much as 25 db of hearing and in some cases, tinnitus gets relieved. Also, the patient gets a discharge free ear. During the period from the Seventeenth to the Nineteenth century, several methods have been attempted at closing the tympanic membrane perforation. At first, closure of the perforation was tried with a prosthesis. Ivory tube (Banzer 1640), rubber disc (Toynbee1853), paper disc (Blake 1887) and various other materials were used. In 1876, Roosa used cauterising agents to promote the healing of tympanic membrane perforations and he used a silver nitrate bead, while trichloroacetic acid was first advocated in 1895 by Okuneff and it still remains the most popular chemical used for this purpose[2,3]. In 1919 Joynt combined both cautery and paper patch technique and Linn used a moist cotton ball with repeated cautery at weekly intervals. Both these techniques were very effective. The method by Linn was modified and popularized by Derlacki and Wright in 1953[4]. Trichloroacetic acid with an aluminum paper foil patch technique is the control group of the present trial as it is a time tested non surgical method for the closure of perforation with a non infected middle ear.

Throughout the history of traditional medical systems, plants have always been used as ingredients in most of the treatment therapies. The use of plants in traditional health care systems is rooted on the exploitation of this biogenic pool of metabolites. *Achyranthes aspera Linn*. is one of the numerous medicinal plant species with a remarkable therapeutic potential that is commonly recognized as Prickly Chaff flower. The species belongs to the Amaranthaceae family and is widely distributed as a weed throughout the tropical and subtropical regions of the world. The plant is popular in folk remedy in traditional systems of medicine in tropical Asia and African countries. Its diverse uses in the various traditional health care systems include the treatment of fever, wound healing, tooth ache, arthritis, gynaecological disorders, urinary disorders, insect and snake bites, abdominal tumor, stomach pain and a number of other ailments[5].

Phenolic compounds of *Achyranthes aspera*, have been shown to enhance tissue regeneration responsible for superficial wounds and burn healing, have demonstrated antiseptic effects (antibacterial and antifungal) and have antioxidant properties (Bruneton, 1995). Phenolic-protein complexes have been implicated in wound healing. The complexes form a film which limits fluid loss and forms a physical barrier to microbial infections and forms insulations on damaged tissue protecting the wound from chemical harm.[6]

Kshara chikitsa (purified alkaline extract) has been in practice since 500 BC. As per Sushruta, the Kshara is prepared from 22 plants such as *Achyranthus aspera, Euphorbia nerifolia, Cassia fistula, Holarrhena antidysentrica, Adhatoda vasica, Calotropis giganteo, Sesamum indicum* etc. There are two types of *Kshara* preparation: one is *Paniya Kshara* for internal use while the other one is the *Pratisaraniya Kshara* for external use. The *Pratisaraniya kshara* is further divided into three types, i.e. *Mridu* (mild in action), *Madhya* (moderate in action) and *Tikshna* (strong in action). In this study, *Tikshna kshara* was taken into consideration for local application directly on the margins of the perforated tympanic membrane. The idea behind selection of *Achyranthes aspera* alkaline extract for the current clinical trial as a trial drug has its proven tissue regeneration property is an indispensible prerequisite in any attempt to restore the integrity of a perforated tympanic membrane, also its antimicrobial and vasoproliferative capacity also compliments the selection of *Achyranthes aspera* alkaline extract[7,8]. *A. aspera* treated animals showed collagen deposition, fibroblast proliferation and formation of epidermis. *A. aspera* showed significant ($p < 0.05$) wound healing, which was evident by wound contraction, elevation of various antioxidant enzymes, catalase, vitamin C and prohealing and biochemical parameters like hydroxyproline and protein content than the control animals.[9,10] *Apamarga Kshara* (*Achyranthes aspera* alkaline extract) is traditionally used for the treatment of hemorrhoid treatment but never has been used for the repair of tympanic membrane and hence this attempt of closure of tympanic membrane perforation by cauterization with *Apamarga Kshara* stands to be a pilot study in every sense.

MATERIALS AND METHODS

The present work is randomised, single blind, prospective, crossover and single centre study comprising patients of either sex in the age group 16-70 years. This clinical study was carried from June 2009 to July 2014. The patients for the research were selected from Department of Shalakya Tantra OPD, M.S.M. Institute of Ayurveda, B.P.S. Mahila Vishwavidyalaya, Khanpur Kalan, Haryana, India. Patients with bilateral or unilateral TMP abiding with the inclusion criterion were selected for the trial and diagnosed patients satisfying inclusion/exclusion and criteria of assessment were divided into two trial groups after having written and informed consent from the patient to participate in the study on a recorded and standardized Performa. The patient was also briefed about the research protocol, intervention, duration of trial, route of administration of drug and possible undesirable effects, prior to the consent. An official permission from institution's research ethical committee and hospital core committee was also taken before the commencement of the trial vide communication letter no. MSM/EC/SKT/2009/11. Eustachian tube patency was assessed by Valsalva's Maneuver. An Otoendoscopy/Otomicrosopy was performed to measure the size of perforation and to rule

out the presence of cholesteatoma, tympanosclerosis, large perforation, subtotal perforation, marginal perforation and pars flaccid perforation.

Statistical Analysis

The calculations performed for the present study was done by spreadsheets developed by Microsoft Excel (2007).

Selection of the patients

40 patients were selected for the clinical study intends to compare the effectiveness of tympanic membrane perforation closure by application of trichloroacetic acid vis-a-vis application of *Apamarga Kshara* (*Achyranthes aspera* alkaline extract) on the margins of the perforation for cauterization. Both unilateral and bilateral tympanic membrane perforations are taken up for the current trial. Six patents were lost in the study/drops out perhaps because of multiple application and discomfort during the procedure and hence a total of 34 patients having participated in the current clinical trial. Both pathological and traumatic perforations are included in the present trial by ramifying them into two underling groups:

1. **Trichloroacetic acid group (TCAG):** in this group a total of 16 patients were treated for the perforation of the tympanic membrane by multiple application of 50% solution of Trichloroacetic acid as a cauterization procedure on tympanic membrane margins. Patching with aluminium foil of the treated margins was done in order to provide a structured support to the newly grown tympanic membrane underneath. A time interval of 1 week is observed between two application and done as an OPD procedure.
2. ***Apamarga Kshara* group (APKG):** in this group a total of 18 patients were treated with cauterization of the tympanic membrane perforation margins repeatedly by multiple application of *Apamarga Kshara* paste. Rest of the procedure including patching and after care is same as of TCAG.

Technique observed in chemical cauterization with paper patching in both groups

The technique was carried out as an OPD procedure. For those who had bilateral perforations, one ear was treated first and the other ear was treated 6 weeks to 3 months later. For the initial application, 4% Xylocaine was used to anaesthetize the tympanic membrane by adding a few drops into a small cotton ball and placing it into the external canal wall over the surface of the tympanic membrane for about 10 min, while subsequent applications did not require local anaesthesia in most of the cases. The rim of the perforation was cauterized using cotton tipped applicator stroke by stroke dipped in 50% trichloroacetic acid until a white cauterized margin 0.5mm in width is created and the excess of the chemical was drained using a dry cotton swab under the operative microscope. Once the blanching of the rim was completed, a small sterile, thin aluminium foil impregnated with antibiotic cream was placed as a patch over the perforation. The patch acts as a splint to bridge the margins of the perforation and give flat surface. The patients were evaluated every 1 week; most of them requiring more than one application and the technique was repeated for a maximum of five times.

In case of *Apamarga Kshara* application group also the same technique is observed to cauterize the margins of the perforation although it takes a little more time to actually blanch the perforated margin rim in an attempt to resurface the tympanic membrane. Patching with aluminium foil was done in APKG also to provide a buttress, underneath of which the neo tympanic membrane is allowed to grow.

Inclusion criteria

- Patients with central perforation of tympanic membrane following Chronic Suppurative Otitis Media or traumatic perforations of tympanic membrane.
- Age group of 16-70 years.
- At least 2 months old dry perforations were included.
- Central perforations not more than 5 mm (35%) by visual assessment were included in the study.

Exclusion criteria

- History of previously done otological surgical intervention.
- Active rhinosinusitis
- Atticoantral disease
- Active discharge
- Nasopharyngeal pathology
- Eustachian tube dysfunction
- History of substance abuse and noise induced hearing loss
- Suggestion of other middle disorders existing, if any.
- Patients hypersensitive to local anaesthetic agent i.e. lignocaine.

Hearing assessment was done by Tuning fork tests and Pure Tone Audiometry (PTA) and an audiometric average was calculated in all the patients before and after the completion of the trial. Nasal Endoscopy was done to rule out any naso- pharyngeal pathology, Eustachian tube dysfunction and rhino sinusitis etc.

OBSERVATIONS AND RESULTS

Total number of 16 cases and 18 cases were registered in the TCAG and APKG respectively. Majority of the TCAG were of unilateral perforation and pathological/CSOM perforation. APKG group statistical observation reflected 13 cases of unilateral perforation and 13 cases of pathological/CSOM perforation out of 18 cases (Column chart 1). Maximum number of patients required application 2 and 3 times application of the trial drugs in both the groups under trial viz. TCAG and APKG.

Average number of application required for the closure/resurfacing of the tympanic membrane was

found to be 3.07 and 3.26 respectively in TCA and APKG (Table 1, 2). Perforation size was < 3 mm in 7 patients of TCAG and 8 patients of APKG. Hearing loss in db was considered by audiometric average parameter (average of 500,100 and 2000 Hz in Pure tone audiometery) and found to be 36 db before intervention in TCAG and 38 db in APKG. The hearing loss average was reduced to 17 db in TCAG and 18 db in APKG (Table 3). 87.5% of the perforations were successfully closed in TCAG and 83.3% in APKG. 2 patients were having residual perforation on TCAG and 3 patients were unable to having resurfacing of the tympanic membrane in APKG. All of the traumatic perforation healed in both the groups under trial (Column chart 2).

DISCUSSION

The Tympanic Membrane (TM) plays a significant role in the physiology of hearing as well as in the pathophysiology of chronic inflammatory middle ear diseases. The tympanic membrane perforations significantly impair the quality of life of patients[11]. A tympanic membrane perforation causes conductive hearing loss due to loss of ossicular coupling which is again due to loss of sound pressure difference across the tympanic membrane which provides the primary drive to the motion of the drum and ossicles. In addition, perforation causes a loss that depends on frequency, perforation size and middle ear space. Perforation induced losses are greatest at lowest frequencies. The volume of middle ear space also affects hearing. Smaller volume results in larger airborne gap. For a given sound pressure in the ear canal and a given perforation, the resulting sound pressure within the middle ear cavity is inversely proportional to the middle ear volume. So the transtympanic sound pressure difference will be smaller with smaller middle ear volumes. Identical perforations in two different ears have conductive losses that can differ by up to 20–30 db if the middle ear space volumes differ. The first recorded use of silver nitrate to stimulate closure of tympanic membrane perforations is by William Wilde in 1848[12].

The office chemical myringoplasty was introduced by Roosa in 1876 and was popularized by Derlacki in the 1950s who reported good results and the procedure came to be known as the Derlacki method[13,14]. In his method he used trichloroacetic acid to cauterize perforation followed by antibiotic sufflation and covering with sterile cotton pledgets and prescribing Euthymol ear drops. Two decades later, Derlacki reported that he and his colleagues (Shambaugh, Harrison and Clemis) at Otologic Professional Associates at Chicago and cumulatively treated 1277 pars tensa perforations between 1953 and 1972 and had successfully healed at least 1027 of them (80.4%)[15].

A large perforation of the tympanic membrane heals often into a thin atrophic scar which is devoid of the middle fibrous elements that provide structural support, having only an outer epidermis and inner mucosal layer. Although such a tympanic membrane is adequate for the purposes of sound conduction, it is vulnerable to repeated perforation by infection or eustachian tube dysfunction. The principle of closure by promotion of healing of the tympanic membrane involves inducing the fibrous layer to close the perforation prior to epithelial closure, restoring the normal anatomy of the tympanic membrane. Histopathological study of a newly formed perforation shows proliferation of squamous epithelium within 12 hours at the edge of the perforation, granulation formation within 36 hours[16], while the inner mucosa of the membrane takes several days to regenerate. As long as there is a suitably flat surface, stratified squamous epithelium grows at the rate of 1 mm a day[17].

Histopathological examination of permanent perforations showed that stratified squamous epithelium grows medially over the edge of the perforation with no raw surface, which appears to arrest the subsequent closure of the perforation[18]. Removal of this medialized epithelium forms the basis of some of the treatments for tympanic membrane perforation. Outer squamous epithelium that has grown inward across the edges must be destroyed repeatedly by cauterization to permit fibroblastic proliferation of the fibrous layer, the rim of the perforation should be kept moist as drying immediately kills the young fibroblasts, hyperemia stimulates fibroblastic proliferation and should be induced by mild irritation.

The present pilot study intends to evaluate *Apamarga Kshara* properties in tympanic membrane regenerative traits as it has traditionally used in wound healing and tissue growth. Alkaline preparations of *A. aspera* can be externally used in such skin diseases like Psoriasis, Taeniasis, Vitiligo, Non-lepromatous lesion, Fistula-in-ano, Tumor, Non-healing ulcer, Sinus, Exfoliative dermatitis, Mole, Non-elevated mole, Localized hyper pigmentation of skin, Nevus, and Abscess and Hemorrhoid. The pharmaceutical study of *Apamarga Kshara* is depicted hereby[19].

Basic chemical traits

Loss on drying at 110°C: 7.25% w/w
pH of 5% by pH paper: 10
Ash value: 67.75% w/w
Water soluble extractive: 78% w/w

Organoleptic characters

Color: Dull white
Touch: Smooth
Taste: Alkaline

Quantitative estimation

Magnesium: Not 0.092-1.045% w/w
Sodium: Not <1.000% w/w, not >28.844% w/w
Potassium: Not <0.05% w/w, not >30.54% w/w
Chloride: Not <15.09% w/w, not >19.81% w/w

Carbonate: Not <13.92% w/w, not >25..583% w/w
Sulfate: Not <12..4755 w/w, not >21.280 w/w
Calcium: Not <1.685 w/w, not >3.5185 w/w
Phosphate: Not <4.211% w/w
Iron: Not >0.263% w/w
Moisture: Not >1.414% w/w
pH value: Not <10.1, not >11.8

Cauterization with *A. aspera* alkaline extract breaks the submucosal fibrosis, excessive necrosed tissue and augments the neovascularization in order to restore the tissue health and integrity. Apart from this the pharmacological active constituents also count and bacterial and fungal growth also. The principle of cauterization is, it breaks up fibrosis, promotes granulation and new tissue formation at the margin of the perforation. The patch acts as a splint to bridge the margins of the perforation and give flat surface.

Histopathological examination of the granulation tissues in the *A. aspera* treated animals showed collagen deposition, fibroblast proliferation and formation of epidermis which probably can explain the successful outcome of the present trial and suggesting *Apamarga Kshara* as an effective and safe substitute to Trichloroacetic acid cauterization.[20]

Genistein is a phytoestrogen isoflavone that is found abundantly in *A. aspera*. Genistein has known anthelmintic, antioxidant properties and has been shown to interact with animal and human estrogen receptors. Apart from these properties, genistein has tyrosine kinase inhibitory activity mostly of the epidermal growth factor receptor (EGFR)[21]. The reason why traumatic perforation has got a 100% closure observed in both the groups can be explained by the fact that traumatic perforations being sterile one in almost all of the cases and these slit like perforation has got less margin to margin distance and the neo generated membrane as a result of cauterization has relatively less distance to cover in order to re approximate and complete the tympanic membrane anatomy. Pathological perforations on the other hand have got an intermittently discharging and an active middle ear mucosa which time and again evert/expedite the healing tympanic membrane margins making it difficult to restore the surface itself. Hearing loss was effectively reduced to less than 20 db (audiometric average of 500, 1000 and 200 Hz in PTA) in both the trail groups because of resurfacing and hence increase in the effective vibratory area of the tympanic membrane and round window shielding effect.

CONCLUSION

Removal of this medialized epithelium, freshening of the perforation margins and prevention of recurrent infection of the cauterized tissue forms the basis of tympanic membrane re-epithelialization which is well taken care of by application of *A. aspera* alkaline extract. *A. aspera* alkaline extract breaks the submucosal fibrosis, excessive necrosed tissue and augments the neovascularization in order to restore the integrity of a perforated tympanic membrane. *A. aspera* alkaline extract can safely advocated as an Ayurvedic cauterization substitute for the resurfacing of the ruptured tympanic membrane with a successful closure rate of 83.3% in comparison to 87.5%, which is observed in trichloroacetic acid, traditionally used as a chemical myringoplasty agent.

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Tables and column charts

Column chart 1: Attributes of perforation in two groups

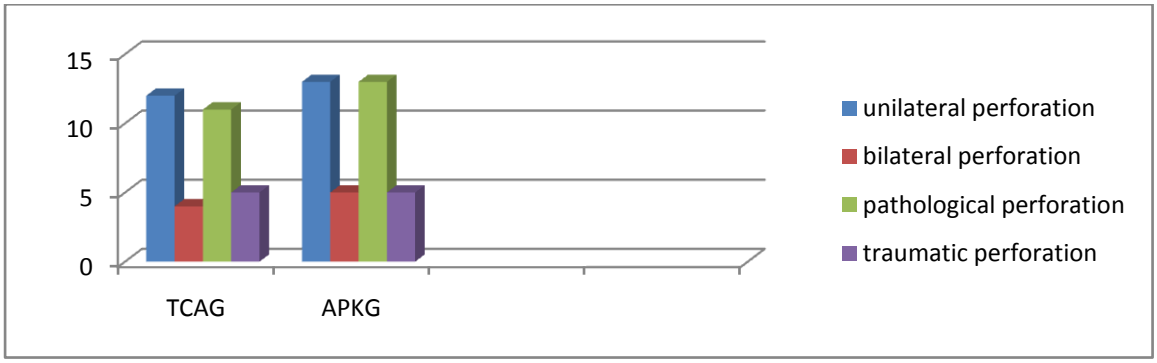

Table 2: Clinical data expression in TCAG

Total no. of application in TCAG for closure of tympanic membrane	No. of cases	Average no. of applications in TCAG
1	1	3.07
2	3	
3	6	
4	2	
5	2	
Non healing/healing failure	2	

Table 3: Clinical data expression in APKG

Total no. of application in APKG for closure of tympanic membrane	No. of cases	Average no. of applications in APKG
1	0	3.26
2	4	
3	6	
4	2	
5	3	
Non healing/healing failure	3	

Table 4: Comparative clinical outcome in hearing loss in TCAG and APKG

Attributes	TCAG	APKG
Total no. of patients	16	18
Perforation size < 3 mm	7	8
Perforation size 3-5 mm	9	10
Audiometric average (average of 500,100 and 2000 Hz in PTA) hearing loss before Trial	36db	38db
Audiometric average (average of 500,100 and 2000 Hz in PTA) hearing after before Trial	17db	18db

Column chart 2: Comparison of successfully closed Tympanic membrane in both groups