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Intralesional penicillamine in the treatment of keloids and hypertrophic scars: Open labeled therapeutic trial

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Abstract--Background: Many therapeutic options have been tried in the treatment of keloid and hypertrophic scars, but no one is superior to others. Penicillamine has been used systemically but not tested as intralesional. Objective: The aim of this study was to evaluate the effectiveness & safety of intralesional penicillamine for treatment of keloids and hypertrophic scars. Patients and Method: this is open labeled therapeutic trial study encountered in Department of Dermatology and Venereology in Al-Yarmouk teaching Hospital, Baghdad-Iraq. Collection of data was carried out during the period between May 2010 – February 2011. This study included 28 patients with keloids and hypertrophic scars. Penicillamine was prepared as a solution for intralesional injection by dissolving (250mg) of Penicillamine (Artamin)[®] by SANDOZ in (10ml) distilled water in sterilized container to obtained (2.5%) concentration of Penicillamine and autoclaved at 121C⁰ for 20 min before use. Penicillamine was administered with 27-gauge needle syringe without anesthesia. Seventy percent ethanol was used as an antiseptic agent before injection. Four sessions of intralesional injection were given at intervals of 15 days each. The size of lesions was assessed before treatment with tape measure and the size detected by taking the largest 2 diameters for irregular shaped lesions and one diameter for regular rounded lesion. Clinical response after treatment was classified according to the following scale: no response (upto10%), minimal response (10-50%), moderate response (50-75%) and very good response (>75%). Result: A total of 28 patients with keloid and hypertrophic scars were seen, their age ranged from 2 - 50 with a mean ± SD (24.82 ± 11.74) years. Female to male ratio was 1.54:1. Patients ranged between 11 and 20 years, were the most common age

group affected. Sixteen (57.14%) patients, in the present study had no symptoms related to scars, 9(32.14%) patients gave history of pruritus, and 3 (10.71%) patients gave history of tenderness. The causes of the scars in this study varied. The most common cause was trauma. This study revealed that the chest was the most commonly affected area. Positive family history accounts for 10.7% of studied cases, all were females. All patients primarily interested in improving the appearance of the scars. The number of sessions required for better response of the therapy ranged from 2-4. Five patients (17.86%) showed very good response, five patients (17.86%) showed minimal response, six patients (21.42%) moderate response and twelve (42.86%) showed no response. The respond to intra-lesional penicillamine enhance by increased the frequencies of sessions except that on ear (after piercing) which show no response to therapy even with 4 sessions. The site of the lesion was correlated to the therapeutic response and the therapeutic response was not correlated with duration of disease. No complications were detected in all patients enrolled in this study. Conclusions: Penicillamine may be an effective therapeutic agent in treating keloid & hypertrophic scars. Penicillamine is low cost, easily soluble and nontoxic.

Keywords---Intralesional penicillamine, Keloid, Hypertrophic scars.

Introduction

Keloids are benign dermal fibroproliferative tumors with no malignant potential. The first description was recorded in the Smith papyrus regarding surgical techniques in Egypt around 1700 BC. ⁽¹⁾ in 1806, Alibert coined the term cancrroid (crab-like) because the lateral extensions of the lesion into normal tissue resembled the legs of a crab. ⁽²⁾ Keloids are found only in humans and occur in 5-15% of wounds. They tend to affect both sexes equally, although a higher incidence exists of women presenting with keloids, possibly secondary to the cosmetic implications associated with the disfigurement. The frequency of keloid occurrence in persons with highly pigmented skin is 15 times higher than in persons with less pigmented skin. The average age at onset is 10-30 years. Persons at the extremes of age rarely develop keloids. ⁽³⁾

Etiology

Despite numerous studies, no uniformly accepted theory or explanation indicates which factor initiates keloid or hypertrophic scar formation. ⁽⁴⁾ Race ⁽³⁾ Genetics ⁽⁵⁾ hormones ⁽⁶⁾ Immunologic alterations have been demonstrated in abnormal scars. ⁽⁷⁾ Additionally, decreased tumor necrosis factor and interleukin 1 levels have been found in these abnormal scars. ⁽⁸⁾

Histology

Keloids have a normal epidermal layer; abundant vasculature; thickened dermis; and increased inflammatory-cell infiltrate when compared with normal scar tissue

histologic features that are consistently found in keloid specimens that are deemed pathognomonic for their diagnosis are: presence of keloidal hyalinized collagen, a tongue like advancing edge underneath normal-appearing epidermis and papillary dermis, horizontal cellular fibrous bands in the upper reticular dermis, prominent fascia like fibrous bands. ⁽⁹⁾

Differential diagnosis

Dermatofibrosarcoma protuberans. ⁽¹⁰⁾ Allergic contact dermatitis secondary to gold earrings may produce keloidal lesions on the earlobes, keloidal blastomycosis. ⁽¹¹⁾

Laboratory Studies

Diagnosis is usually based on clinical findings. Biopsy may confirm the diagnosis in equivocal cases. ⁽¹²⁾

Treatment of Keloids and Hypertrophic Scars:

Keloid and hypertrophic scars have affected patients and frustrated physicians for centuries. ⁽¹³⁾ Over the past four decades, there has been considerable clinical and experimental research looking at the biological nature and therapeutic response of keloid and hypertrophic scarring. ⁽¹⁴⁾ No single therapeutic modality is best for all keloids. The location, size, and depth of lesion; the age of the patient; and the past response to treatment determine the type of therapy used. ⁽¹²⁾

Prevention is key, but therapeutic treatment of hypertrophic scars and keloids includes occlusive dressings, compression therapy, intralesional corticosteroid injections, cryosurgery, excision, radiation therapy, laser therapy, interferon (IFN) therapy, 5-fluorouracil (5-FU), doxorubicin, bleomycin, verapamil, retinoic acid, imiquimod 5% cream, tamoxifen, tacrolimus, botulinum toxin, and over-the-counter treatments (onion extract; combination of hydrocortisone, silicon, and vitamin E). ⁽¹²⁾

Other promising therapies include antiangiogenic factors, including vascular endothelial growth factor (VEGF) inhibitors (bevacizumab), phototherapy (photodynamic therapy [PDT], UVA-1 therapy, narrowband UVB therapy), transforming growth factor (TGF)-beta3, tumor necrosis factor (TNF)-alpha inhibitors (etanercept), and recombinant human interleukin (rhIL-10), which are directed at decreasing collagen synthesis. ⁽¹²⁾

Penicillamine

Dr. John Walshe (1956) first described the use of penicillamine in Wilson's disease. ⁽¹⁵⁾ Penicillamine is an effective chelator of copper, mercury, zinc, and lead, and other heavy metals to form stable, soluble complexes that are readily excreted in the urine. ⁽¹⁶⁾

Mechanism of Inhibition of Collagen Cross linking by Penicillamine

It is known that the cross-linking of collagen fibrils can be prevented by the use of agents which have their effect either by inhibiting the enzyme, lysyl oxidase, or by binding to the aldehydes produced as a result of its action. Beta-aminopropionitrile (BAPN), aminoacetonitrile, beta-mercaptoethylamine, dithiothreitol, isoniazid, iproniazid, carbonyl reagents, disulfhydryls and diamines are all known to cause in vitro inhibition of lysyl oxidase. ⁽¹⁷⁾ D-penicillamine is known to have two effects on the collagen cross-linking process: to reversibly inhibit the lysyl oxidase; and to irreversibly bind to the aldehydes generated as a result of its action. BAPN and d-penicillamine apparently have the best in vivo effects of the above mentioned agents, and, therefore, have been the most closely investigated. ⁽¹⁷⁾ The interest in penicillamine and its potential involvement in the metabolism of collagen were prompted by two factors: (1) its resistance to metabolic degradation, and its strong capacity to chelate metals observed by Walshe (1962), which caused him to use it in Wilson's disease; and (2) the fact that penicillamine administration was shown by Scheinberg to be responsible for a number of side reactions which affected the connective tissue structures (Scheinberg 1964).⁽¹⁸⁾

Patients and method

Collection of data was carried out during the period between May 2010 – February 2011. Twenty-eight patients complaining of keloid and hypertrophic scars were enrolled in this study. It includes patients attending the Department of Dermatology and Venereology in Al-Yarmouk Hospital, Baghdad-Iraq. The questionnaire form includes socio-demographic information like; name, age, sex, address, occupation, marital status, date of examination, onset of the lesion, symptoms (itching, pain, or tenderness, erythema, hardness), site of lesion, number of lesions, family history of similar conditions. The nature and target of this study were explained for each patient and formal consent was taken for each patient before starting the therapy, after explanation about the nature of the disease, the procedure of treatment, follow up, prognosis and the need for pre and post treatment photographs by using Sony optical steady shot DSC-H55 were taken in the same place with fixed illumination and distance. Also, ethical approval was given by the scientific committee of the Scientific Council of Dermatology and Venereology-Arab Board for Medical Specializations. Inclusion criteria included: more than one year duration lesion, the last modality of treatment used at least 6 months ago with either no response or recurrence. Exclusion criteria included: pregnant women or if want to be pregnant, lactating women, active infection. Technique of injection Seventy percent ethanol was used as a topical antiseptic agent before injection. Disposable insulin syringe with 27-gauge needle syringe was inserted into the substance of keloid and the solution pushed with adequate pressure till minimal blanching was seen. This was repeated at multiple sites on the keloid.

The study sample consists of (17) females (60.71%) and (11) males (39.28%), their age range were (2-50) mean \pm SD age was (24.82 \pm 11.74 years), they were generally in good health, a part from the subjects of the study. Patients have at least one keloid or hypertrophic scar, with no modification in size, color and

symptoms. The size of lesions was assessed before treatment with tape measurement graduated with (centimeter) unite and the size detected by taking the largest 2 diameters for irregular shaped lesions and one diameter for regular rounded lesion.

Penicillamine was prepared as a solution for intralesional injection by dissolving (250mg) of Penicillamine (Artamin) ®by SANDOZ in (10ml) distilled water in sterilized container to obtained (2.5%) concentration of Penicillamine and autoclaved at 121C0 for 20 min before use. The scar to be injected is sponged off with a mild antiseptic solution. Penicillamine was administered through multiple superficial puncture technique without anesthesia. After the first treatment is completed, the patient is advised to return again in 2 weeks, at which time a second injection is done. Four sessions of intralesional injection were given at intervals of 15 days each. Most patients experience minimal pain when the solution is injected into the scar but they tolerate it. Results were evaluated according to change in size as follows: no response (upto10%), minimal response (10-50%), moderate response (50-75%) and very good response (>75%). Reduction in signs & symptoms was subjective & no other way to assess them:

A-Complete disappearance of pain & or pruritus

B-No change of tenderness& or pruritus

Results

A total of 28 patients with keloid and hypertrophic scars were seen. their age ranged from 2 - 50 with a mean \pm SD (24.82 \pm 11.74) years. Females represented 17 (60.71%) of patients while males were 11 (39.28%), with female to male ratio was1.54:1(Figure1). Patients ranged between 11 and 20 years, were the most common age groups affected (Figure 2).

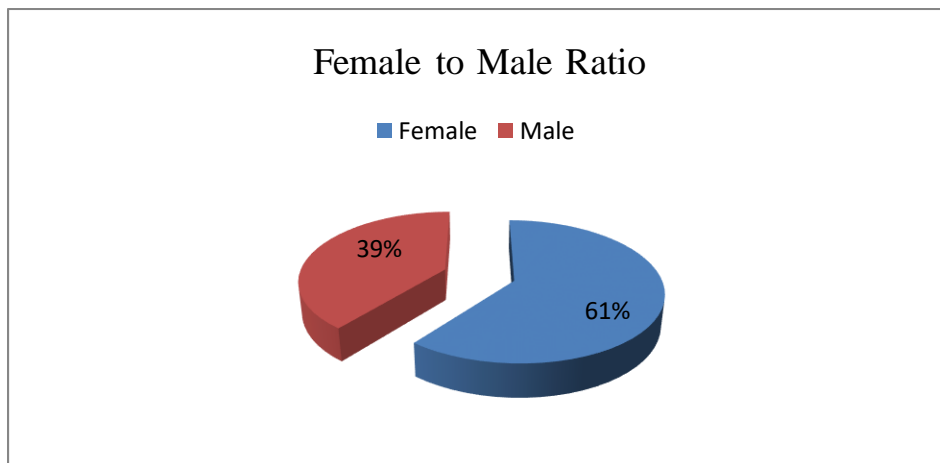


Figure1: Sex distribution of 28 cases of keloid and hypertrophic scars

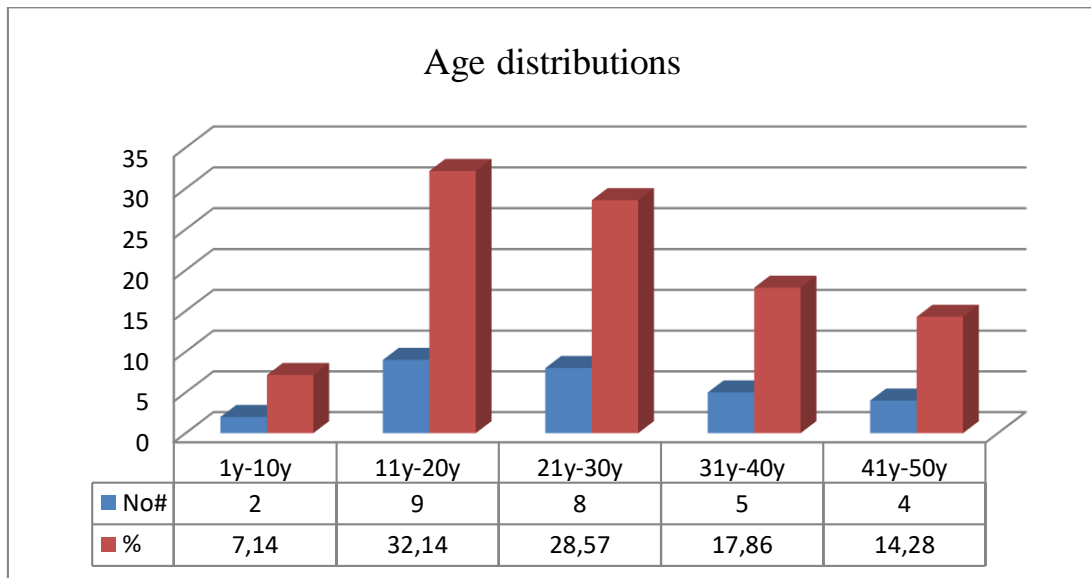


Figure2: Age distribution of 28 cases with keloid and hypertrophic scars under the study

The mean \pm SD durations of all lesions were (31.46 \pm 34.26) months, and the mean \pm SD duration of the very good response lesions were (34.4 \pm 36.48) months, mean \pm SD duration of the minimal response (20.8 \pm 16.34) months, mean \pm SD duration of the moderate response (21.66 \pm 13.99) and non-responded (39.58 \pm 46.38) months, shown in (Tab. 1).

Table1: The duration of lesion in months and their response to therapy

Response to treatment	No. of Patients	Total Duration\months	Mean \pm SD
No response	12	475	39.58 \pm 46.38
Minimal response	5	104	20.8 \pm 16.34
Moderate response	6	130	21.66 \pm 13.99
Very good response	5	172	34.4 \pm 36.48
Total	28	881	31.46 \pm 34.26

Sixteen (57.14%) patients, in the present study had no symptoms related to scars, 9(32.14%) patients gave history of pruritus, 3(10.71%) patients gave history of tenderness. (Tab.2)

Table 2: The signs & symptoms of 28 cases before and after therapy

Signs & symptoms	No. of Patient		
	Before treatment	After treatment	% of Reduction
Asymptomatic	16	16	100
Pruritus	9	1	88
Tenderness	3	1	66.6

The causes of the scars in this study varied. The most common cause was trauma 10 (35.71%) patients were seen, 5 (17.85%) patients were unrecognized, 5 (17.85%) patients from infection due to acne, 2 (7.14%) patients after ear piercing, 2 (7.14%) patients resulted after surgical excision of benign lesions (cyst), 1 (3.57%) patient resulted from tattoo, 1 (3.57%) patient from a burn, 1 (3.57%) patient resulted from operative incision, and 1 (3.57%) patient was the result of vaccinations. (Tab. 3).

Table 3: The causes of keloid and hypertrophic scars and the number of patients with their percentage

Causes	No. of patients	%
Trauma	10	35.71
Acne	5	17.85
Unrecognized	5	17.85
Cyst	2	7.14
Ear piercing	2	7.14
Post vaccination	1	3.57
Burn	1	3.57
Tattoo	1	3.57
Post operation	1	3.57
Total	28	100

Regarding body site involved by lesions, this study revealed that the chest was the most commonly affected area, 11 (39.28%) patients, followed by the upper back 6 (21.43%) and the extensor hand was the third site affected (Figure 3). All patients had single lesion. Positive family history accounts 3 (10.7%) patients of studied cases, all were females. Most of the patients involved in the present study were females. All patients primarily interested in improving the appearance of the scars.

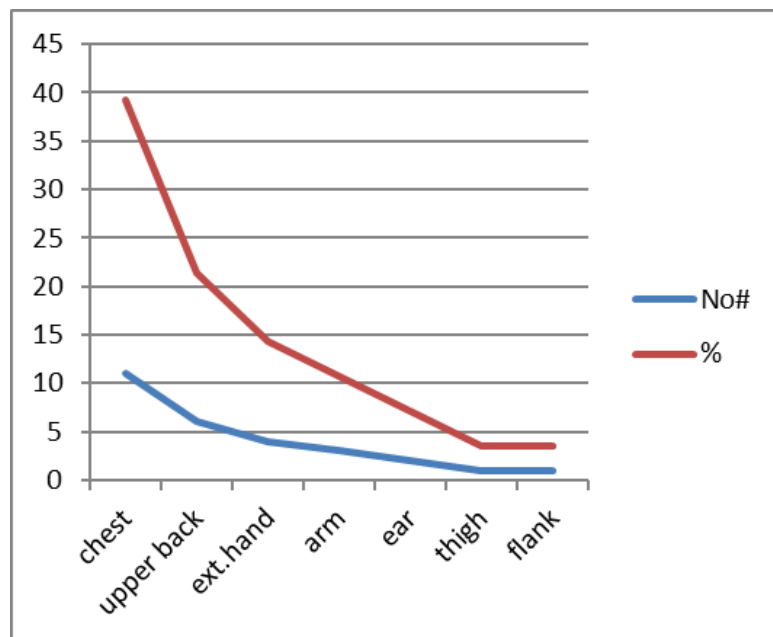


Figure 3: The frequency distribution of cases by their sites of lesion presented in numbers and percentages

Regarding therapeutic results, the response to treatment according to the sessions was:

Session one: no response (100%).

session two: (2) minimal response, (6) no response.

session three:(2) very good response, (2) minimal response & (5) moderate response.

session four:(3) very good response, (1) minimal response, (1) moderate response and (2) no response (Tab.4).

Table 4: The response to treatment according to the sessions

Sessions	No response	Minimal response	Moderate response	Very good response	Total No.
One	4	0	0	0	4
Two	6	2	0	0	8
Three	0	2	5	2	9
Four	2	1	1	3	7
Total	12	5	6	5	28

The response to treatment according to the site of lesion was: In the chest (2) lesions very good response apart of (11), upper back (2) lesions very good response and the lesion of flank was very good responded. (Tab.5).

Table 5: The response to treatment according to the site of lesion

Anatomical site of lesion	No response	Minimal response	Moderate response	Very good response	Total No.
Chest	6	2	1	2	11
Upper back	0	1	3	2	6
Extensor of hand	2	1	1	0	4
Arm	1	1	1	0	3
Ear	2	0	0	0	2
Flank	0	0	0	1	1
Thigh	1	0	0	0	1
TOTAL	12	5	6	5	28

The final result of this study was (42.86%) patients no response, (17.86%) minimal response, (21.42%) moderate response and (17.86%) patients very good response. (Figure4)

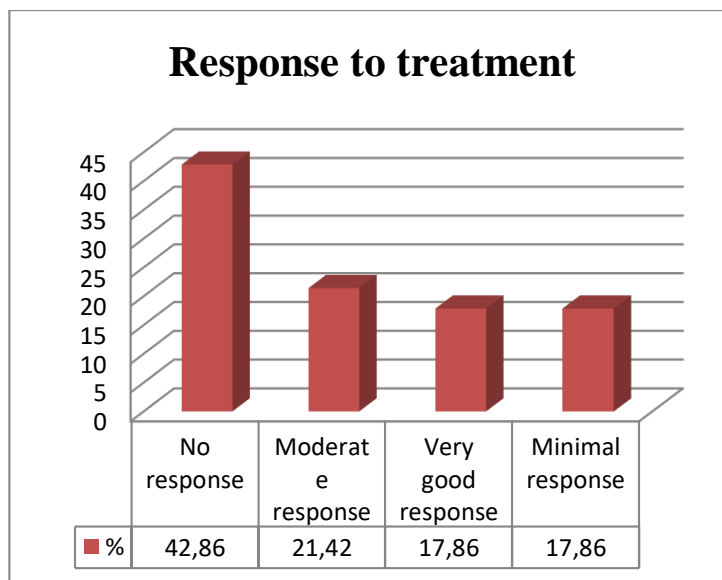


Figure 4: percentage of response to treatment of all cases

Significant improvements obtained in patients' subjective complaints of pruritus and tenderness, between two to four sessions. The respond to intralesional penicillamine enhance by increased the frequencies of sessions except that on ear (after piercing) which show no response to therapy even with 4 sessions. Duration of lesion seem to have no effect on their response to intralesional penicillamine.

Discussion

Hypertrophic scars and keloids are overgrowths of dense fibrous tissue that develop after trauma to the skin. Normal wound healing requires a delicate balance between collagen synthesis and degradation and is regulated by various growth factors. Inflammation or an alteration of these growth factors may contribute to scar formation. ⁽¹⁹⁾ Numerous treatments have been attempted with variable results using either single or combination modalities for treatment of keloids and hypertrophic scars. ^(12, 20, 13,21) The interest in penicillamine and its potential involvement in the metabolism of collagen were prompted by two factors: One; its resistance to metabolic degradation, and strong capacity to chelate metals observed by Walshe (1962), ⁽¹⁸⁾ That lead to use it in Wilson's disease; and Second: The fact that penicillamine administration was shown by Sheinberg to be responsible for a number of side reactions which affected the connective tissue structures. ⁽¹⁸⁾ D-penicillamine is known to have two effects on the collagen cross-linking process: to reversibly inhibit the lysyl oxidase; and to irreversibly bind to the aldehydes generated as a result of its action. ⁽¹⁷⁾

BRYAN J. MAYOU used Long term low- dosage systemic penicillamine in two prospective trials, and concluded from that study that low dosage, long term penicillamine treatment of keloids is ineffective. ⁽²²⁾ This study represented a trial to evaluate the effectiveness and safety of intralesional penicillamine that would avoid systemic side-effect.

It showed that mean \pm SD age of patients were (24.82 \pm 11.74) years, indicating that either; a younger age group affected with the disease or that this age group is more vulnerable for appearance of the lesion (cosmetic reasons). Female showed higher rate than males 1.54:1 this may have association with the same reason with age those females more caring for the cosmetic aspect than males, rather than a real higher rate than males these results were seen by others. ^(12,21,23) but, it is in contrast with other studies show equal incidence of keloids in male and female subjects. ⁽²⁴⁾ The symptoms of the patient were trivial since most of them was symptomless few have pruritus and very few have just tenderness and this goes with reality of the disease. ⁽²⁰⁾ More than 57% of the cases were symptomless, the rest 43% were divided into 75% pruritus & 25% tenderness; after treatment the reduction in pruritus was prominent 88% while the reduction of tenderness was 66%. Regarding the etiology of the disease more than 35% of the cases the reason behind the causes was traumatic which were the actual reasons in most of the studies; and never forget a spontaneous reason without history of any cause although some blame acne as the reason in unrecognized causes. ⁽²⁰⁾ The commonest part of the body affected were the chest & upper back 60.71% (trunk) followed by extensor aspects of hands & arms 25%, ear was also affected in 7% these results were more or less similar to most of the studies with slight difference in the rates. ⁽²⁵⁾ Family history was positive in about 11% of the cases were more or less similar results were seen by other different studies. ⁽²⁶⁾ The reasons for attending the clinic were mainly cosmetic for all patients & this goes with most of the studies. ^(12, 20,27)

The response to intralesional penicillamine in relation to duration of disease & location of the lesion, the study showed no relation between duration of skin

lesion and response to therapy, similar findings were noticed by Fatemi. ⁽²⁸⁾ The responses in relation to location were noticed to be affected in this study (ear piercing cases not responding all the sessions) a fact was denied by the Iranian researcher Fatemi. ⁽²⁸⁾

The response to penicillamine was unnoticed in the first 2 sessions which was disappointing; any how after 15 days from the second session the results became encouraging, in the session three (17.85%) show moderate response with (7.1%) show very good response. However, for the successive sessions the results were more encouraging giving final results of more than 39% have either moderate or very good response, these results were good compared with other results achieved by different researchers. ^(29,30,31) Penicillamine probably exerts their effects in these conditions through the inhibition of collagen synthesis a fact that initiate the researchers to conduct this study. ⁽¹⁷⁾ Increasing the session to more than 4 may initiate new responders that would increase the rate of response to these new therapeutic models. However, 42% of the patients have no response. The study on penicillamine was the first to the best of our knowledge. There was little adverse effect from this treatment modality as face only mild pain at site of injection these were tolerance by patients, which was expected to happen in any studies that use a penetrating object. ^(32,33) no other complications were reported.

Conclusions

Penicillamine may be an effective therapeutic agent in treating keloid & hypertrophic scars. Penicillamine is low cost, easily soluble and nontoxic.

Recommendations

- 1 Different concentrations of penicillamine should be tried like 1%, 5%, & 10% for futures studies.
- 2 More frequent session should be tried & a longer duration of follow-up should be achieved.

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