

# Evaluating serum level of thymidylate synthase in post burn keloid patients before and after intralesional injection of 5-fluorouracil

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## Abstract

**Background:** Keloids are fibrous lesions formed at the site of trauma due to types I and III collagen irregular production. The presence of thymidylate synthase (TS) is a must for DNA synthesis and repairs causing cell death. 5-fluorouracil (5-FU) is a fluorinated pyrimidine analogue acting as an anti-metabolic agent that inhibits thymidylate synthase and interferes with ribo-nucleic acid (RNA) synthesis.

**Objectives:** we aimed to evaluate the level of thymidylate synthase in post burn keloid patients before and after intralesional injection of 5-fluorouracil.

**Methods:** The study included 20 keloid patients and 20 healthy subjects as a control. Serum TS was estimated using commercially available enzyme-linked immunosorbent assay (ELISA) kits before and after treatment with 5-fluorouracil.

**Results:** There was a statistically significant difference in TS levels before and after 5-FU treatment ( $p < 0.05$ ). Also, results have shown that 5-FU injection has good satisfactory results in treatment of keloid causing reduction in scar volume and symptoms improvement (90% of the patients improved). On the other hand, there was no statistically significant difference in TS levels and the outcomes of the treatment.

**Conclusion:** Our findings suggest that intralesional 5-FU injection in keloid has very satisfactory results. However, thymidylate synthase enzyme has a minimal role in evaluating the treatment of keloid, so further studies are required to elaborate the relation between this enzyme and keloid scars.

## Keywords

keloid, thymidylate synthase, 5-fluorouracil

## Lay summary

Keloids are recurrent resistant skin scars that are known for their chronic attitude. 5-FU is a type of medicine that is used in treatment of cancer and keloid by acting on thymidylate synthetase which is an enzyme helping the cell multiplicity process.

## Introduction

Keloid is a fibroproliferative tumor with accumulation of extracellular matrix (ECM) components, especially collagen, resulting from over expression of growth factors and cytokines.<sup>1</sup>

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Keloid etiology is still not clear. Keloids are probably associated with an abnormal wound-healing process as a result of skin injury such as piercings, abrasions, burning, surgery, tattoos, insect bites, or any processes causing skin inflammation.<sup>2</sup>

Keloid scar management is clinically challenging. Many treatment lines have been advocated either alone or in conjunction with others, including surgical excision, cryotherapy, radiotherapy, intralesional chemotherapeutic injection, laser therapy, topical silicone, systemic chemotherapy and pressure therapy.<sup>3</sup>

5-fluorouracil (5-FU) a pyrimidine analogue that is classified as an antineoplastic agent inhibiting normal DNA and RNA synthesis, thereby decreasing thymidylate synthase activity.<sup>4</sup>

5-FU stops fibroblast proliferation in tissue culture. Thus, it is important to pay attention when using low-dose intralesional 5-FU to treat an undesirable scar.<sup>5</sup> Thymidylate synthase, the main intracellular target of 5-FU, is a key enzyme in nucleotide biosynthesis.<sup>6</sup> 5-fluorouracil inhibits deoxyribonucleic acids synthesis by irreversible inhibition of thymidine synthase that is responsible for converting uridine to thymidine. Additionally, 5-FU hinders type I collagen gene expression and tumor growth-beta 1 effects.<sup>7</sup>

The role of TS in patients with keloid was not studied; additionally, the impact of treatment with 5-FU on the TS was not assessed. We aimed to evaluate the level of thymidylate synthase in post burn keloid patients before and after intralesional injection of 5-FU.

## Subjects and methods

The present study was a case control cross sectional study conducted on 40 subjects during the period from 28 January 2019 to 9 December 2019. The subjects consisted of 20 keloid patients not suffering from any other skin disorders and 20 apparently healthy controls.

### Inclusion criteria

Patients with keloid scars aged (15–50) years.

### Exclusion criteria

We excluded patients with other diseases that may affect the level of thymidylate synthase such as tumors. Patients with primary skin diseases other than keloid and patients with hematological disorder were excluded, children and pregnant women were also excluded.

## Informed consent

After approval from the Research Ethical Committee (REC), Faculty of Medicine, Fayoum University, informed consent was obtained from all participants.

*Examination.* Examination of keloid including site, size and the number of scars, tenderness, itching and erythema of the keloid lesion. Full general examination to exclude any systemic illness associated with TS level was also done.

*Specimen collection.* Four ml of blood was collected from each subject in the study from the antecubital vein by sterile 5 ml syringe. Blood samples were collected in plain vacutainer tubes for serum separation. They were incubated at 37°C for 10–15 min then were centrifuged at 3000 rpm to separate serum using 800 D centrifuge. Serum samples were frozen at –20°C for later measurements of thymidylate synthase. Another sample was taken from patients after sessions of intralesional 5-fluorouracil injection.

*5-fluorouracil injection.* Patients received weekly intralesional injections of 0.2–0.5 mg/cm<sup>2</sup> of 50 mg/ml 5-fluorouracil/ (Utoral®) which were administered over an average of 4–12 sessions using 1 ml insulin syringe.

*Evaluation of response to treatment.* Photos were obtained at baseline, before each session and after the final session. Response after each injection session is documented as a percentage improvement from baseline, based on improvement in redness, thickness and pruritus. Response to treatment was classified as follows: no, mild, moderate, marked and complete improvement (0, from 0–29, from 30–59, from 60–94 and from 95–100%) respectively. The assessment of improvement Vancouver scar scale.<sup>8</sup>

## Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median and inter-quartile range (IQR) when non-parametric distribution of the data. Also, qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data were done by using Chi-square

test and/or Fisher exact test only when the expected count in any cell found less than 5.

The comparison between two independent groups with quantitative data and parametric distribution were done by using independent t-test.

The comparison between two independent groups with quantitative data and non-parametric distribution were done by using Mann-Whitney test while the comparison between two paired groups regarding quantitative data with non-parametric distribution were done by using Wilcoxon Rank test

Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

p > 0.05: Non significant, p < 0.05: Significant and p < 0.01: Highly significant.

## Results

The present case-control cross-sectional study included 40 individuals in the period from January to December 2019 (20 keloid scar patients) not suffering from any other skin disorders. Data were compared to 20 healthy controls who were age and sex matched.

There was no statistical significance with p-value > 0.05 between the case and control groups as regards age and sex.

There was a statistically significant difference between TS levels before and after treatment with (p-value < 0.05) (Table 1).

The response to treatment was shown in Figure 1: 10% of patients showed no response, 30% of patients had a mild response to treatment, 25% of patients had a moderate response to treatment, 25% of patients had a marked response while 10% of the patients had a complete response.

There was no statistically significant difference (p-value > 0.05) in TS levels between case and control groups (Table 2).

In our patients, positive correlation was found between percentage of improvement and dose of drug (Figure 2).

There was no statistically significant difference (p-value > 0.05) in TS levels before treatment and degree of improvement (Table 3).

There was no statistically significant difference (p-value > 0.05) in TS levels between different study groups as regards other parameters (Table 4).

Figures 3, 4 and 5 showed some of the studied patients: improvement was noted in the visual assessment.

## Discussion

Our study was conducted on 40 subjects. They were classified into two groups: the patient group ages ranged between 15 and 50 years with a mean value of 27.70 years  $\pm$  10.60 and the control group ages ranged between 18 and 50 years with a mean value of 29.25  $\pm$  9.30 which indicate proper matching of the two groups.

Patients received weekly intralesional injections of 0.2–0.5 mg/ml of 50 mg/ml 5-fluorouracil administered over an average of 4–12 sessions using 1 ml insulin syringe.

Results have shown 10% of the patients with complete improvement, 30% marked improvement, 25% mild and moderate improvement and only 10% showed no improvement.

This was in agreement with results of a similar study conducted by Kontochristopoulos et al. who studied the efficacy of intralesional 5-FU in treating keloids in 20 patients.<sup>9</sup>

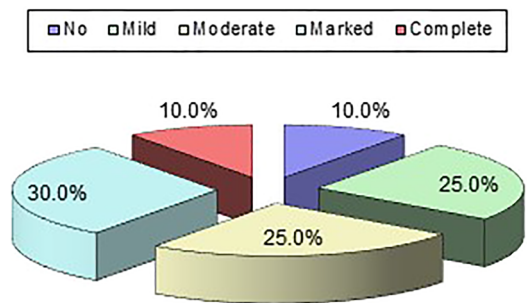
Along the same lines was a prospective study by Haurani et al. conducted on patients with keloids and hypertrophic scars. Keloid patients underwent post excision 5-FU injections. They found a 65% reduction in scar volume noted in all patients at the end of the treatment. Moreover, 86% of patients had partial or complete resolution of symptoms, which included purities, pain and pressure.<sup>10</sup>

To our knowledge, this is the first study to be done to measure the level of thymidylate

**Table 1.** Comparison between the level of TS (mg/ml) before and after treatment.

TS (mg/ml)	Before	After	% of improvement	p-value	Sig.
Median (IQR)	1.53(1.16 – 1.85)	1.32(1.17 – 1.44)	–5.88 (–18.33–0.72)	0.013	S
Range	0.96–12.16	1.10–9.93	–28.86–23.65		

TS, thymidylate synthase.



**Figure 1.** Percentage of improvement among the case group.

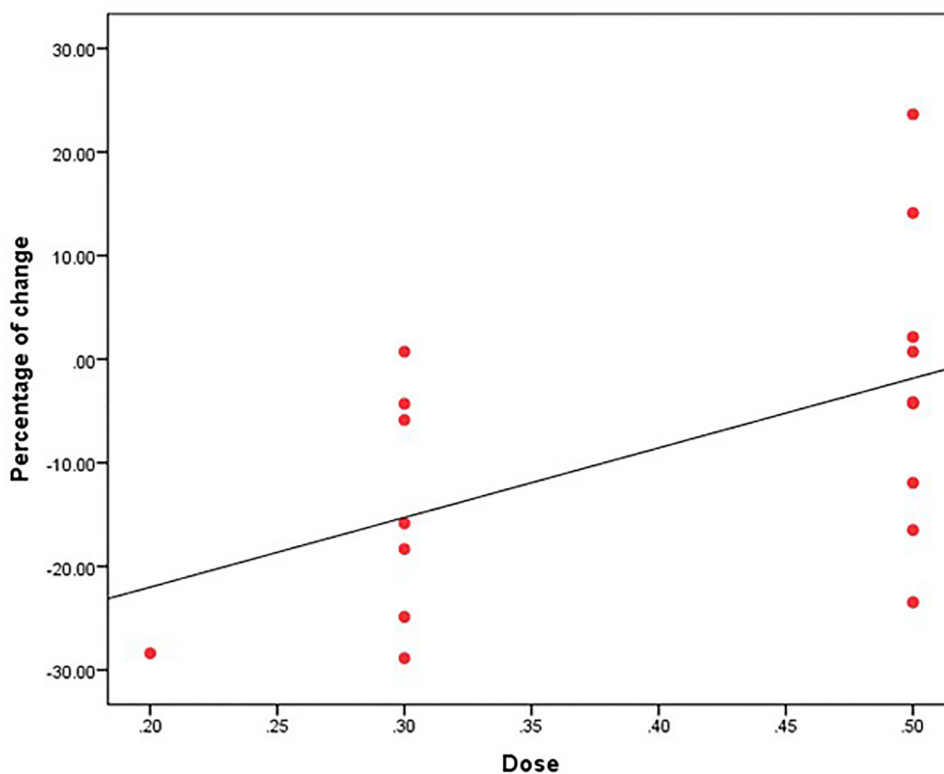
synthase in keloid patients. Our results have shown a statistically significant difference between TS level before and after 5-FU sessions ( $p$ -value  $< 0.05$ ). Thymidylate synthase is a key enzyme in nucleotide biosynthesis and is the main intracellular target to 5-FU.<sup>6</sup>

On the other hand, there was no statistically significant difference ( $p$ -value  $> 0.05$ ) in TS levels between case and control groups. This may be due to dilution of the enzyme concentration in the case serum, so advanced research with tissue sampling may be more beneficial.

**Table 2.** Comparison between the level of TS (mg/ml) in cases and control.

TS (mg/ml)		Cases	Controls	p-value	Sig.
Before	Median (IQR)	1.53 (1.16 – 1.85)	1.32 (1.07–1.93)	0.222	NS
	Range	0.96–12.16	0.62–6.98		
After	Median (IQR)	1.32 (1.17 – 1.44)	1.32 (1.07–1.93)	0.879	NS
	Range	1.10–9.93	0.62–6.98		

TS, thymidlate synthase.



**Figure 2.** Correlation of percentage of improvement and dose of drug.

**Table 3.** Correlation of TS before and degree of improvement.

		TS before mg/ml					
		Mean $\pm$ SD	Range	Test value	p-value	Sig.	
Improvement	No	5.08 $\pm$ 2.68	3.19–6.97	1.043	0.418	NS	
	Mild	1.31 $\pm$ 0.21	1.1–1.64				
	Moderate	24.69 $\pm$ 42.1	0.96–99.5				
	Marked	1.45 $\pm$ 0.33	1.08–1.85				
	Complete	1.46 $\pm$ 0.09	1.4–1.53				

TS, thymidilate synthase.

**Table 4.** Relation of percentage of change of TS with the other parameters.

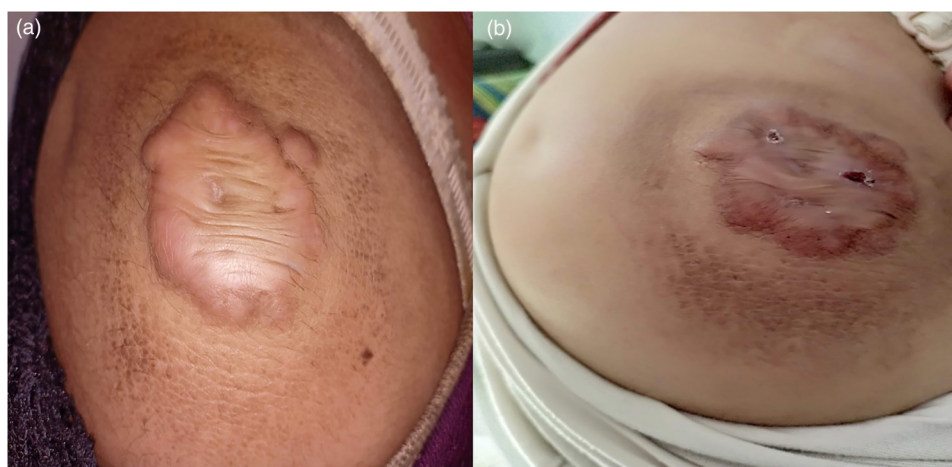
		Percentage of change				
		Median (IQR)	Range	Test value	p-value	Sig.
Sex	Female	–5.88 (–24.89 – 0.72)	–28.86 – 2.14	0.603	0.546	NS
	Male	–10.07 (–16.5 – 14.11)	–23.47 – 23.65			
Cause	Burn	–4.21 (–15.85 – 0.73)	–28.86 – 14.11	1.655	0.437	NS
	Trauma	–14.59 (–24.89 – –4.3)	–24.89 – –4.3			
	Idiopathic	–16.5 (–23.47 – –11.94)	–28.39 – 23.65			
Site	Back	14.11 (14.11 – 14.11)	14.11 – 14.11	9.121	0.332	NS
	Thigh	–4.15 (–4.15 – –4.15)	–4.15 – –4.15			
	Forearm	–12.1 (–21.61 – –2.57)	–24.89 – 0.73			
	Arm	–22.36 (–28.86 – –15.85)	–28.86 – –15.85			
	Shoulder	–4.28 (–11.94 – 2.14)	–11.94–2.14			
	Chest	0.72 (–16.5–23.65)	–16.5 – 23.65			
	Forehead	–4.3 (–4.3 – –4.3)	–4.3 – –4.3			
	Head	–23.47 (–23.47 – –23.47)	–23.47 – –23.47			
	Abdomen	–28.39 (–28.39 – –28.39)	–28.39 – –28.39			
Itching	No	–4.3 (–24.89 – 2.14)	–28.86 – 23.65	0.488	0.626	NS
	Yes	–13.89 (–18.33 – –4.15)	–28.39 – 14.11			
Erythematic	No	–5.09 (–18.33–0.72)	–28.86 – 23.65	0.195	0.845	NS
	Yes	–11.94 (–23.47 – 0.73)	–28.39 – 14.11			
Tenderness	No	–5.09 (–18.33 – 0.72)	–28.86 – 23.65	0.504	0.614	NS
	Yes	–16.5 (–24.89 – 0.73)	–24.89 – 0.73			
Treatment history	No	–8.12 (–24.89 – 0.72)	–28.86 – 23.65	0.506	0.777	NS

(Continued)

**Table 4.** (Continued)

		Percentage of change		Test value	p-value	Sig.
		Median (IQR)	Range			
	Topical treatment	-5.01 (-12.1 - 4.98)	-18.33 - 14.11			
	ILCs	-16.5 (-23.47 - 0.73)	-23.47 - 0.73			
Complication	No	-15.85 (-24.89 - 0.72)	-28.86 - 2.14	0.976	0.329	NS
	Yes	-5.08 (-16.5 - 0.73)	-28.39 - 23.65			
Pain	No	-17.09 (-24.18 - -1.79)	-28.86 - 2.14	1.347	0.178	NS
	Yes	-4.28 (-11.94 - 0.73)	-28.39 - 23.65			
Ulcer	No	-16.17 (-24.18 - -1.79)	-28.86 - 23.65	1.687	0.092	NS
	Yes	-4.15 (-4.28 - 0.73)	-11.94 - 14.11			
Hyperpigmentation	No	-5.09 (-19.98 - 0.73)	-28.86 - 23.65	0.816	0.414	NS
	Yes	-18.33 (-18.33 - -18.33)	-18.33 - -18.33			
Improvement	No	-2.58 (-5.88 - 0.72)	-5.88 - 0.72	1.967	0.742	NS
	Mild	-4.28 (-16.5 - 0.73)	-28.39 - 23.65			
	Moderate	-11.32 (-18.33 - -4.3)	-18.33 - -4.3			
	Marked	-8.04 (-23.47 - 2.14)	-28.86 - 14.11			
	Complete	-20.37 (-24.89 - -15.85)	-24.89 - -15.85			

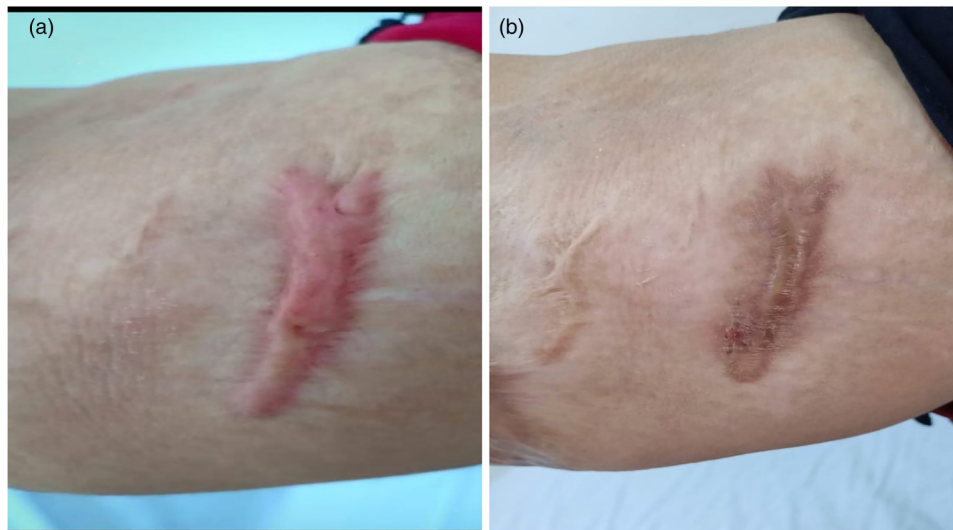
TS, thymidilate synthase.



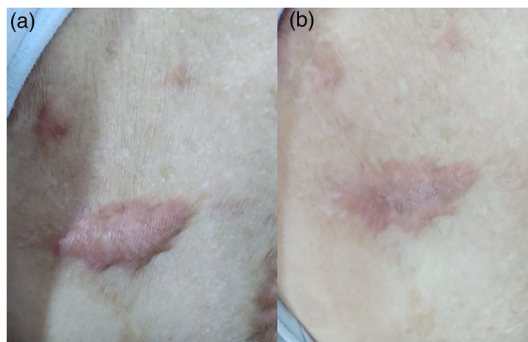
**Figure 3.** Improvement was noted in patient with keloid after intralesional injection of 5-FU in the shoulder.

The present study also showed that there was no statistically significant difference in TS levels with respect to the patient age, size of lesion and the disease duration among keloid patients.

There are no other studies that discuss a relationship between serum TS level and keloid cases, but there are many studies conducted on the role of this enzyme in other tumors.<sup>11-12</sup>



**Figure 4.** Improvement was noted in patient with keloid after intralesional injection of 5-FU in the arm.



**Figure 5.** Improvement was noted in patient with keloid after intralesional injection of 5-FU in the chest.

In conclusion, 5-FU injection has good satisfactory results in treatment of keloid. The serum level of thymidylate synthase in patients with keloid decreased after intralesional injection of 5-FU in the scar. However, there was no significant difference in TS levels between case and control groups. Thus, further studies are required to elaborate the relation between thymidylate synthase enzyme and keloid scar.


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