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Efficacy of Picosecond Laser in Clinical, Histopathology, and Immunohistochemistry Examination in Indonesian Wrinkled Skin

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Abstract

Introduction: The elderly population wants to improve skin function and maintain a youthful appearance without wrinkles. Laser is one of the highly developed and widely used therapeutic modalities of skin aging by stimulating dermal collagen formation. Picosecond laser therapy is performed on skin with wrinkles to determine the mechanism of wrinkle improvement with a picosecond laser through an increasing in tissue inhibitor of metalloproteinases 1 (TIMP-1), and a decrease in matrix metalloproteinase-1 (MMP-1), which was confirmed by collagen density.

Methods: The study was started from March 2021 to July 2021 at URJ Dr. Soetomo Hospital Surabaya and Surabaya Skin Centre. Twenty patients meeting the inclusion criteria of being 36-55 years of age and having a wrinkle in the forearm were enrolled by random sampling. Each sample data was recorded, the average TIMP-1, MMP-1, collagen density, and clinical wrinkles were calculated, and the laser was performed by using a picosecond 755 nm laser.

Results: All patients showed a moderate pre-test wrinkle scale. The figure for TIMP-1 and MMP-1 after therapy was higher. The MMP-1/TIMP-1 ratio in wrinkles treated by using a picosecond laser was lower but insignificant than before. The picture of collagen density indicates that collagen density after laser therapy was higher than before.

Conclusion: The result of clinical examination, histopathology, and immunohistochemistry in this research explained that a picosecond 755 nm laser with DLA is an effective therapy for wrinkles in Indonesian patients without any adverse effects.

Keywords: Laser; Photoaging; Picosecond; Rejuvenation; Wrinkle.

Introduction

The number of elderly people in Indonesia in 2020 is estimated at 27.08 million, and it is expected to multiply to 48.19 million people by 2035.[1] The increase in life expectancy will be followed by various changes, which is a challenge for dermatologists. Aging skin has two distinct types of aging, intrinsic and extrinsic. Intrinsic aging is physiologic changes that occur over time, and it is influenced by genetics and hormones. Extrinsic aging is a change in structure and function due to exogenous factors, mainly sun exposure.[2] The major sign of aging is the wrinkles that appear as a line because of skin depressions, commonly appearing on the face, neck, hands, and other areas that are frequently exposed to the sunlight.[3,4] Reactive oxygen species (ROS) accumulation increases with age and sun exposure, leading to an increase in matrix metalloproteinase-1 (MMP-1). Matrix metalloproteinases (MMPs) are endopeptidases that can degrade extracellular matrix proteins. MMP-1 is the main protease that initiates collagen fragmentation, which is mainly type I and III. MMPs are physiologically regulated by tissue inhibitors of metalloproteinases (TIMPs). The main sources of MMPs and TIMPs in the skin are keratinocytes in the epidermis and fibroblasts in the dermis.[5] MMPs and TIMPs are coordinated in balance to control the overactivity of MMPs. The increase in MMPs in aging skin is not accompanied by an increase in TIMPs, and the amount of TIMP-1 in both intrinsic and extrinsic skin aging skin even decreases. The imbalance between MMPs and TIMPs accelerates progressive collagen fragmentation and skin aging.[1,5]

The elderly population wants to improve skin function and maintain a youthful appearance without wrinkles.[6] Laser is one of the highly developed and widely used therapeutic modalities of skin aging for skin rejuvenation by stimulating dermal collagen formation. The basic concept of dermal rejuvenation by a laser is the controlled wound healing process induced by a laser. Studies using nonablative lasers have shown dermal remodelling by decreasing MMP-1 and increasing TIMP-

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1, type I and III collagen visible on histopathological and immunohistochemical examinations.[5,7]

In 2014, The Food and Drug Administration (FDA) gave clearance for a picosecond 755 nm laser with a diffractive lens array (DLA) in the treatment of wrinkles. A retrospective study in 2016 by Haimovic et al showed that a picosecond 755 nm laser with DLA in patients with Fitzpatrick skin type IV-VI is safe, with no adverse effects in all cases.[8] Research by Weiss et al in 2017 showed that the picosecond 755 nm laser is highly effective for the treatment of wrinkles, confirmed by histologic examination showing an increase in dermal collagen.[9] Furthermore, research by Tanghetti and Tartar in 2016 explained epidermal damage by the picosecond 755 nm laser (Alexandrite) in contrast to previous lasers. The picosecond laser induces laser-induced optical breakdown (LIOB), thus forming focal vacuoles in the epidermis that can stimulate an inflammatory process followed by a dermis response that causes the production of collagen, elastin, and mucin.[10]

In this study, picosecond laser therapy was performed on human forearm skin that had wrinkles. Complaints of wrinkles are often on the face, but the forearm is preferred over the face which is more cosmetically sensitive when a biopsy examination is required. Then clinical, histopathological, and immunohistochemical examinations were employed to determine the mechanism of wrinkle improvement with a picosecond laser through an increase in TIMP-1 as a regulator of MMP-1 and a decrease in MMP-1 that initiates collagen fragmentation and then is confirmed by collagen density and wrinkle improvement.

Methods

The study was started from March 2021 to July 2021 at Dr. Soetomo General Academic Hospital Surabaya and Surabaya Skin Centre. The total number of patients was 20 people, and research sampling was carried out by random sampling. The sample size was calculated based on the pre-test and post-test formula (paired numerical comparative analysis of 2 groups)[11]: $n = ((Z\alpha + Z\beta)s/(x1\text{-}x2))^2$ n = sample size, $z\alpha = 1.64$ at a significance level $\alpha = 0.05$ (the magnitude of type 1 error or pseudo-positive results), $z\beta = 1.28$ (power or type 2 error or false negative result), s = 1.31, x1-x2 = 0.89,[12] to anticipate drop out, 10% was added, so the sample size = 20.[13] The inclusion criteria were as follows: patients with a wrinkle in the forearm, patients whose age ranged between 36 and 55 years, and patients who agreed to participate in this research and then signed the informed consent form. Exclusion criteria included the patients who were pregnant, were in menopause, had a history of skin malignancy or keloid, were photosensitive, were in an immunosuppressive state, or had an active infection. The patients who received previous therapy within 6 months like chemical peeling, microdermabrasion, Botox, filler, laser or other types of light therapy were also excluded. Each sample data was recorded, biopsies were taken, and the average TIMP-1, MMP-1, collagen density, and clinical wrinkles were calculated and analyzed with SPSS 22 software. Statistical analysis was carried out to compare the results of clinical, histopathological, and immunohistochemical examinations before and after receiving therapy. The Shapiro-Wilk test was used to check the normality of the data because the sample size was smaller than 30. If the data were normally distributed, a parametric statistical test with a paired sample t-test was used, and if they were not normally distributed, the Wilcoxon test was utilized. The laser was performed by using a nonablative laser with a pulse duration of picosecond and a wavelength of 755 nm + DLA (PicoSure, Cynosure, USA) with fluence parameters of 0.71 mJ/cm^2, spot size of 6 mm with the tip of the laser device against the skin at an angle of 90° up to 5000-6000 pulses (3-4 passes) until erythema appeared.

Results

Twenty patients were enrolled in this research, with the youngest and oldest age being 36 and 55 years respectively. Most of the patients were in the age group of 36-45 years (55%). The patients were dominantly female patients (95% females and 5% males). The clinical assessment of wrinkles using the assessment form scaled from 0-9 (0: no wrinkles, 1-3: mild, 4-6: moderate, and 7-9: severe). In this study, all patients showed a moderate pre-test wrinkle scale with the most wrinkle scale being scale 5 (55%).

The results indicate that TIMP-1 in wrinkles treated with a picosecond laser was higher (6.30) than that before therapy (4.33). Analysis with a paired t-test showed there was a significant difference between the groups ($P < 0.05$) (Table 1 and Figure 1). Table 1 shows the distribution of MMP-1 where the E0 group obtained a mean of 2.56 and the E1 group a mean of 3.21. The paired t-test showed that the difference in the MMP-1 figure between the groups was not significant ($P > 0.05$). These results indicate that MMP-1 in wrinkles treated with a picosecond laser was higher but not significant compared to that before therapy

Table 1. Data Analysis Results on TIMP-1, MMP-1, and MMP-1/TIMP-1 Ratio

Group	n	Mean ± SD	Min-Max	*P* Value
TIMP-1				
E0	20	4.33 ± 1.24	2.6 – 6.8	0.000
E1	20	6.30 ± 1.39	3.9 – 9.7	
MMP-1				
E0	20	2.56 ± 0.89	0.20 – 4.00	0.079
E1	20	3.21 ± 1.16	1.60 – 5.10	
MMP-1/TIMP-1 Ratio				
E0	20	0.65 ± 0.31	0.04 – 1.36	0.215
E1	20	0.53 ± 0.20	0.16 – 0.91	

(Figure 2). The MMP-1/TIMP-1 ratio is shown in Table 1 (the MMP-1/TIMP-1 ratio in group E0 with a mean of 0.65 and group E1 with a mean of 0.53). Analysis with the paired t-test showed there was a difference in the ratio of MMP-1/TIMP-1 which was not significant between the groups ($P > 0.05$).

The picture of collagen density in histopathology examination shown in Figure 3 indicates that collagen density in wrinkles after therapy was higher than that before therapy (Table 2). Analysis with the paired t-test showed there was a significant difference in collagen density between the groups ($P < 0.05$).

Figure 4 shows a comparison of wrinkles before and after therapy using a skin analyzer. It appears that the wrinkles before therapy indicated by red arrows are more than those after therapy. Table 3 shows the wrinkle scale values in which the E0 group had a median of 5 and the E1 group had a median of 3. The analysis using the Wilcoxon test showed that there was a significant difference in the wrinkle scale between the groups ($P < 0.05$).

Discussion

Susceptibility to skin aging is mediated by genetic factors, age, gender, increased sun exposure, and geographic

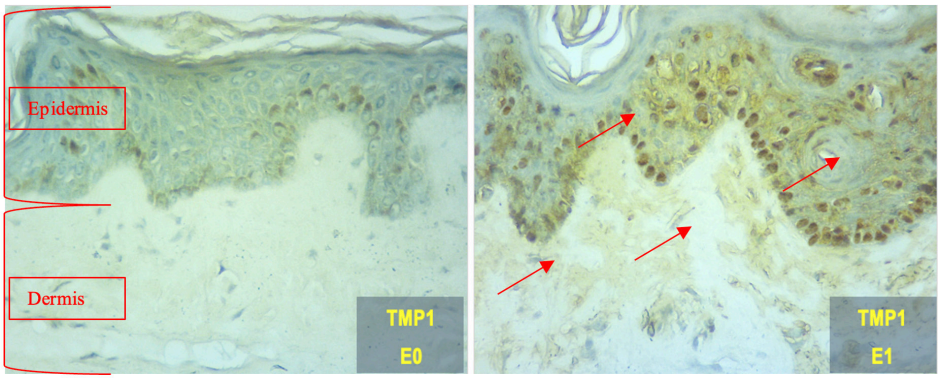

Figure 1. Comparison of TIMP-1 Between the Groups. The chromogenic brown color in group E1 was stronger than E0. (Immunohistochemical staining, Magnification 400x; inlet 1000x; Nikon H600L microscope; DS Fi2 300 megapixel camera).

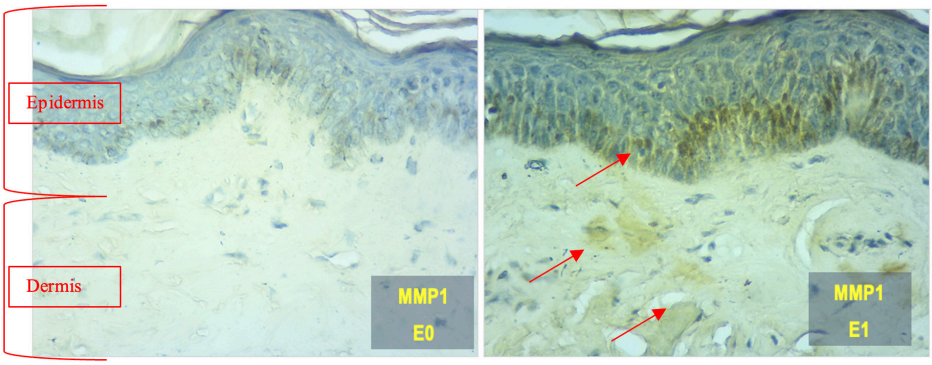

Figure 2. Comparison of MMP-1 Figure Between the Groups. The figure of MMP-1 in cells is characterized by a chromogen brown color, whereas in the slide above it appears that the figure in group E1 was stronger than E0. (Immuno histochemical staining, Magnification 400x; inlet 1000x; Nikon H600L microscope; DS Fi2 300 megapixel camera).

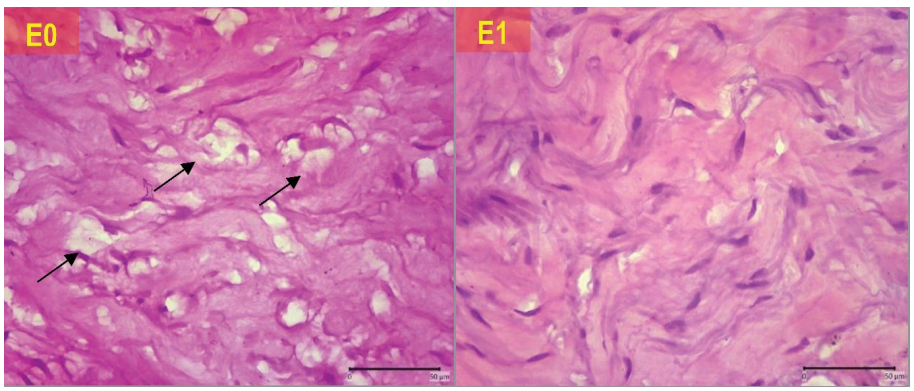

Figure 3. Comparison of Collagen Density Between the Groups. (HE staining, Magnification 400×; microscope Nikon H600L; camera DS Fi2 300 megapixel)

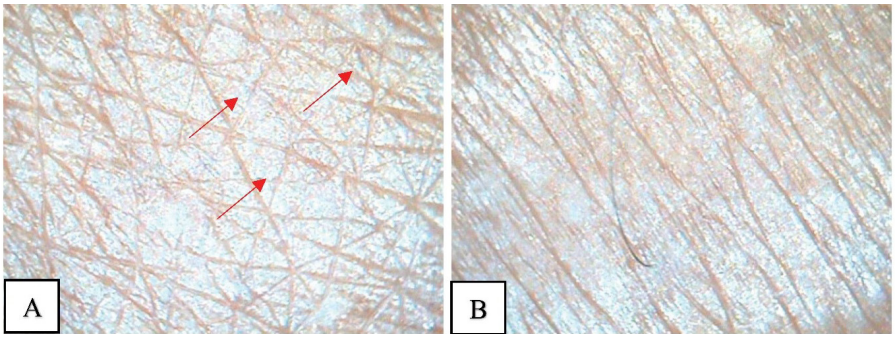

Figure 4. Comparison of Wrinkles Using a Skin Analyzer. A: before therapy. B: after therapy

Table 2. Results of Data Analysis on Collagen Density

Group	n	Collagen Density		*P*
		Mean ± SD	Min-Max	
E0	20	82.72 ± 6.38	72.19 – 94.78	0.000
E1	20	92.41 ± 3.86	83.70 - 99.17	

Table 3. Results of Data Analysis on the Wrinkle Scale

Group	n	Wrinkle Scale		*P*
		Median	Min-Max	
E0	20	5	4.00 – 6.00	0.000
E1	20	3	2.00 – 4.00	

location with strong solar irradiation. Wrinkles are more common in patients with light skin (mainly phototype I and II skin) compared to the more pigmented skin of Asians (Fitzpatrick skin types III-V). A prospective study explained that the onset of wrinkles in Asian women occurs 10 years later than in white women. Wrinkles are most prevalent in women in their 40s compared to those in their teens or early 60s with moderate to severe wrinkle severity. The severity of wrinkles varies by skin type and increases with age.[14-16] During the aging process, various cells secrete endopeptidases that can degrade extracellular matrix proteins called MMPs. MMP-1 is the major protease in human skin which degrades collagen types I and III, regulated by TIMPs. During the aging process, an imbalance of TIMPs with MMPs occurs, accelerating skin aging. Collagen, MMPs, and TIMPs are important markers for studying skin rejuvenation using nonablative lasers.[17]

In this study, there was a significant increase ($P<0.05$) in TIMP-1 after therapy. This research was in line with various other studies. A study on human skin fibroblasts cultured after being irradiated twice at 1-month intervals using QS Nd: YAG 532 nm and 1,064 nm lasers with a fluence of 1.5 J/cm^2 showed that both lasers significantly increased TIMP-1 figure at 24 and 48 hours post-irradiation using RT-PCR examination. The increase using 1064 nm QS Nd: YAG laser was higher than 532 nm. The confirmatory results of this study showed that the modulation of TIMP figures resulting from laser irradiation led to clinical improvement.[18]

The result of this study was that there was a non-significant increase in MMP-1 ($P>0.05$) in wrinkles treated with the picosecond laser. Another study with the fractional CO_2 laser as a therapeutic modality for skin rejuvenation showed a significant increase in MMP-1, -3, and -9, followed by the induction of procollagen types I and III.[19] In a study on 20 patients (mean age: 58 years) with photodamage treated focally on the dorsal arm with a nonablative fractional laser, skin samples were taken at baseline and after treatment. Proinflammatory cytokines are known to induce MMPs. MMP-1 mRNA levels significantly increased 1 day after laser therapy (153 and 276-fold using 15 and 70 mJ energy, respectively), and MMP-1 mRNA levels returned to baseline one week after laser exposure.[20] In a study, six female rats were treated with the 1064 nm QS Nd: Yag laser with a spot size of 6 mm and three fluences, namely 0.6, 1.5, and 2.5 J/cm^2. The treatment was carried out four times with an interval of one day, and the samples were taken on the 30th day. The results explained that the MMP level in the treatment group with a fluence of 2.5 J/cm^2 was higher than the groups treated with fluences of 0.6 and 1.5 J/cm^2.[17] It can be concluded that MMP can increase at the beginning of laser therapy and decrease over time. The basic concept of laser skin rejuvenation is laser-induced wound healing. The release of various immune cells and the increase of inflammatory mediators after laser-induced injury induce MMPs. Skin injury caused by a laser with high fluence may be the main reason for the increase in the MMP. The increase in MMP can degrade the old abnormal extracellular matrix and stimulate the production of the new extracellular matrix to reorganize collagen in the dermis.[17-19,21]

MMP and TIMP are considered as the ratio of MMP/TIMP; an increase in MMP and a decrease in TIMP can reflect aging. The balance between MMPs and TIMPs occurs under normal circumstances and is important during tissue remodeling.[17] In this study, the MMP-1/TIMP-1 ratio in wrinkles treated with the picosecond laser was lower with a non-significant difference ($P<0.215$). There was a decrease in the MMP-1/TIMP-1 ratio which occurred due to an insignificant increase in MMP-1 but

a significant increase in TIMP-1. The decrease in the MMP-1/TIMP-1 ratio may reflect MMP-1 activity being inhibited by the increased TIMP-1. This study showed that collagen degradation after therapy can be inhibited not only by decreasing the MMP but also by increasing the TIMP. In addition to the upregulation of collagen figure, the inhibition of collagen degradation also contributes to the increase of collagen content in laser-irradiated skin.[17,18,22]

There was a significant increase in collagen density ($P < 0.05$) in this study. By the histological examination of the skin 3 months after therapy using the QS Nd: YAG laser performed on 6 patients with skin type II-IV, each patient received therapy with a fluence of 7 J/cm^2 and a spot size of 3 mm. Histological analysis showed that the treated area had a thickened upper papillary collagen zone with increased regularity of collagen fiber arrangement.[5] Research with a 755 nm picosecond laser using DLA on 6 research subjects that received 4 times therapy on the face with an interval of 1 month. Biopsy at one month after therapy showed mild vacuolization seen in the basal layer of keratinocytes with increased dermal collagen (larger volume and denser) with normal architecture. Three months after therapy, the collagen architecture continued denser. Furthermore, 6 months after therapy, a histology examination found a significant increase in collagen, which was more pronounced in the mid and lower dermis but increased throughout the sample thickness. The histology examination correlated with wrinkle reduction.[23]

In this study, there was a significant decrease in the wrinkle scale ($P < 0.05$). Lower scales show fewer wrinkles. The side effects that occurred were mild and disappeared without leaving a trace. Side effects that occurred were mild erythema and edema that occurred in just a few hours, scabs in 1 patient, and 3 patients with urticarial reactions which disappeared shortly after therapy. There were no reports of scarring, post-inflammatory hyperpigmentation, or hypopigmentation, and no reports of any other serious side effects. A study of 40 Caucasian women with an average age of 58 years with 85% percent of subjects having Fitzpatrick skin types II and III, 4 subjects were skin types I, and 2 subjects were IV skin type. Subjects received laser therapy on the face 4 times with an interval of 1 month, using a 755 nm picosecond laser + DLA with a spot size of 6 mm and fluence of 0.71 J/cm^2, 10 Hz. The average score of wrinkles before therapy using the Fitzpatrick wrinkle scale was 5.48. Six months after therapy, evaluation showed that the mean score decreased to 3.47 ($P < 0.05$). Lower scores indicate fewer wrinkles.[9] The 755 nm picosecond laser with DLA is currently FDA-approved for reducing wrinkles with scientific evidence to be a useful tool for skin rejuvenation (Level 2A).

Various studies have demonstrated safety with good efficacy due to the unique mechanism of fractionated picosecond lasers that produce more intense tissue injury that is localized, focused, and precise in the epidermis and papillary dermis.[24] The laser uses a wavelength of 755 nm with the main target of melanin in the epidermis. It uses DLA which will redistribute and focus laser energy by delivering 70% of the energy to 10% of the tissue. In contrast, 30% of the energy will be delivered to the rest of the tissue to maintain energy and remain low so that no damage occurs in the surrounding tissue. Laser power is delivered very quickly because it uses a picosecond pulse duration; a very fast pulse duration will also cause the peak energy to be greater. The incoming energy will be absorbed by melanin as a good mediator for LIOB, and the next incoming energy will enter LIOB, causing a photomechanical effect that will cause a wound-healing response to the dermis. The formation of LIOB makes therapy with a picosecond laser safe because the laser power is absorbed by the LIOB so that the photothermal effect will not spread to the surroundings.[10,23] Various scientific pieces of evidence show that laser therapy is effective in increasing collagen density, which is consistent with the results of this study. The mechanism of improving wrinkles can be through increasing TIMP-1 and decreasing the MMP-1/TIMP-1 ratio which causes an increase in collagen density. Treatment using a picosecond laser is a good treatment option for skin rejuvenation, especially in skin of color where laser options are more limited due to possible side effects.[10,22,23,25] However, there are several limitations in this study. Observation in this study was carried out for only 4 weeks, not periodically, considering the dynamic wound healing process. This study also was not compared with a control group who received standard therapy. Further research on the face as an area where rejuvenation procedures are often performed is needed with a longer observation period, and it is also required to compare the results of such a treatment with those of standard therapy.

Conclusion

The result of clinical examination, histopathology, and immunohistochemistry in this research explained that a picosecond 755 nm laser with DLA is an effective therapy for Indonesian patients with wrinkles without any adverse effects.

Authors' Contribution

Conceptualization: Putri Hendria Wardhani, Cita Rosita Sigit Prakoeswa, Muhammad Yulianto Listiawan.
Data curation: Putri Hendria Wardhani.
Investigation: Putri Hendria Wardhani, Cita Rosita Sigit Prakoeswa, Muhammad Yulianto Listiawan.
Methodology: Putri Hendria Wardhani, Cita Rosita Sigit Prakoeswa.
Project administration: Putri Hendria Wardhani, Cita Rosita Sigit Prakoeswa.
Software: Putri Hendria Wardhani.
Supervision: Cita Rosita Sigit Prakoeswa, Muhammad Yulianto

Listiawan.
Validation: Cita Rosita Sigit Prakoeswa, Muhammad Yulianto Listiawan.
Writing–original draft: Putri Hendria Wardhani.
Writing–review & editing: Putri Hendria Wardhani, Cita Rosita Sigit Prakoeswa, Muhammad Yulianto Listiawan.

Competing Interests
None declared.

Ethical Approval
Ethics was approved by the Dr. Soetomo General Academic Hospital Review Board in Surabaya, Indonesia with number: 0161/KEPK/III/2021

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