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The Effects of Topical Ketamine Gel on Saliva and Serum Level of Interlukin-6 in Patients with Recurrent Aphthous Ulceration

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الخلاصة

الهدف من الدراسة: تهدف الدراسة الى تقييم فعالية هلام الكيتامين الموضعي(٠,٥:) في اللعاب والمصل ومستوى (٦- Interlukin) في المريض الذي يعاني من تقرح القلاعية المتكررة (RAU) ومقارنة النتائج مع مجموعة صحية ليس لديها تقرح القلاعية المتكررة. **المواد وطرائق العمل:** تم إجراء الدراسة على(٣٦) شخصا بأعمار تراوحت على شكل متوسط حسابي بين (٢٥,١١±٦,٣١)سنة ولكلا الجنسين(٢٠ ذكر، ١٦أنثى) وتم تقسيمهم الى ثلاث مجاميع. المجموعة(١) هي (مجموعة الدراسة) تكونت من (١٢) مريضا يعاني من تقرح القلاعية المتكررة الطفيفة (Min AU) استعمل هلام كيتامين موضعيا, المجموعة(٢) هو (مجموعة علاج مموّه) تكونت من(١٢)مريضا يعاني من تقرح القلاعية المتكررة الطفيفة استعمل الهلام لوحده موضعيا. المجموعة(٣)(مجموعة المراقبة) تكونت من (١٢)شخصا يتمتعون بصحة جيدة وعدم وجود تقرح القلاعية المتكررة. تم جمع عينات من الدم الوريدي المحيطي وعينة لعاب من كل المجاميع قبل العلاج،وأربعة أيام بعد العلاج من كل مريض من المجموعة (١)والمجموعة(٢)فقط،لتقييم مستوى (٦-IL) في مصل الدم واللعاب بواسطة جهاز((ELISA، أجري تقييم ذاتي للمرضى،وسجلت شدة الألم قبل وبعد(٤)أيام من العلاج في المجموعة (١)والمجموعة (٢)باستخدام مقياس الألم اللفظي (VPS): تم تحليل البيانات باستخدام paired t-test و One way ANOVA و Wilcoxon Signed Ranks Test. **النتائج:** أظهرت النتائج أن معدل تركيز (٦-IL)في المصل ازداد في المجموعة الأولى والثانية (١٨,٤٩ ± ٥,٤pg/ml) , (١٨,٩٧ ± ٧,٤٧pg/ml) على التوالي قبل العلاج مقارنة مع تركيزه في مصل المجموعة الثالثة (٩,٦٣ ± ٢,٧٧pg/ml)،كما وازداد تركيز(٦-IL) في لعاب المجموعة الأولى والثانية (٦٣,٠٣ ± ٤٧,٢٨pg/ml) (٦٢,٢٥ ± ٤٥,١٤pg/ml) على التوالي قبل العلاج مقارنة مع متوسط تركيزه في لعاب المجموعة الثالثة (١٣,١ ± ٥,٠٨pg/ml). وقد انخفضت تركيز(٦-IL) في المصل واللعاب بشكل ملحوظ في المجموعة الأولى بعد (٤) أيام من العلاج بواسطة هلام الكيتامين الموضعي إلى (١١,٣١ ± ٤,٤٩pg/ml) ، (٢٢,٦١ ± ١٦,٨١pg/ml)على التوالي، بينما نلاحظ في المجموعة الثانية المعاملة بالهلام لوحده أن تركيز (٦-IL) في مصل الدم واللعاب قد ارتفع الى (٩٧,٣٦ ± ٢٥,٨٧pg/ml) و (٨٥,٦٣ ± ٦٧,٢٩pg/ml) على التوالي بعد اربعة أيام من العلاج. إن شدة الألم أنخفضت بشكل معنوي من مرتبه(٣)في المجاميع المعاملة بهلام الكيتامين وبالهلام لوحده الى (٠) ، (١,٣٣ ± ١,٣) على التوالي بعد (٤) أيام من العلاج. **الخلاصة:** أشارت البيانات إلى أن هلام الكيتامين له تأثير مضاد للالتهابات الذي يمكن أن يقلل من مستوى (٦-IL) في مصل الدم واللعاب في المريض الذي يعاني من تقرح القلاعية المتكررة (RAU) وتقليل كل من الالتهاب والألم حتى بعد الأستعمال الموضعي.

ABSTRACT

Aims: To evaluate the effect of topical application of ketamine gel (0.5%) and gel alone(without ketamine) on salivary and serum level of human interlukin-6(IL-6)in patient with recurrent aphthous ulceration (RAU) and compare the results with control group having no RAU. **Materials and methods:** The investigation was carried out on(36) subjects with mean ± SD ages (25.11±6.31)years and different sex (20 males,16 females). These subjects were divided into three groups. Group1: The study group consisted of (12)patients having minor RAU received topical ketamine gel (0.5%) applied on the lesion. Group2: The placebo group consisted of (12)patients having minor RAU received topical gel alone. Group3: The control group consisted of (12) healthy subjects having no RAU.Peripheral venous blood and saliva samples were collected from all subjects before treatment, and four days after treatment from group1and group2 only, to assess the levels of serum and salivary (IL-6) by Enzyme Linked Immuno Sorbent Assays (ELISA) method. Patients were subjectively assessed for the intensity of pain which was recorded pre and four days post treatment in group1 and 2 by using Verbal Pain Scale (VPS). Data were analyzed using paired t-test, One way ANOVA and Willcoxon Signed Ranks test. **Results:** The results showed that the mean of serum IL-6 concentrations were high in the study and placebo groups (18.49±5.4pg/ml) and (18.97± 7,47 pg/ml) respectively before treatment comparing with mean of serum IL-6 concentration of control group (9.63±2.77pg/ml), and the mean of salivary IL-6 concentrations were high in the study and placebo groups (63.03±47.28pg/ml) and (62.25±45.14pg/ml) respectively before treatment comparing with mean of saliva IL-6 concentration of control group (13.1±5.08pg/ml).

Treatment by topical ketamine gel (0.5%) had led to significant decrease in the means of serum and salivary IL-6 level in the study group to (11.31±4.49pg/ml) and (22.61±16.81pg/ml) respectively, whereas the means of serum and salivary IL-6 level were increased to (36.97±25.87pg/ml) and (85.63±67.29pg/ml) respectively 4 days after treatment by topical gel alone in placebo group. The intensity of pain was significantly decreased from (3) in study and placebo groups to (0) and (1.33±1.3) respectively after 4 days of treatment. **Conclusions:** Data denote that ketamine gel posses anti inflammatory effect by decrease the levels of serum and salivary IL-6 in patients with RAU that may reduce inflammation and pain after topical application.

Key words: Ketamine, Interlukine-6(IL-6), Verbal Pain Scale (VPS), Recurrent Aphthous Ulceration (RAU).

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INTRODUCTION

RAU is defined as a disorder of unknown causes with many etiological predisposing factors associated with clinically significant morbidity[1]. These ulcerations are periodic painful, single or multiple ulcers which heal spontaneously, thay are round or ovoid , shallow and surrounded by inflammation that chiefly involves the nonkeratinized mucosa[2,3].RAU is mostly located on the peripheral borders of the tongue, lips, buccal mucosa, or palate. It is divided into three clinically distinct groups which consists of minor, major and herpetiform lesions[3].

(IL-6) is known to cause pro- and anti-inflammatory effects during different stages of inflammation[4], due to it3s multiple stimulatory effects on cells of the immune system and vascular endothelial cells, it is believed that excess IL-6 plays a pathogenic role in the development of inflammation, resulting in hyperalgesia and edema[5], RAU is correlated with elevated pro-inflammatory cytokines such as IL-6[6,7].

The treatment of RAU remains unsatisfactory, as most therapies only reduce the severity of the ulceration and do not stop recurrence[8]. Topical application, this term is used to describe medicine that has effects only in a specific area, not throughout the body, particularly medicine that is put directly on the mucosa, dental, gingival, or pulpal tissues[9].

Ketamine is a phencyclidine anesthetic, increasingly used in sub anesthetic doses as an analgesic in a wide range of pain settings[10]. The analgesic effect of ketamine is primarily based on the antagonism of the N-methyl-D-aspartate (NMDA) receptor[11] .The topical ketamine applications as being useful for pain management in cases of post-surgical neuropathic pain, complex regional pain syndrome, lumbar radiculopathy, post-herpetic neuralgia, and idiopathic proctodynia.[12,13] , so the aims of present study are to evaluate the efficacy of topical ketamine gel on serum and salivary level of human IL-6 as anti-inflammatory medication in patients with RAU and to assess the analgesic effect of topical ketamine gel in patients with RAU.

Materials and Methods

The study was carried out on a total number of forty five patients (nine patients were excluded due to failure the follow up or did not use the recommended drugs). Mean age ± SD was (25.11±6.31) years with a range of (13-42) years and sex (20 males,16 females). All patients were healthy and they had no history of any systemic diseases.

Subjects were divided into three groups each group consisted of (12) patients, group 1 (study group) and group 2 (placebo group) having minor RAU received topical ketamine gel (0.5%) and gel without ketamine respectively on the lesion three time daily for four days, group 3(control group) having no (RAU). (5) ml of peripheral venous blood and saliva sample were collected from each subject of (group3). Also (5) ml of peripheral venous blood and saliva samples were collected from each subject of (group1) and (group2) before and 4 days after treatment, then store them in deep freeze. Serum and salivary IL-6 level were evaluated by Enzyme Linked Immuno Sorbent Assays (ELISA) using a quantitative sandwich immunoassay technique according to (Boster Biological Technology, LTD. USA, 2013). Patients subjectively assessed the intensity of pain pre and 4 days post treatment in group1 and 2 and recorded by using Verbal Pain Scale (VPS).The scale consists of four degree: 0: no pain; 1: Mild pain; 2: Moderate pain; 3: Severe pain; 4: Very severe pain[14]. Data were analyzed using Paired t-test, One way ANOVA and Wilcoxon Signed Ranks test.

Results

The results showed that the mean of serum (IL-6) concentration were high significantly in the study and placebo group (18.49± 5.4pg/ml) and (18.97±7.47pg/ml) respectively before treatment comparing with serum (IL-6) concentration of control group (9.63±2.77pg/ml) at P≤0.01 (Figure (1)).

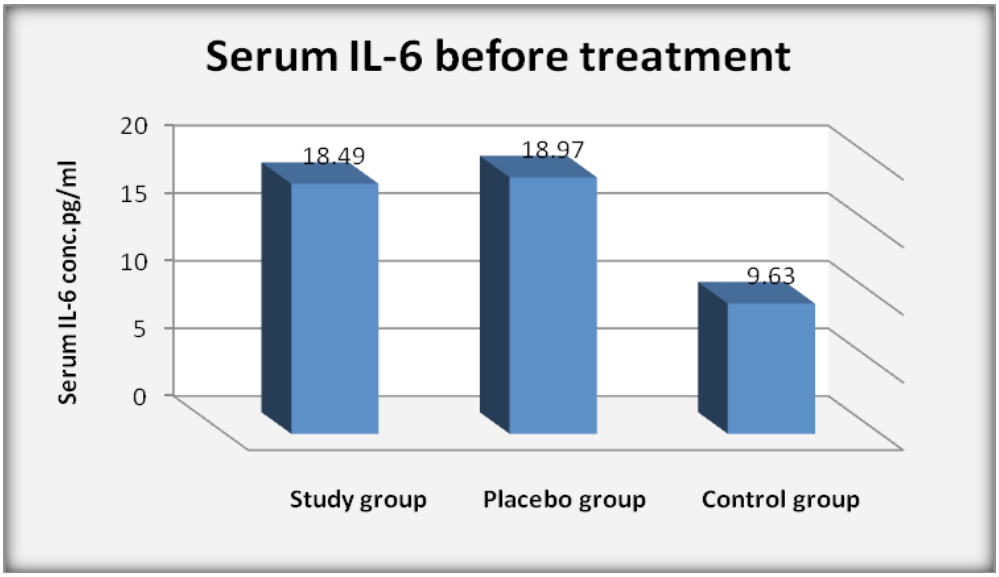

Figure (1): Comparison between the mean of serum IL-6 concentration before treatment in study, placebo and control group.

The result also reveals that the mean of salivary (IL-6) concentration were high significant in the study and placebo group (63.03±47.28pg/ml) and (62.25±45.14pg/ml) respectively before treatment comparing with salivary (IL-6) concentration of control group (13.1±5.08pg/ml) at P≤0.01 (Figure (2)).

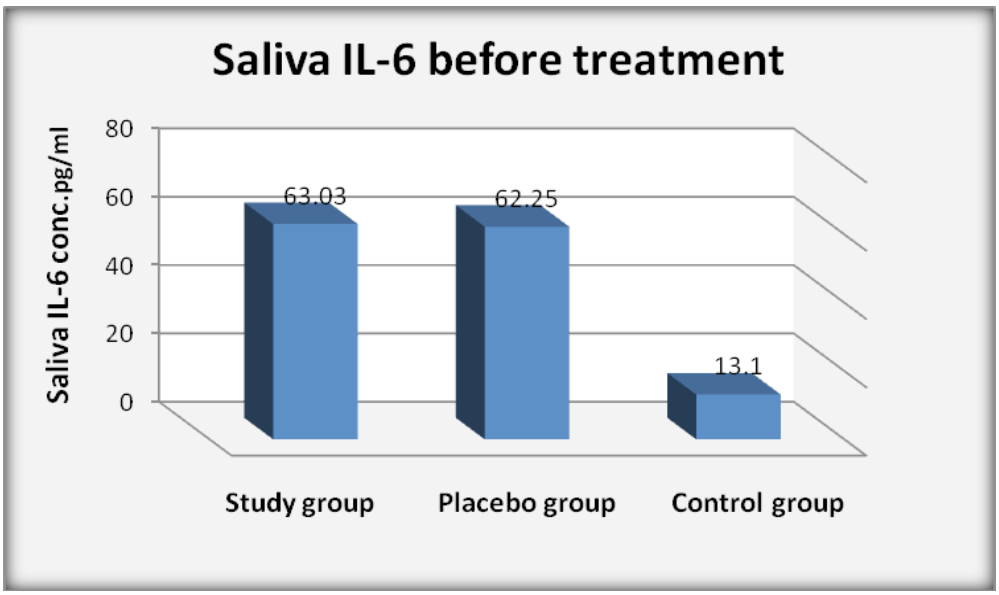

Figure (2): Comparison between the mean of saliva IL-6 concentration before treatment in study, placebo and control group.

The mean of serum (IL-6) concentra-tion were decreased significantly in the study group from (18.49± 5.4pg/ml) to (11.31±4.49 pg/ml) 4 days after treatment by topical ketamine gel at P≤0.01, whereas the mean of serum (IL-6) concentration increased significantly from (18.97±7.47pg/ml) to (36.97±25.87pg/ml) 4 days after treatment by topical gel alone in placebo group at P≤0.05,(Table (1)).

Table (1): Comparison between the mean of serum IL-6 concentration in study and placebo group before and after treatment.

	Mean ± SD of serum IL-6 before treatment	Mean ± SD of serum IL-6 after treatment	t	df	Sig. (2-tailed)
Study group	18.49±5.4	11.31±4.49	3.35	11	0.006**
Placebo group	18.97±7.47	36.97±25.87	-2.3	11	0.042*

** P ≤ 0.01 * P ≤ 0.05

(Table (2)) shows that the mean of salivary (IL-6) concentration were decreased significantly in the study group from (63.03±47.28 pg/ml pg/ml) to (22.61±16.81 pg/ml) 4 days after treatment by topical ketamine gel at P≤0.05, whereas the mean of salivary(IL-6)concentration increased but not significantly from (62.25±45.14pg/ml) to (85.63±67.29pg/ml) 4 days after treatment by topical gel alone in placebo group.

Table (2): Comparison between the mean of saliva IL-6 concentration in study and placebo group before and after treatment.

	Mean±SD of saliva IL-6before treatment	Mean±SD of saliva IL-6after treatment	t	df	Sig. (2-tailed)
Study group	63.03±47.28	22.61±16.81	2.69	11	0.021*
Placebo group	62.25±45.14	85.63±67.29	-1.91	11	0.082

P ≤ 0.05 *

This indicated that the level of IL-6 was decrease in study group after treatment in comparison to the same time of treatment in placebo group where IL-6 level is increased.

The mean of pain intensity significantly decreased from (3) in study and placebo groups to (0) and (1.33±1.3) respectively after 4 days of treatment at p ≤ 0.01 (Table (3)).

Table (3): Comparison between the mean of intensity of pain in study and placebo group before and after treatment.

	Mean±SD of pain intensity before treatment	Mean±SD of pain intensity after treatment	Z	df	P-value
Study group	3±0	0	-3.464	11	0.001**
Placebo group	3±0	1.33±1.3	-2.72	11	0.006**

** P ≤ 0.01

Discussion

IL-6 is secreted by T cells and macrophages to stimulate immune response, eg. during infection and after trauma, especially burns or other tissue damage leading to inflammation[15].

In this study the IL-6 level high significant in patients having RAU, this result is in agreement with a previous studies which suggested that the RAU is correlated with elevated pro-inflammatory cytokines, such as IL-6[6,7] and agree with another study which suggested that the earliest lesion of recurrent aphthous ulceration is a pre ulcerative inflammatory focus within the oral epithelium that is characterized by an influx of T lymphocytes[16], inflammation results in rapid elevation of the secretion of proinflammatory mediators, chemokines and cytokines, such as IL-1, IL-6 and TNF-α[17].

IL-6 level was decreased significantly 4 days after treatment by topical ketamine gel (0.5%), this mean that the ketamine have anti-inflammatory effects because IL-6 is known to cause proinflammatory effects during different stages of inflammation[4], this result is in agreement with a previous study which suggested that ketamine has anti-inflammatory effects on macrophages stimulated with lipopolysaccharide (LPS) in vivo and in vitro[18].

In this study it had been found that the decreased IL-6 level by ketamine associated with significantly decreased pain, this result is in agreement with a previous study which suggested that deregulation of IL-6

expression causes the synthesis and release of many inflammatory mediators, which may result in pain[19].

Inflammatory substances play a role in pain modulation by interfering with nociceptive transduction, conduction and transmission[17]. The IL-6 has multiple stimulatory effects on cells of the immune system and vascular endothelial cells, and their excess plays a pathogenic role in the development of inflammation, resulting in hyperalgesia and edema[5]. The analgesic effect of ketamine in the present study is coincide with previous studies which suggested that one of the custom compounds useful for some patients is a topical ketamine, because peripheral N-methyl-D-aspartate (NMDA) receptors have been implicated in nociception[20,21,22]. Ketamine blocks NMDA and 5HT receptors, Na+ and Ca+2 channels, and the edema response associated with inflammation[10,23,24,25].

Topical ketamine applications have documented success with neuropathic pain in terms of providing some direct analgesia and in terms of inhibiting sympathetically maintained pain, these different mechanism of action of ketamine for blocking the pain may explain the decrease of pain intensity in the present study, however, the most important use of custom-compounded ketamine cream is the reduction of hyperalgesia, the allodynia, and as a tolerance-protective agent[10,23,24,25]. While a decrease in pain by using gel alone may be due to providing a protective cover which reduce pain, this result is in agreement with previous study which suggest that the dressing agent will lead to decrease pain, promote wound healing and improves the quality of patient's life[26].

Conclusion

Besides the classical action of ketamine as a general anesthetic drug it may play an important role in immune system as anti-inflammatory by reducing secretion of IL-6, cytokine and reduce pain of RAU.

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