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The Effect of Vit D3 (1,25 dihydroxy cholicalciferol) on Serum Level of Human Tumor Necrosis Factor Alpha (TNF α) Following Apicoectomy

Faris E Mohammad
B.PH.SC

Bin Sina Teaching Hospital
Ninavah Health Directorate MOH Iraq

Tahani A Alsondook
BDS, MSc (Prof.)

Department of Dental Basic Sciences
College of Dentistry, University of Mosul

Attalla F Rejab
BDS, MSc, FIBMF (Lec.)

Department of Oral and Maxillofacial Surgery
College of Dentistry, University of Mosul

الخلاصة

الاهداف: يهدف البحث إلى تقييم فعالية فيتامين D3 على مستوى TNF α في مصل الدم للمرضى الذين يعانون من خراج مزمن في جذر السن والذي يعالج عن طريق استئصال قمة الجذر ومقارنة نتائجهم مع المجموعة الأخرى(السيطرة) المعاملة بالماء المقطر **المواد وطرائق العمل:** أجريت هذه الدراسة على (٥٠) شخص بأعمار مختلفة (١٦-٤٥) سنة ومن كلا الجنسين (٢٧ ذكرا و ٢٣ أنثى) يعانون من خراج مزمن في جذر السن تم تقسيم المرضى الى مجموعتين (مجموعة الدراسة ومجموعة السيطرة) مجموعة الدراسة شملت (٢٥) مريضا تم إعطائهم فيتامين D3 أمبولة (٣٠٠٠٠٠ وحدة دولية) فمويا قبل (٢٤) ساعة من العملية ، و مجموعة السيطرة شملت (٢٥) مريض تم إعطائهم الماء المقطر بالفم قبل (٢٤) ساعة من إجراء العملية وجميع المرضى. تم سحب الدم من جميع المرضى لكلا المجموعتين قبل إجراء العملية وبعد (٣ و٢٤)ساعة من إجراء العملية .ومن ثم تم تحديد مستوى TNF ألفا بالدم بواسطة جهاز (ELISA). تم تقييم شدة الألم للمرضى ذاتيا باستخدام مقياس (VAS) وقد تم تحليل البيانات باستخدام اختبار t وطريقة تحليل التباين ANOVA. **النتائج** :وأظهرت النتائج انخفاض مستويات TNF α بعد(٣) ساعات من العملية في مجموعة الدراسة إلى (٢٢.٦٢ ± ٤.٥٨ pg/ml) بعد العلاج بفيتامين D3 مقارنة مع ما قبل المعالجة (٢٨.٣٣ ± ١٠.١٦pg/ml)، والمجموعة الثانية (٣) ساعات بعد العمل الجراحي (٥١.١١ ± ٩.٠٥pg/ml) ،كما انخفض مستوى TNF بعد٢٤ساعة من العملية إلى حد كبير (٢٨.٢٤ ± ٧.٣٥ (pg/ml) مقارنة بالوقت نفسه مع مستواه في مجموعة السيطرة (٦٦.١٥ ± ١٣.٠٢pg/ml). ولقد لوحظ انخفاضا ملحوظا في مجموعة الدراسة بعدد حبوب الباراستول إلى(١.٢٤ ± ١.٠١حبة) مقارنة مع مجموعة السيطرة (٢.٢٤ ± ١.٢٣ حبة) كما لوحظ زيادة في فترة التسكين من الألم بعد العملية (٢٤٠.٥٦ ± ١٥٦.٥٥ دقيقة) لمجموعة الدراسة مقارنة مع مجموعة السيطرة (١٥١.١٢ ± ١٠٢.٨٠ دقيقة) كما وتبين من دراستنا الحالية انخفاضا واضحا في شدة الألم بعد (٨ و١٢) ساعة من إجراء العملية في مجموعة الدراسة (١.١٦ ± ٢.٢٧) (٠.٤٤ ± ٠.٩٦) على التوالي بالمقارنة بنفس الأوقات في مجموعة السيطرة (١.٨٧±٢.٩٢) (١.٥٢ ± ١.٧٨) على التوالي. **الاستنتاجات:** تشير النتائج التي تم الحصول عليها إلى أن فيتامين D3 له تأثير مضاد للالتهابات وذلك عن طريق خفضه من مستوى TNF α مما يقلل بدوره من الالتهاب والألم بعد عملية قطع ذروة الجذر.

ABSTRACT

Aims: To evaluate the efficacy of Vit D3 on serum level of human TNF α in patients with chronic periapical lesion that intended to undergo apicoectomy and compare the results with a group of placebo control. **Materials and Methods:** The investigation was carried out on 50 subjects of different age(16-45) and sex(27 male,23 female) complain of chronic periapical lesion were divided into two groups (study and placebo group). Study group consist of 25 subject received Vit D3 ampoule(300000 IU) orally 24 hrs before apicoectomy procedure and placebo group consist of 25 subject received distal water ampoule orally 24 hrs before apicoectomy procedure. Peripheral venous blood samples were collected preoperatively , 3hrs postoperatively and 24 hrs postoperatively from each subject of both group to evaluate serum TNF α level by Enzyme Linked Immunosorbent Assays (ELISA) device. Patients subjectively assessed the strength the post operative pain by using Visual Analogue Scale (VAS). Data were analyzed using paired t-test and A NOVA test. **Results**: The results showed that the serum TNF α levels were decreased significantly in the study group after 3hrs of surgical procedure to (22.62 ± 4.58 Pg/ml) following Vit D3 treatment comparing with TNF α level pretreatment (28.33 ± 10.16 Pg/ml) whereas the TNF α level were increased from (33.38 ± 23.92 Pg/ml) preoperative to (51.11 ± 9.05 Pg/ml) 3hrs postoperative in placebo group. Serum TNF α level after 24hrs of surgical procedure in study group decreased significantly to(28.24± 7.35Pg/ml) pg/ml) comparing with the postoperative 24 hrs TNF α of placebo group (66.15 ± 13.02 pg/ml),this indicated that the level of TNF α were increased in placebo group after 3hrs and 24hrs postoperatively in comparison to the same time of opera-

tion in study group. The number of analgesic tablet (acetaminophen) significantly decreased in study group to (1.24 ± 1.01) comparing with placebo group (2.24 ± 1.23). Duration of analgesia post operatively increase significantly to (308.20± 144.43) min in study group compared with placebo group (228.44 ±123.02) min. The intensity of postoperative pain decrease significantly after 8hrs (VAS1), 12 hrs (VAS2) of surgical operation in study group (1.16 ± 2.27), (0.44 ± 0.96) compared with placebo group (2.92 ± 1.87), (1.52 ± 1.78) respectively. **Conclusion**: data indicated that Vit D3 have anti inflammatory effect that can decrease level of TNF α so reduced pain and inflammation after apicoectomy .

Key words: Vit D3 , Tumor necrosis factor (TNF α), Visual analog scale (VAS), Apicoectomy.

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INTODUCTION

Periapical inflammatory lesions are frequent a consequence of dental caries. This type of lesion develops as an immune reaction triggered by the presence of bacteria and toxins in the root canal of periapical region.[1] After microbial invasion of periapical tissues, both nonspecific and specific immunologic responses persist in the host tissues. This inflammatory process ultimately results in destruction of the alveolar bone surrounding the tooth. It is characterized by the presence of immunocompetent cells producing a wide variety of inflammatory mediators. TNF-alpha is a soluble mediator release from immune-competent cells in inflammatory processes. TNF-alpha plays an important role in initiating and coordinating the cellular events that make up the immune system's response to infection. The biological effects of TNF-alpha include activation of leukocytes such as lymphocytes (T and B cells), macrophages, and natural killer cells; fever induction; acute-phase protein release; cytokine and chemokine gene expression; and endothelial cell activation.[2] TNF α belongs to the group of main pro inflammatory cytokines play a major role in inflammatory and immune responses initiated by infection or injury.[3,4] The inflammatory response in the persisting apical lesion protects the host from further microbial invasion.[5]

Besides its classical actions in calcium homeostasis and bone metabolism vitamin D plays an important role in the immune system.[5,6] Current studies have related vitamin D deficiency with several autoimmune disorders, including insulin dependent diabetes mellitus, multiple sclerosis, inflammatory bowel disease, systemic lupus erythema- tosus,and rheumatoid arthritis. [7-10] Among the immunomodulatory effects of Vit D3 a reduction in the production of interleukin-2 (IL-2), gamma interferon (INFγ), and tumor necrosis factor (TNF α); inhibition of the expression of IL-6; and inhibition of the secretion and production of auto antibodies by B lymphocytes. [11,12]

MATERAILS AND METHODS

The investigation was carried out on 50 subjects of different age (16-45) and sex (27 male, 23 female) complain of chronic periapical lesion of upper anterior and premolar (single tooth) attended to the Oral and Maxillofacial Surgery Department in College of Dentistry at University of Mosul, diagnoses were established according to the clinical and radiological findings. All patient were medically fit and they had no history of any disease, fifty patients with chronic periapical lesion were divided into two groups of study and placebo (control). Study group consist of 25 subjects received Vit D3 ampoule (300000 IU/1ml) orally 24 hrs before apicectomy procedure and placebo group consist of 25 subjects received distal water ampoule orally 24 hrs before apicectomy procedure. Blood sample taken from each subject of both groups 24 hrs pre operatively, 3hrs and 24 hrs postoperatively in serum isolated tube, blood sample stay for 30 min in room temperature for clotting then centrifuge at 2000 rpm for 10 min (china). Serum TNF alpha level were determined by Enzyme Linked Immunosorbent Assays (ELISA) (Assaypro ,USA) using a quantitative sandwich immunoas-

say technique as manufacture instructions. The intensity of pain was recorded using a Visual Analogue Scale at 8th, and 12th hours post operation. Data were analyzed using paired T-test and A NOVA test.

RESULTS

The results (Tables 1, 2, 3) showed that the serum TNF α levels were decreased significantly in the study group after 3hrs of surgical procedure to (22.62 ± 4.58 Pg/ml) following Vit D3 treatment comparing with TNF α level pretreatment (28.33 ± 10.16 Pg/ml) whereas the TNF α level were increased from (33.38 ± 23.92 Pg/ml) preoperative to (51.11 ± 9.05 Pg/ml) 3hrs postoperative in placebo group.

Table (1): TNF α concentration in study and placebo group

GROUP		N	Mean(pg/ml)	SD±	Std. Error
control	preoperative	25	33.38	23.92	4.784862
	postoperative 3hr	25	51.11	9.05	1.810605
	postoperative 24hrs	25	66.15	13.04	2.607346
	Total	75	50.21	21.19	2.447075
Study	preoperative	25	28.33	10.16	2.031780
	postoperative 3hr	25	22.62	4.58	.915986
	postoperative 24hrs	25	28.24	7.35	1.469513
	Total	75	26.39	8.06	.931098

GROUP=placebo

Table (2): ANOVA Test comparison for TNF α Within placebo groups

		Duncan's Grouping		
TIME	**N**	**A**	**B**	**C**
preoperative	25	33.38 ± 23.92		
postoperative 3hr	25		51.11 ± 9.05	
postoperative 24hrs	25			66.15 ± 13.04

GROUP=study

Table (3): ANOVA test comparison for TNF α Within study group

		Duncan's Grouping	
TIME	**N**	**A**	**B**
postoperative 3hr	25	22.62 ± 4.58	
postoperative 24hrs	25		28.24 ± 7.35
preoperative	25		28.33 ± 10.16

Table (4) showed that Serum TNF α level after 24hrs of surgical procedure in study group decreased significantly to (28.24± 7.35Pg/ml) pg/ml) comparing with the postoperative 24 hrs TNF α of placebo group (66.15 ± 13.02 pg/ml), this indicated that the level of TNF α were increased in placebo group after 3hrs and 24hrs postoperatively in comparison to the same time of operation in study group (Table 4).

Table (4): t–test comparison for TNF between study and placebo groups

Time	Group	No.	Mean pg/ml	± SD	t-value	df	*p*-value
Pre-operative	Study	25	28.33	10.159	0.972	48	0.336
	Control	25	33.38	23.92			
Post-operative 3 hrs	Study	25	22.62	4.58	14.041	48	0.000*
	Control	25	51.11	9.05			
Post-operative 24 hrs	Study	25	28.24	7.35	12.665	48	0.000*
	Control	25	66.15	13.04			

*Differences were statistically significant at $p \leq 0.05$

The number of analgesic tablet (acetaminophen) significantly decreased in study group to (1.24 ± 1.01) comparing with placebo group (2.24 ± 1.23) Table (5).

Table (5) : t- test comparison of mean analgesic tablet consumption and duration of analgesia between study and placebo group.

	group	N	Mean ± SD	Std. Error Mean	t	df	*p*- value
Number of analgesic tablet	placebo	25	2.24 ± 1.23	.247	3.113	48	0.003*
	Study	25	1.24 ± 1.01	.202			
duration of analgesia	Placebo	25	228.44 ± 123.02min	24.60	-2.102	48	0.041*
	Study	25	308.20 ± 144.43min	28.89			

* Differences were statistically significant at $p \leq 0.05$

Duration of analgesia post operatively increase significantly to (308.20± 144.43) min in study group compared with placebo group (228.44 ±123.02) min (Table 5).The intensity of postoperative pain decrease significantly after 8hrs (VAS1),12hrs (VAS2) of surgical operation in study group (1.16 ± 2.27), (0.44 ± 0.96) compared with placebo group (2.92 ± 1.87), (1.52 ± 1.78) respectively (Table 6).

Table (6) : t- test comparison of VAS between study and placebo group.

	group	N	Mean ± SD	Std. Error Mean	*p* value
VAS1	placebo	25	2.92 ± 1.87	.374	0.004*
	study	25	1.16 ± 2.27	.453	
VAS2	placebo	25	1.52 ± 1.79	.356	0.01*
	study	25	0.44 ± 0.96	.192	

* Differences were statistically significant at $p \leq 0.05$, VAS1:Intensity of pain 8hrs postoperatively, VAS2:Intensity of pain 12hrs postoperatively

DISCUSSION

Periapical lesions usually result from a persistent inflammatory response induced by prolonged exposure of periapical tissues to various microbial agents, evoking an immunological reaction. In this local defense mechanism, various inflammatory mediators, in particular inflammatory cytokine TNF-alpha, play a complex and central role in regulation of the immune response, the immune complex is formed by cells whose main function is to recognize antigen that penetrate the organism and to neutralize and/or destroy them[15]

Secretion of cytokines is initiated with the purpose of activating immunological response to irritants and increasing local concentrations of inflammatory cells in order to prevent further colonization of bacteria within the tissues. Enhanced reaction of the the host to various antigens results bone resorption and formation of granulomatous tissue, which are the typical features of periapical lesion.[16] Systemic cytokine response induced by surgical damage has been reported and is thought to have an important effect on the immune system. TNF-α a major mediators of the acute-phase protein response to tissue damage caused by surgical intervention.[21-23]

In the present study we found that the administration of *Vit D3* significantly decrease the serum concentration of *TNF α* after 3, 24 hrs of surgical operation in *study group* compared with *TNF -α* concentration pre operative and after 3 , 24 hrs of surgical operation in *placebo group*, this result agreement with previous study suggested that the Vit D3 reduce the inflammation by decrease the secretion of pro inflammatory cytokine (TNF *α*).[11,14] All surgical procedures produced secondary effects such as pain , the intensity of which depending on the degree of tissue damage, the pain is acute and the consequence of the nociceptive stimulation produced by the surgical aggression, distension of ligaments, muscular spasms, swelling of tissue, and in general all situations related to surgical manipulation.[18] In the present study the result show by using VAS to evaluate pain intensity the maximum pain will be during the first 24 postoperative hours, this agreement with previous study.[17-20]

In the present study we found that the VAS significantly lower in *study group* at 8th, 12th hours postoperatively compared with *placebo group* and the Duration of analgesia significantly longer in *study group* with decreased in paracetamol tablet consumption. The reason for this effect may be due to the role of *TNF α* as a proinflammatory cytokine cause production of small mediators, also induce cyclooxygenase 2 which generates prostaglandin E2 (or prostacyclin from endothelium), which causes vasodilation and enhances perception of pain. Also TNF α induce NO synthase which contributes to vasodilation.[24] Recently, several studies have provided evidence that TNF α may act on primary afferent neurons and induces hypersensitivity also TNF α elicits ectopic activity if applied to (dorsal root ganglia) DRG.[25, 26]

In addition to enhancing tetrodotoxin-resistant (TTX-R) Na channels in nociceptive DRG neurons,[27] TNF-α can also increase membrane K+ ion conductance in a non-voltage-gated fashion,[28] leading to overall neuronal hyper-excitability and pain. So Vit D3 reduced pain after operation by reduced secretion of TNF α cytokine.

CONCLUSION

Besides its classical actions in calcium homeostasis and bone metabolism, vitamin D3 plays an important role in the immune system that has anti- inflammatory effect by reducing secretion of TNF α cytokine also reduced pain and inflammation after periapical surgery.

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