

Evaluation the Effect of Folic Acid on Some Inflammatory Mediators; IL-6 and TNF- α in Chronic Gingivitis Patients

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الخلاصة

الأهداف: تهدف الدراسة الى دراسة تأثير المضاد للالتهاب لحمض الفوليك وعلاقته بصحة الفم على المرضى المصابين بالتهاب اللثة المزمن. **المواد وطرائق العمل:** تم اختيار " ٤٠ " مريض حيث تم تصنيفهم الى مجموعتين: الاولى تتكون من " ٢٠ " مريضاً مصابين بالتهاب اللثة المزمن ولم يتناولوا اي نوع من العلاج (مجموعة السيطرة) ، والمجموعة الثانية تتكون من " ٢٠ " مريضاً (مجموعة العلاج) الذين تناولوا حمض الفوليك بجرعة ١ ملغرام / يوم لمدة ٤٢ يوماً ، على شكل حبوب عن طريق الفم . في بداية الدراسة تم اجراء عمليتي ازالة الترسبات السنوية و اجراء التلميع لكل مريض للوصول الى نقطة الاساس لمعايير اللثة والصحة الفموية . في اليوم التالي تم قياس هذه المعايير لكل المرضى المشاركين وايضا تم قياسها بعد ٢١ يوماً واخيراً بعد ٤٢ يوماً من تلقي العلاج. و في كل زيارة من هذه الزيارات تم جمع ٥ ملليلتر من لعاب المرضى بدون استخدام أي تحفيز من اجل قياس وسطاء الالتهاب IL-6 و TNF- α في اللعاب بطريقة ELISA . **النتائج:** بمقارنة مجموعة العلاج مع مجموعة السيطرة أظهرت النتائج وجود فرق معنوي في مستوى TNF- α بعد (٢١ و ٤٢) يوم , وانه ليس هناك فرق معنوي في مستوى IL-6 في اللعاب بالإضافة الى انه لا يوجد اختلاف معنوي بمعايير اللثة وصحة الفم بين مجموعة السيطرة ومجموعة العلاج. **الاستنتاجات:** ان استخدام حمض الفوليك لمدة قصيرة ممكن ان يحسن من مؤشرات الالتهاب في اللعاب مثل TNF- α .

ABSTRACT

Aims: To evaluate the anti-inflammatory effect of systemic treatment with folic acid and relation to oral health indices of patients with chronic gingivitis. **Materials and Methods:** Forty patients were classified into two groups; Group 1: (n:20) chronic gingivitis patients, did not receive any medications (control group), Group 2: (n:20) chronic gingivitis patients (treatment group), received 1 mg/day oral tablet of folic acid for 42 days. Scaling and polishing have been carried out for each volunteer to reach the base-line for the gingival index and oral hygiene index. In the next day, these indices were measured for all participants, then measured after 21 days then after 42 days of treatment. In each visit, five milliliters of unstimulated saliva were collected for measurement of salivary IL-6 and salivary TNF- α by human salivary ELISA kits. **Results:** There is a significant decrease in salivary TNF- α level between treatment group and control group, while there is a non-significant decrease in levels of salivary IL-6 at 21st and 42nd day. There is no significant difference in gingival index and oral hygiene index between treatment and control group. **Conclusions:** sub-acute using of oral supplementation of 1mg / day folic acid in chronic gingivitis patients improves their gingival health by reducing TNF- α level in their saliva.

Key words: Folic acid, oral health, saliva, Inflammatory mediators

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INTRODUCTION

Periodontal disease is highly prevalent in world population¹. Gingivitis is a form of periodontal disease in which gingival tissues are inflamed but their destruction is mild and reversible. Usually, gingival inflammation is caused by a pathogenic infection which triggers the innate immune system^{2,3} causing inflammation and oxidative stress in the oral tissues. It is a reversible disease in which the gum becomes red, swollen and bleed easily while touching, or brushing. It is important to treat gingivitis as early as possible to prevent the periodontitis⁴, which is an oral inflammatory disorder results in tissue damage and loss of bone⁵. Stimulation of pro-inflammatory cytokines as IL-6 and TNF- α will participate as an integral part of periodontal disease pathogenesis^{6,7}. These inflammatory cytokines are detectable in oral fluids^{8,9}. Saliva is an easily collectable oral biologic fluid that contains number of inflammatory cytokines that are produced locally or emerged from the vascular beds in the gingival tissues¹⁰. Folic acid supplementation was found to improve the gingival inflammation^{11,12} by decreasing the expression of cytokines and chemokines¹³. It has been reported that low folate level in serum is associated with periodontal disease in non-

institutionalized older adults¹⁴. This study is hypothesized that folic acid supplementation might be associated with gingival inflammation improvement and to evaluate its effect on some inflammatory mediators; IL-6 and TNF- α in the saliva.

MATERIALS AND METHODS

The study was approved by Research Ethics Committee board (University of Mosul, College of Dentistry, REC reference No. D.B.S./4/2432019-4)

Forty patients included, their ages ranged between 20 to 40 years, diagnosed and treated in the dental private clinics in Mosul city. They were divided into 2 groups: The first group consisted of "20" chronic gingivitis patients, did not receive any medication (control group). The second group, consisted of "20" chronic gingivitis patients (treatment group), who received 1 mg/day oral tablet of folic acid (SDI Company/Iraq). The choice of each volunteer depended on inclusion criteria (systematically and orally healthy individuals except for chronic gingivitis, Non-pregnant or lactating females, non-consuming any drug or supplements, a complement of more than "20" teeth, non-smoking, non-alcoholic). Scaling and polishing had been carried out for each volunteer to reach the base line

gingival index (GI) according to Loe and Silness, 1963 and oral hygiene index (OHI) according to Greene and Vermilion, 1960. In the next day, these indices were measured for all participants and measured again after 21 days then after 42 days from treatment start. At the same visits, five milliliters of unstimulated saliva were collected for measuring of IL-6 by salivary IL-6 ELISA kit (Salimetric®, USA) and measurement of TNF-α by human TNF-

α ELISA kit (MyBioSource®, USA). Statistical analysis was done by Microsoft Excel-2010. P-value is < 0.05.

RESULTS

The results are showing that there is no significant difference in GI and OHI between treatment group and control group at the beginning of the study by using Independent T-test of two means.(Table 1).

Table (1): Comparison of oral health scores between treated and control group at the beginning of the study.

Oral health scores	Treatment group [n = 20] Mean ± SD	Control group [n = 20] Mean ± SD	P-value
Gingival index	0.458 ± 0.436	0.550 ± 0.329	0.457
Oral hygiene index	0.233 ± 0.348	0.433 ± 0.380	0.091

There is no significant difference in GI and OHI between treatment and control group at the 21st day of folic

acid administration, as shown in (Table 2). Independent T-test of two means was used

Table (2): Comparison in oral health scores between treated and control group at the 21st day of the study.

Oral health scores	Treatment group [n = 20] Mean ± SD	Control group [n = 20] Mean ± SD	P-value
Gingival index	0.350 ± 0.346	0.541 ± 0.338	0.086
Oral hygiene index	0.398 ± 0.394	0.558 ± 0.493	0.085

Also there are no significant differences found in GI and OHI between treatment group and control group at

the day 42nd day of the study. Independent T-test of two means was used (Table 3).

Table (3): Comparison in oral health scores between treated and control group at the 42nd day of the study

Oral health scores	Treatment group	Control group	P-value
	[n = 20] Mean ± SD	[n = 20] Mean ± SD	
Gingival index	0.400 ± 0.317	0.566 ± 0.406	0.156
Oral hygiene index	0.441 ± 0.307	0.624 ± 0.515	0.180

In the treatment group, there is no significant difference in means of the GI and OHI during all the study period as illustrated in **Table 4**. One-way

ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share (A) letter are significantly different.

Table (4): The effect of systemic folic acid treatment on the oral health scores during the study period.

Oral health Scores	Treatment group			P-value
	1 st day Mean ± SD	21 st day Mean ± SD	42 nd day Mean ± SD	
Gingival index	0.458 ± 0.436 ^A	0.350 ± 0.346 ^A	0.400 ± 0.317 ^A	0.653
Oral hygiene index	0.233 ± 0.348 ^A	0.398 ± 0.394 ^A	0.441 ± 0.307 ^A	0.175

At the beginning of study, the results showed that there are no significant changes in the level of salivary IL-

6 and salivary TNF- α between control group and treated group. Independent T-test of two means was used (Table 5).

Table (5): Comparison in mean of salivary parameters between treatment group and control group at the beginning of the study.

Salivary parameters	Treatment group	Control group	P-value
	[n = 20] Mean ± SD	[n = 20] Mean ± SD	
IL-6 (Pg/ml)	3.35 ± 2.65	2.68 ± 1.64	0.345
TNF- α (Pg/ml)	16.65 ± 1.38	17.21 ± 1.55	0.228

There is a significant difference in the salivary level of TNF- α treated group and control group, while there is no significant difference in salivary IL-

6 between treated group and control group in salivary IL-6 at the 21st day, by using Independent T-test of two means as illustrated in table 6.

Table (6): Comparison in mean of salivary parameters between treatment group and control group at the 21st day of the study.

Salivary parameters	Treatment group	Control group	P-value*
	[n = 20] Mean ± SD	[n = 20] Mean ± SD	
IL-6 (Pg/ml)	2.59 ± 2.11	3.45 ± 2.22	0.213
TNF-α (Pg/ml)	16.26 ± 0.56	17.75 ± 1.84	0.001

* P-value is < 0.05

There is a significant difference in the salivary level of TNF-α between treated group and control group, while there is no significant difference in salivary IL-6 between treated group and

control group at the 42nd day of folic acid administration by using Independent T-test of two means, as illustrated in table 7.

Table (7): Comparison in mean of salivary parameters between treatment group and control group at the 42 day of the study.

Salivary parameters	Treatment group	Control group	P-value*
	[n = 20] Mean ± SD	[n = 20] Mean ± SD	
IL-6 (Pg/ml)	2.00 ± 1.79	2.91 ± 1.75	0.116
TNF-α (Pg/ml)	16.11 ± 0.05	19.56 ± 5.12	0.005

* P-value is < 0.05

The comparisons of salivary parameters levels as a result of the effect of systemic folic acid treatment during the study period are showing that there is no significant difference in the level

of the salivary parameters during all the study period by using one-way ANOVA-test with Tukey's Pair wise comparisons. Means that do not share (A) letter are significantly different. (Table 8).

Table (8): Effect of folic acid on the levels of salivary biochemical parameters during the study period.

Salivary parameters	Treatment group			P-value
	1 st day Mean ± SD	21 st day Mean ± SD	42 nd day Mean ± SD	
IL-6 (Pg/ml)	3.35 ± 2.65 ^A	2.59 ± 2.11 ^A	2.00 ± 1.79 ^A	0.167
TNF-α (Pg/ml)	16.65 ± 1.38 ^A	16.26 ± 0.56 ^A	16.11 ± 0.05 ^A	0.132

DISCUSSION

The regular plaque removal by mechanical cleaning of the teeth is a simple and cost-effective method that has approved efficient in the control of gingivitis¹⁵, but the inability of some people to perform adequate mechanical tooth cleaning has been induced the researchers for finding effective chemotherapeutic agents that can improve gingivitis¹⁶.

Folic acid is essential for the proper maturation of the rapidly proliferating cells that are needed during repair of the periodontium, thus folic acid deficiency is associated with severe gingival inflammation^{17,18}.

The results (table 4) showing that there is a slight but non-significant improvement in GI at the end of the study in comparison with the first day due to the intake of folic acid supplement. Our study is in agreement with the study of *Khan et al* (2020) which is cited a study that reported use of 2 mg folic acid, twice daily for 30 days and its effect on gingivitis. Results indicate that folic acid leads to a reduction in gingival inflammation¹⁹. The dose of folic acid used in this study is pharmacologically compatible with tolerable upper intake limit/dose (TUL) that was recommended by Institute of Medicine (IOM) in 1998 which is 1mg once daily²⁰.

Gingivitis could be a non-plaque induced disease, the constant host inflammatory immune reactions against the periodontopathogens could lead to increase vascularization i.e angiogenesis, increased permeability of blood vessels, and migration of white blood cells to the site of infection^{21,22}. Cytokines are among the host mediators produced after microbial invasion. TNF- α , and IL-6 were the first to have their role in periodontal disease pathogenesis²³⁻²⁶.

These cytokines are produced by epithelial cells and fibroblasts, neutrophils and macrophages in the chronic phases of inflammation²⁷.

Periodontopathogens secrete lipopolysaccharides (LPS) by their cell wall that induce an inflammatory response by binding with Toll-like receptors (TLRs); which are class of immune receptors present at the cell surface of macrophages, also are considered the major activator of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) which is a transcription factor presents in the cytoplasm of all human cells, and is a key regulator of both innate and adaptive responses by controlling genes of cytokines which involved in the inflammatory process. It is found to be active in many chronic inflammatory diseases. NF- κ B is normally found as inactive in

the cytoplasm, associated with an inhibitory protein called I κ B. Upon stimulation by LPS-TRL complex, instant phosphorylation of I κ B and releasing of NF- κ B into nucleus will happen to start transcription of inflammatory cytokines genes²⁸⁻³².

Some studies supposed that folic acid subsides the inflammatory response that mediated by IL-6 and TNF- α through inhibition of phosphorylation of I κ B^{33,34}.

Results are showing that there is no significant difference in the level of IL-6 and TNF- α in saliva between treatment and control group at the beginning of the study, subsequently their levels were decreased in response to the folic acid treatment at the 21st day and there was a significant difference in TNF- α at 42nd day comparing to control, also there is a decrease in IL-6 level but in non-significant manner which could be related to the low dose of folic acid used during the current study. The levels of both IL-6 and TNF- α in control group were increased throughout the study days.

Results are in line with a recent study that concluded sub-acute, 5 weeks- oral intake of 1 mg/day of folic acid has the ability to decrease in T-cell proliferation, so that decrease cytokines production in dermatitis treatment³⁵. Another study has been

evaluated the effect of 1.25 mg/day of folic acid supplementation for six months on newly diagnosed patients with Alzheimer disease, they found that folic acid improved cognition and decreased markers of inflammation³⁶. Considering the limitations of the current study, including the small sample size and the low dose of folic acid.

CONCLUSION

It was concluded that folic acid supplementation at dose of 1 mg/day for 42 days may increase host resistance to gingival inflammation by decreasing TNF- α level in saliva and a little decrease in IL-6 level in saliva. Further research with higher doses and longer durations of treatment with folic acid are needed.

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