The Role of Vitamin C in Wound Healing in Periodontal Flap Surgery in Patients with Chronic Periodontitis: A Randomized Controlled Trial

Suman Mukherjee*, Vinayak S. Gowda

Department of Periodontics and Oral Implantology, VS Dental College and Hospital, Bangalore, India

Corresponding Author*

Suman Mukherjee
Department of Periodontics and Oral Implantology
VS Dental College and Hospital
Bangalore
India
E-mail: smjee234@gmail.com

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Abstract

Aim of the Study: The study aimed to investigate the role of vitamin C in wound healing and post-operative pain management after flap surgery in chronic periodontitis patients.

Methodology: A triple-blind, randomized clinical controlled trial was conducted on 60 patients with chronic generalized periodontitis. After periodontal flap surgery, the test group received 1g vitamin C supplementation for 14 days. The wound healing index, VAS score, GI, PI, and periodontal parameters were assessed, recorded and evaluated.

Results: Mann Whitney test showed a significant difference in the Laundry wound healing index and VAS score between the two groups at day -3 and 7 follow up. The clinical parameters (GI, PD and CAL) were also significant for the test group.

Conclusion: Our study concludes that vitamin C plays a significant role in post-operative wound healing and the reduction of post-operative pain.

Keywords: Periodontitis • Vitamin C • healing • pain

Clinical Relevance:

Scientific rationale for the Study: Antibiotics and analgesics are commonly prescribed for wound healing and post-operative pain management. Vitamin C has shown dual properties of effect on analgesia and healing.

Principal findings: Our observation showed that Supplementation of vitamin C caused significant decrease in wound healing time and a significant reduction in post-operative pain.

In the 6 months follow-up, it significantly improved the clinical parameters like Lobene Gingival Index, pocket depth and clinical attachment loss.

Practical Implications: Supplementation of vitamin C post periodontal treatment would reduce the toxicity of excess-medications post-therapy. It also improved the clinical parameters in long term.

1. Introduction:

Periodontitis is defined as a microbial-associated, host-mediated inflammatory disease that affects the tooth and its supporting structures. Progression of periodontal disease leads to the destruction of tooth-supporting structures leading to tooth loss. As the sixth most prevalent disease, it affects 90% of the global population. In India, its prevalence has been reported between 96.3 – 100% in different states.

Therapies for periodontal disease include cause-related therapies and non-surgical interventions. Surgical intervention is indicated when non-surgical therapy is unable to restrain the progression of periodontitis. Post-surgery patients usually complain of various types of discomfort including pain, swelling, bleeding and transient mobility.

Previous studies1, 2 have established that host nutrition critical for periodontal health and fundamental for recovery after therapy. Lack of specific nutrients leads to disturbed wound- healing process, and a prolonged nutrient deficiency could increase the risk of wound-related complications. As such, the supplementation of adequate nutrition following periodontal surgery may enhance the postsurgical healing process while minimizing post-operative pain.

Historically, severe periodontal pathos, including gingival hemorrhage, tooth mobility and loss of connective tissue attachment, has been a classical clinical feature of ascorbic acid deficiency.

Vit C has been used for modulating periodontal disease, although its exact role is not known. Studies have shown that vitamin C supplementation lowers hypertension, endothelial

Dysfunction, chronic inflammation, and Helicobacter pylori infection, which are independent risk factors of periodontitis.

In a comprehensive review 3, it was concluded that while the relationship between periodontal health and vitamin C was not well documented, available studies suggested that even though ascorbic acid deficiency may adversely affect the periodontium, among other organ systems, these changes in a plaque-free dentition do not include severe clinical gingivitis and are not consistent with the histological characteristics of advanced periodontal disease.

Even more inconclusive are studies that address the periodontal benefit of ascorbic acid supplementation. Clinical reports of scorbutic gingivitis, combined with studies suggesting that ascorbic acid supplements may promote improvement in gingival health4,5, have led some investigators to advocate the inclusion of vitamin C supplements in an oral-hygiene- based program for maintenance of periodontal health. Investigations on the benefits of ascorbic acid supplementation on gingival or periodontal health are still controversial.

To the best of our knowledge, this is the first study to investigate that whether vitamin C supplementation (Ascorbic acid) helps in the healing process of patients with Chronic Generalized Periodontitis (CGP) after periodontal flap surgery. So, this present research aimed to explore and determine the effects of Vitamin C supplementations in patients with chronic periodontitis following periodontal flap surgery. Also, the effects of vitamin C supplementation on postoperative pain and the gingival and periodontal parameters had been probed.

2. Objective of the Study

The primary objective of this study was to evaluate the role of vitamin C in wound healing and postoperative pain after flap surgery in patients with chronic generalized periodontitis.

The secondary objective is to investigate the effect of vitamin C on the gingival and periodontal parameters after periodontal surgery.

3. Methods & Methodology

3.1. Study Design and Patient Selection:

A triple-blind, randomized clinical controlled trial was conducted with 60 subjects who met the selection criteria were selected following an informed verbal and written consent.

3.2. Ethical Clearance:

The study was approved by local ethical committee in accordance with the Declaration of Helsinki.

3.3. Study Group:

Following verbal and written consent, 60 patients were randomly divided into two groups of 30 each by coin toss. After initial examination and evaluation, patients received phase 1 therapy, and were recalled after 3-4 weeks. On recall, patients with satisfactory oral hygiene maintenance were further included for this study. The Gingival and Periodontal parameters were evaluated and recorded as baseline on the day of surgery.

3.4. Selection Criteria

3.4.1. Inclusion Criteria

- Patients should be >18 years of age and below 70 years of age
- At least 4-5 teeth in a quadrant with average probing pocket depth >5 mm.

3.4.2. Exclusion Criteria

- Presence of any systemic disease or chronic medical condition, apart from periodontitis which can impair wound healing post periodontal surgery e.g. anaemia, uncontrolled diabetes.
- History of presence of any infectious disease like Tuberculosis, Hepatitis, HIV, etc
- Any oral procedure under local anesthesia (e.g. Tooth extraction) in the past 3 months.
- Patients on anxiolytics, analgesics or any other chronic medications.
- Smokers, lactating women, and pregnant women.
- Teeth with crowns or restorations without CEJ reference were excluded.
- Patients who had received periodontal surgical interventions in the past.

3.5. Surgical Procedure:

Following routine blood and radiographic investigations, the gingival and periodontal parameters were recorded on the day of surgery.

A Kirkland flap (sulcular incision) design was performed for all the patients. Following surgical therapy, the test groups subjects were prescribed ascorbic acid supplementations [Limcee 500mg, Abbott Healthcare Pvt. Ltd.] twice daily for the next 2 weeks. The patients were recalled on the 7th day post-operative for suture removal.

3.6. Post-operative Evaluation:

Interpolation algorithms forecast iron supplementation coverage in unseen areas based on available data in both deterministic and probabilistic ways. Ordinary kriging, universal kriging, and empirical Bayesian kriging are the most commonly used probabilistic types of interpolation methods for prediction.

We conducted and compared the three procedures utilizing residuals and root mean square error based on this evidence. We picked ordinary kriging as the interpolation technique for this study since it has the lowest residuals and root mean square error value based on the parameters. Kriging spatial interpolation is a technique that uses observable measures to anticipate the percentage of iron supplementation coverage among women aged 15 to 49 in unstamped parts of the country [22].

Finally, because it contains spatial autocorrelation and statistically optimizes the weight, the ordinary kriging spatial interpolation approach was used to forecast iron supplementation coverage among women aged 15 to 49 years in unobserved areas of Ethiopia [23].

4. Statistical Analysis:

4.1. Sample Size Estimation

The sample size has been estimated using the G Power software v. 3.1.9.2

Considering the effect size to be measured (d) at 80% for the Two-tailed hypothesis, power of the study at 80% and the margin of the error at 5%, the total sample size needed is 52. Anticipating 10% of attrition loss among study patients during follow-up periods, the sample size is increased to 60. Hence each study group will comprise 30 samples [30 samples x 2 groups = 60 samples].

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses.

4.2. Descriptive Statistics:

Descriptive analysis of all the explanatory and outcome parameters was done using frequency and proportions for categorical variables, whereas in Mean & SD for continuous variables.

4.3. Inferential Statistics:

Mann Whitney Test was used to compare the mean values of different clinical parameters including VAS scores between the Test and Control group at different time intervals.

Chi-Square Test was used to compare the healing index scores & Quadrant Involved between test and control at different time intervals.

Friedman's test followed by the Wilcoxon Signed-Rank test was used to compare the clinical parameters between different time Intervals in the test and control group.

The level of significance [P-Value] was set at $P<0.05$.

5. Results:

5.1. Demographic data: Age and Gender

In the present study, Mann Whitney and Chi-Square test showed no statistically significant difference in the mean age [Figure S1] (TM =42.0, TSD = 9.3; CM= 42.3, CSD = 6.8) and

Gender [Figure S2][males (n=14) and females (n=16)] differences between the two groups. [Table S1]

5.2. Quadrant involved:

Comparison of Quadrant Involved for Treatment among Test and Control group using Chi- Square Test showed no statistically significant difference. [Table S2, Figure S3]

5.3. Wound Healing:

We measured the laundry wound healing index compare the wound healing between the two groups. With the Chi-Square Test, a statistically significant difference between the two groups was found at the 1st week follow up. [Table 1, Figure S4]

5.4. Effect of vitamin C on postoperative pain

The VAS score was used to compare the postoperative pain between the two groups. Using Mann Whitney test, a significant difference was found at the 3rd-day follow-up. No statistical difference was found at 1 week follow up. [Table S3, Figure S5]

Using the Wilcoxon Signed Rank Test to compare the VAS scores showed a statistically significant difference at both 3rd day and 1- week follow up. [Table S4, Figure S8]

One-week post-operative, none of the patient's complaint of pain and discomfort and had VAS 0 (at 2 weeks, 3 weeks and 1- month follow-up). Hence, no statistical analysis was done post 7 days follow-up.

5.5. Effect of vitamin C on Plaque and Gingival Index scores [Figure S-10, 11]

At baseline no statistically significant difference was found between the PI and GI scores using Mann Whitney test. [Table 2, Figure 1]

At 7th day [Table 3, Figure S6] and 1-month [Table S5, Figure S7] follow-ups, Mann Whitney test showed a statistically significant difference in GI scores for the test group, but no significant difference in PI scores were seen [Figure S9].

Mann Whitney test used to compare both groups showed no statistically significant difference for the PI and GI Scores at the 3 – and 6- months' period. [Table S6, S7]

Multiple Intra-group comparisons of each groups at different time intervals using Friedman's Test and Wilcoxon Signed Rank Post hoc Test showed a statistically significant difference in PI scores for the test group. [Table S8, S9]

Friedmann test used to compare the PI scores of both groups at different time intervals showed a statistically significant difference in both groups. Multiple comparisons of mean PI scores between different time intervals using Wilcoxon Signed Rank Post hoc Test were significant for some time intervals in both groups. [Table S10, S11]

5.6. Effect of Vitamin C on Periodontal Parameters [Table S12, S13, Figure S-11, 12]

At the baseline, a comparison of the periodontal parameters (pocket depth and clinical attachment loss) using the Mann Whitney test showed no statistically significant difference. [Table 2, Figure 1]

At the 3-months follow-up, Mann Whitney test used to compare the clinical attachment loss of both groups showed a statistically significant difference in the test group only. The pocket depth was statistically significant for both the groups. [Figure 2]

At the 6-months follow up, Mann Whitney test used to compare pocket depth and clinical attachment level showed a statistically significant difference in both the groups. [Figure 3]

Friedman's test followed by the Wilcoxon Signed-Rank Post hoc Test showed a statistically significant difference in both the intra- and intergroup comparisons of PD and CAL at all-time intervals.

However, the Wilcoxon Signed-Rank Test didn't show any statistically significant difference in comparison between the 3- and 6-month intra-group values of PD and CAL in the control group. [Table S12, S13]

Figure 1: Mean values of different clinical parameters between Test and Control group at Baseline period

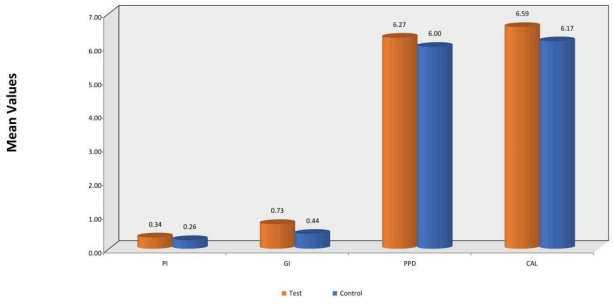


Figure 2: Mean values of different clinical parameters between Test and Control group at 3 Month

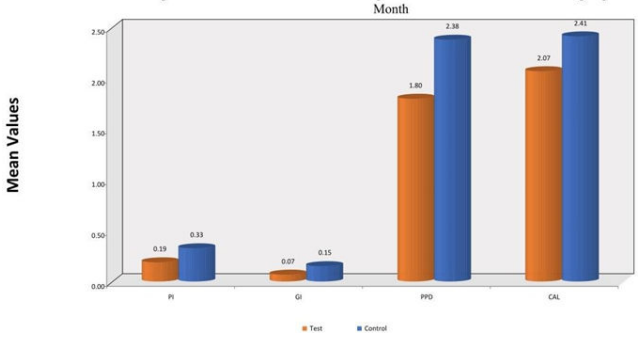


Figure 3: Mean values of different clinical parameters between Test and Control group at 6 Months period

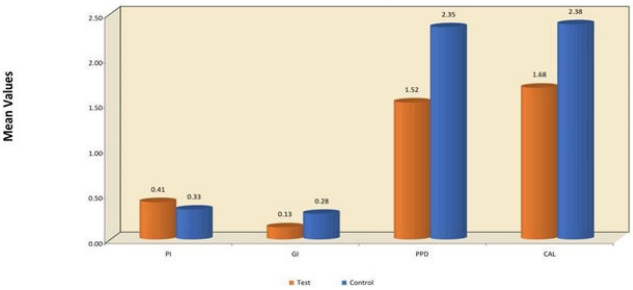


Table 1: Comparison of Healing Index scores between 2 groups using chi square Test

Time	Healing	Test		Control	Value	P-value	
		n	%	n			%
Day 7	Good	0	0%	3	10%	60.0-00	<0.0-01•
	Very Good	0	0%	27	90%		
	Excellent	30	100%	0	0%		
Day 14	Good	0	0%	0	0%	..	..
	Very Good	0	0%	0	0%		
	Excellent	30	100%	30	100%		
Day 21	Good	0	0%	0	0	..	..
	Very Good	0	0%	0	5		
	Excellent	30	100%	30	100%		
1 Month	Good	0	0%	0	0%	..	.

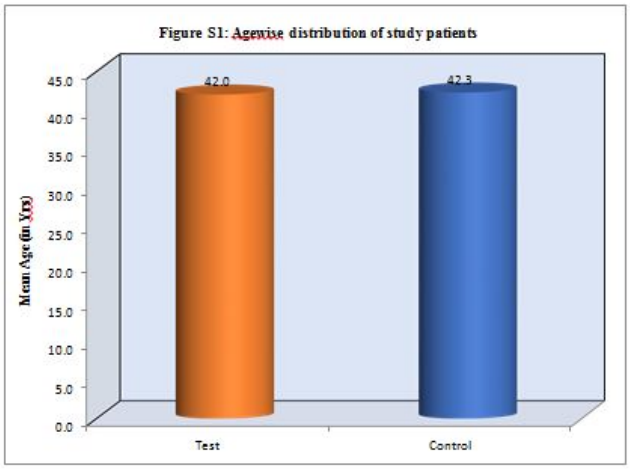
Very Good	0	0%	0	0%
Excellent-Statistically Significant	30	100	30%	100%

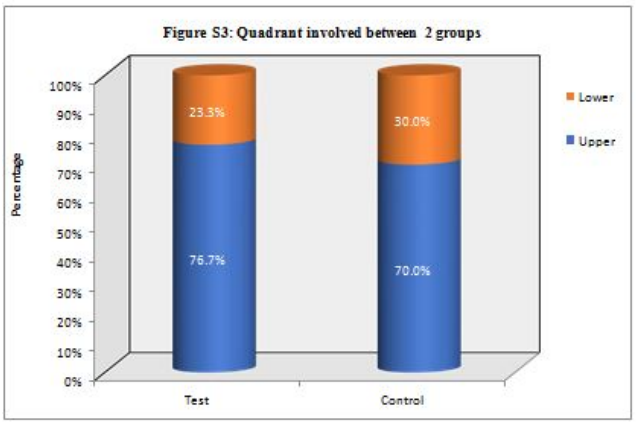
Table 2: Comparison of mean values of different clinical parameters between Test and Control group at Baseline period using Mann Whitney Test

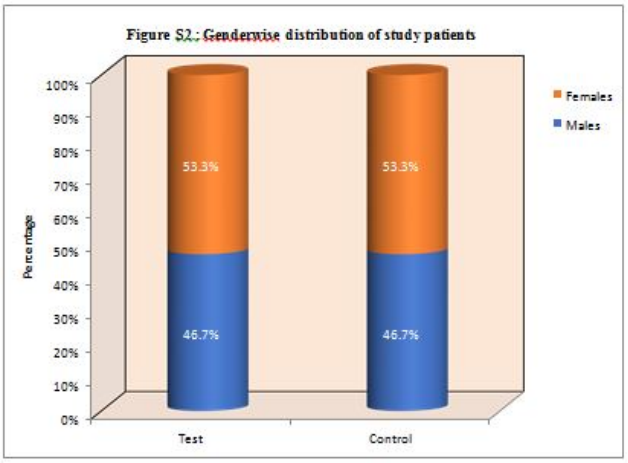
Parameters	Group	N	Mean	SD	Mean Diff	P-Value
PI	Test	30	0.34	0.2	0.08	0.1 7
	Control	30	0.26	0.2		
GI	Test	30	0.73	0.87	0.29	0.24
	Control	30	0.44	0.58		
PD	Test	30	6.27	0.86	0.27	0.22
	Control	30	6	0.74		
CAL	Test	30	6.59	1.12	0.42	0. 1 7
	Control	30	6.17	0.75		

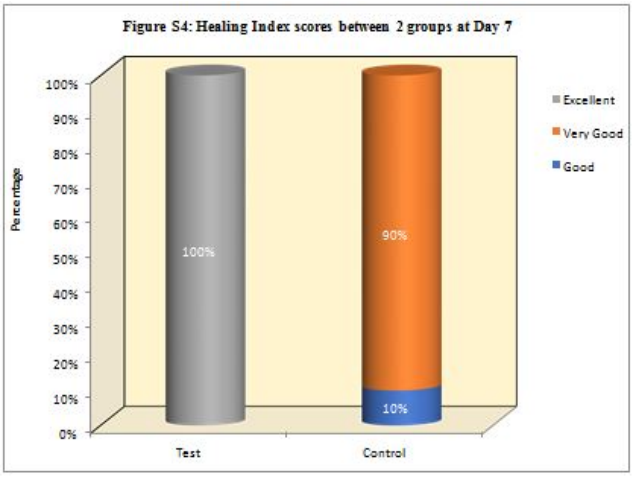
Table 3: Comparison of mean values of different clinical parameters between Test and control group at 7 days period using menn whitney Test

Parameters	Group	N	Mean	SD	Mean Diff	P-Value
PI	Test	30	0.33	0.31	-0.01	0.69
	Control	30	0.34	0.29		
GI	Test	30	0.15	0.35	·0.68	<0.001•
	Control	30	0.83	0.24		









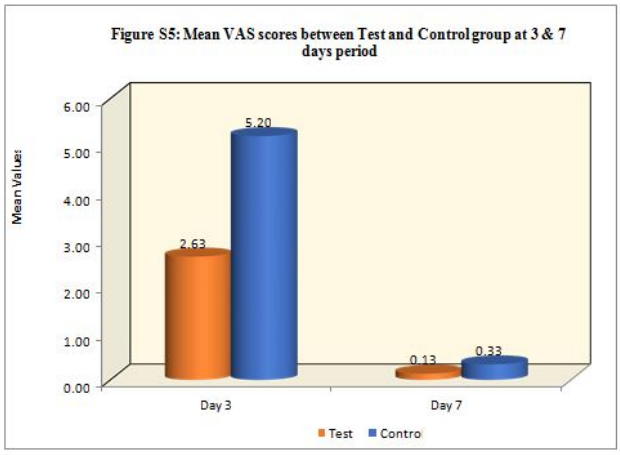


Figure S5: Mean VAS scores between Test and Control group at 3 & 7 days period
Mean Values
6.00
5.00
4.00
3.00
2.00
1.00
0.00
5.20
2.63
0.13
0.33
Day 3
Day 7
Test
Control

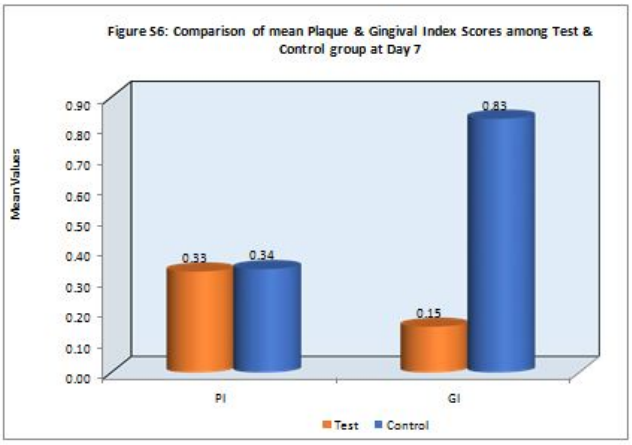


Figure S6: Comparison of mean Plaque & Gingival Index Scores among Test & Control group at Day 7
Mean Values
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.33
0.34
0.15
0.83
PI
GI
Test
Control

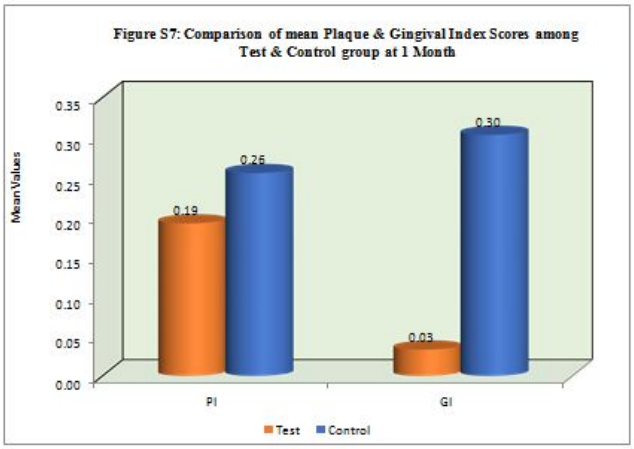


Figure S7: Comparison of mean Plaque & Gingival Index Scores among Test & Control group at 1 Month
Mean Values
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.19
0.26
0.03
0.30
PI
GI
Test
Control

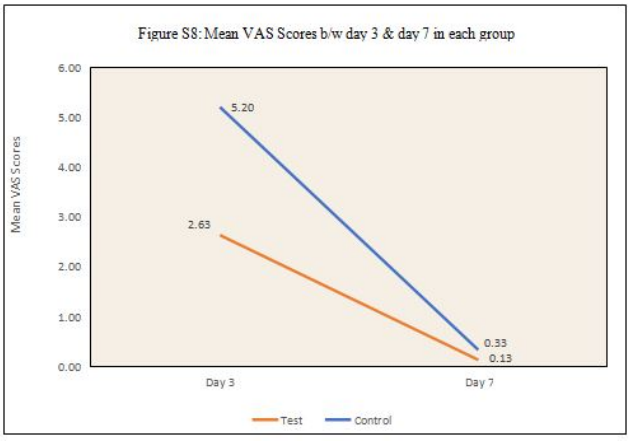


Figure S8: Mean VAS Scores b/w day 3 & day 7 in each group
Mean VAS Scores
6.00
5.00
4.00
3.00
2.00
1.00
0.00
5.20
2.63
0.33
0.13
Day 3
Day 7
Test
Control

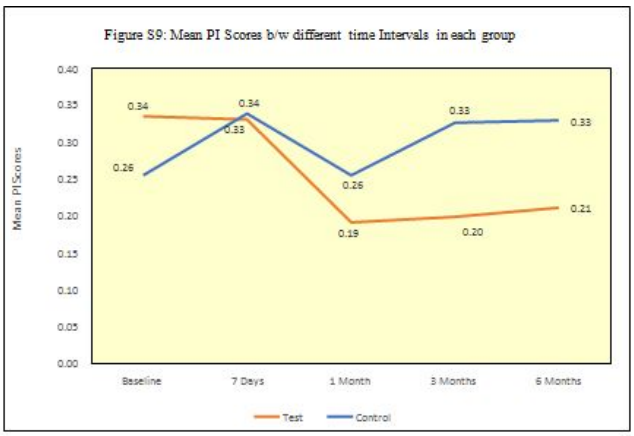


Figure S9: Mean PI Scores b/w different time Intervals in each group
Mean PI Scores
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.34
0.33
0.26
0.34
0.26
0.19
0.33
0.20
0.33
0.21
Baseline
7 Days
1 Month
3 Months
6 Months
Test
Control

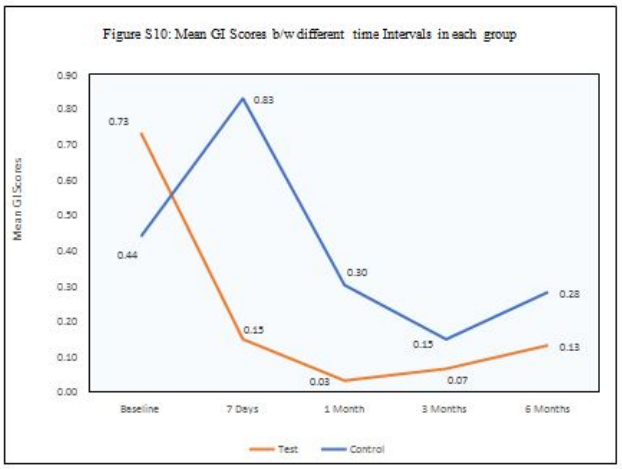


Figure S10: Mean GI Scores b/w different time Intervals in each group
Mean GI Scores
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.73
0.44
0.83
0.15
0.03
0.30
0.15
0.07
0.28
0.13
Baseline
7 Days
1 Month
3 Months
6 Months
Test
Control

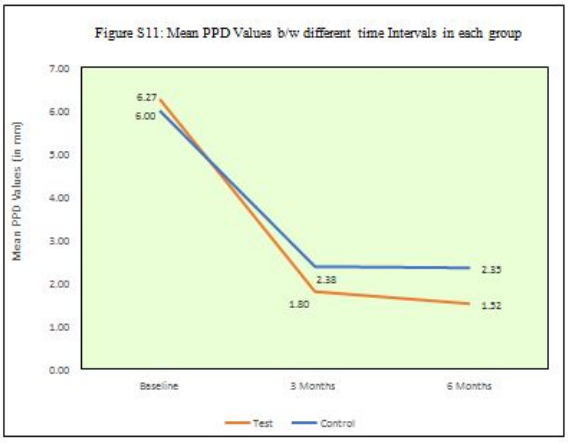


Figure S11: Mean PPD Values b/w different time Intervals in each group
Mean PPD Values (in mm)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
6.27
6.00
2.38
1.80
2.35
1.52
Baseline
3 Months
6 Months
Test
Control

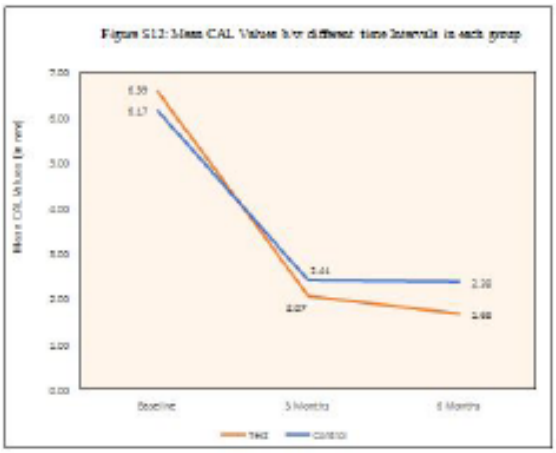


Figure S12: Mean CAL Values b/w different time Intervals in each group
6.39
6.17
2.44
2.30
2.07
1.88
Baseline
3 Months
6 Months

Table S1:Age and Gender distribution among 2 study groups						
Variables	Values	Test		Control	P-Value	
		Mean	SD	Mean		SD
Age	Mean & SD	42	9.3	42.3	6.8	0.91a
	Range	22 - 62	27 - 56			
		n	%	n	%	
Gender	Males	14	46.70%	14	46.70%	0.11b
	Females	16	53.30%	16	53.30%	

*** - Statistically Significant** **Note:** a. Mann Whitney Test; b. Chi Square Test

Table S2: Comparison of Quadrant Involved for Treatment among Test and Control group using Chi Square Test						
Quadrant	Test		Control	▢2 va1ue	P-Value	
	n	%	n			%
Upper	23	76.7%	21	70.0%	0.341	0.56
Lower	7	23.3%	9	30.0%		

* - Statistically Significant

Table S3: Comparison of mean VAS scores between Test and Control group at 3- & 7-days period using Mann Whitney Test						
Time	Group	N	Mean	SD	Mean Diff	P-Value
3 Days	Test	30	2.63	0.67	-2.57	<0.001*
	Control	30	5.20	1.1		
7 Days	Test	30	0.13	0.43	-0.20	0.16
	Control	30	0.33	0.66		

* - Statistically Significant

Note: At Day 14 & 1 Month, both groups should VAS score of 0, hence no statistics was done for those time periods.

Table S4:Comparison of mean VAS scores between day 3 & day 7 in Test and Control group using Wilcoxon Signed Rank Test						
Group	Time	N	Mean	SD	Mean Diff	P-Value
Test	3D	30	2.63	0.67	2.50	<0.001*
	7D	30	0.13	0.43		
Control	3D	30	5.20	1.10	4.87	<0.001*
	7D	30	0.33	0.66		

* - Statistically Significant

Table S5: Comparison of mean values of different clinical parameters between Test and Control group at 1Month period using Mann Whitney Test

Parameters	Group	N	Mean	SD	Mean Diff	P-Value
PI	Test	30	0.19	0.18	-0.06	0.34
	Control	30	0.26	0.32		
GI	Test	30	0.03	0.18	-0.27	<0.001*
	Control	30	0.30	0.33		

*- Statistically Significant

Table S6: Comparison of mean values of different clinical parameters between Test and Control group at 3 Months period using Mann Whitney Test

Parameters	Group	N	Mean	SD	Mean Diff	P-Value
PI	Test	30	0.19	0.17	-0.14	0.70
	Control	30	0.33	0.45		
GI	Test	30	0.07	0.25	-0.08	0.29
	Control	30	0.15	0.35		
PD	Test	30	1.80	0.85	-0.58	0.007*
	Control	30	2.38	0.59		
CAL	Test	30	2.07	1.02	-0.34	0.34
	Control	30	2.41	0.59		

*- Statistically Significant

Table S7: Comparison of mean values of different clinical parameters between Test and Control group at 6Months period using Mann Whitney Test

Parameters	Group	N	Mean	SD	Mean Diff	P-Value
PI	Test	30	0.41	0.47	0.08	0.34
	Control	30	0.33	0.45		
GI	Test	30	0.13	0.35	-0.15	0.13
	Control	30	0.28	0.45		
PD	Test	30	1.52	0.75	-0.83	<0.001*
	Control	30	2.35	0.68		
CAL	Test	30	1.68	0.84	-0.70	0.001*
	Control	30	2.38	0.70		

*- Statistically Significant

Table S8: Comparison of mean PI Scores between different time Intervals using Friedman's Test

Group	Time	N	Mean	SD	Min	Max	P-Value
Test	Baseline	30	0.34	0.20	0.2	0.8	0.005*
	7 Days	30	0.33	0.31	0.0	1.3	
	1Month	30	0.19	0.18	0.0	0.5	
	3 Months	30	0.20	0.16	0.0	0.5	
	6 Months	30	0.21	0.25	0.0	0.8	
Control	Baseline	30	0.26	0.20	0.0	0.6	0.59
	7 Days	30	0.34	0.29	0.0	1.3	
	1Month	30	0.26	0.32	0.0	1.5	
	3 Months	30	0.33	0.45	0.0	1.6	
	6 Months	30	0.33	0.45	0.0	1.6	

*- Statistically Significant

Table S9: Multiple comparison of mean PI scores between different time intervals in Test Group using Wilcoxon Signed Rank Post hoc Test

Time	BL vs 7D	BL vs 1M	BL vs 3M	BL vs 6M	7D vs IM
P-Value	0.94	0.004*	0.006*	0.01*	0.02*
Time	7D vs 3M	7D vs 6M	1M vs 3M	1M vs 6M	3M vs 6M
P-Value	0.03*	0.07	0.88	0.73	0.82

*- Statistically Significant

Table S10: Comparison of mean GI Scores between different time Intervals using Friedman's Test

Group	Time	N	Mean	SD	Min	Max	P-Value
Test	Baseline	30	0.73	0.87	0.0	3.0	<0.001*
	7 Days	30	0.15	0.35	0.0	1.0	
	1Month	30	0.03	0.18	0.0	1.0	
	3 Months	30	0.07	0.25	0.0	1.0	
	6Months	30	0.13	0.35	0.0	1.0	
Control	Baseline	30	0.44	0.58	0.0	2.0	<0.001•
	7 Days	30	0.83	0.24	0.4	1.0	
	1Month	30	0.30	0.33	0.0	1.0	
	3 Months	30	0.15	0.35	0.0	1.0	
	6Months	30	0.28	0.45	0.0	1.0	

*- Statistically Significant

Table S11: Multiple comparison of mean GI scores between different time intervals in Test & Control Group using Wilcoxon Signed Rank Post hoc Test

Test	Time	BL vs 7D	BL vs lM	BL vs 3M	BL vs 6M	7D vs 1M
	P-Value	<0.001*	<0.001*	<0.001*	0.001*	0.09
	Time	7D vs 3M	7D vs 6M	1M vs 3M	1M vs 6M	3M vs 6M
	P-Value	0.33	0.81	0.57	0.18	0.42
Control	Time	BL vs 7D	BL vs lM	BL vs 3M	BL vs 6M	7D vs 1M
	P-Value	<0.001*	0.29	0.02*	0.30	<0.001*
	Time	7D vs 3M	7D vs 6M	1M vs 3M	1M vs 6M	3M vs 6M
	P-Value	<0.001*	<0.001*	0.07	0.84	0.26

*- Statistically Significant

Table S12:Comparison of mean Pocket depth (in mm) between different time Intervals using Friedman 's Test followed by Wilcoxon Signed Rank Post hoc Test

Group	Time	N	Mean	SD	Min	Max	P-Valuea	Sig. Diff	P-Valueb
Test	BL	30	6.27	0.86	5.0	8.0	<0.001*	BL vs 3M	<0.001*
	3M	30	1.80	0.85	0.2	3.0		BL vs 6M	<0.001*
	6M	30	1.52	0.75	0.2	3.0		3M vs 6M	<0.001*
Control	BL	30	6.00	0.74	5.0	7.0	<0.001*	BL vs 3M	<0.001*
	3M	30	2.38	0.59	1.0	3.3		BL vs 6M	<0.001*
	6M	30	2.35	0.68	0.0	3.0		3M vs 6M	1.00

***- Statistically Significant**

Note: a. P-value obtained by Friedman's Test; b. P-value obtained by Wilcoxon Signed Rank Test

Table S13: Comparison of mean CAL (in mm)between different time Intervals using Friedman's Test followed by Wilcoxon Signed Rank Post hoc Test

Group	Time	N	Mean	SD	Min	Max	P-Valuea	Sig.Diff	P-Valueb
Test	BL	30	6.59	1.12	5.0	9.0	<0.001*	BL vs 3M	<0.001*
	3M	30	2.07	1.02	0.2	4.0		BL vs 6M	<0.001*

	6M	30	1.68	0.84	0.2	3.0		3M vs 6M	<0.001*
Control	BL	30	6.17	0.75	5.0	7.0	<0.001*	BL vs 3M	<0.001*
	3M	30	2.41	0.59	1.0	3.3		BL vs 6M	<0.001*
	6M	30	2.38	0.70	0.0	3.5		3M vs 6M	1.00

***- Statistically Significant**

6. Discussion

The role of vitamin C in periodontal health and wound healing has been extensively studied in literature yet the results have been inconclusive. To the best of our knowledge, this is the first study exploring the role of vitamin C in wound healing after a flap surgery in patients with chronic periodontitis. The primary outcome of this current clinical trial was that the postoperative administration of vitamin C improved the healing potential in patients suffering from chronic periodontitis. The use of vitamin C supplementation also significantly reduced the immediate post-operative pain following flap surgery. The secondary outcome of the study was that vitamin C showed a significant reduction in gingival index scores and improvement in periodontal parameters (PD, CAL).

Vitamin C is a water-soluble, versatile vitamin that cannot be synthesized and/or stored in the body, as such has to be supplemented daily. Although, the RDA for vitamin C ranges between 45 – 200 mg6, 7, in this study we supplemented the subjects with 1g/d. It was Nobel Prize winner Linus Pauling, who first suggested the use of mega doses of vitamin C for therapeutic benefits.8 A study had reported a 42 percent reduction in circulating WBCs ascorbic acid level on the third day postoperative, indicating there is a convincing need for supplementation of vitamin C post-surgery.9

The serum vitamin C level measurement tests are a specific but not sensitive marker of deficiency states and may be normal even in states of severe depletion.10, 11 besides being quite expensive, these tests are technique sensitive and unreliable. It is also unlikely that the dietary habits of this population had changed much during the 6 months. Thus, considering all the aspects pre- and post-operative vitamin C level wasn't assessed and taken into consideration in our study.

The oral wound healing comprises three consecutive and overlapping stages, which include inflammation, new tissue formation, and remodelling. Distinct cascades of events and varied internal and external factors influence the wound healing process. Thus, there are three primary factors involved in wound healing, including angiogenesis, immune response, and epithelialization. The healing process for oral wounds closely mimics the wound healing of other connective tissues in the body. 12

Wound healing is also adversely affected by dietary deficiencies and the subsequent metabolism profile of macro and micronutrient changes. According to Brown and Phillips (2010)13, there are no specific guidelines for ascorbic acid in wound healing, although Demling R (2009)14 recommends between 500 mg and 2 g for support of energy production in the hypermetabolic state, which constitutes more than 10 times the recommended daily intake as suggested by FSA (2006)15.

Vitamin C is a cofactor involved in the hydroxylation of type IV collagen and facilitation of oxidative protein folding, further leading to the maturation of collagen. Therefore, we hypothesize that wound healing is disturbed when plasma concentrations of vitamin C reach low levels. Previous studies have shown that the plasma level of ascorbic acid directly influences periodontal indices16, 17 and periodontal disease severity18, 19.

Studies by Mohammed and colleagues20 showed that vitamin C could promote wound healing through a variety of mechanisms. For example, vitamin C protects the function of vascular endothelium, increasing the expression of vascular endothelial growth factor (VEGF), which promotes cell division and secretion of matrix proteins. VEGF also promotes angiogenesis, which is an essential element for the regeneration of damaged tissues. Finally, vitamin C has been shown to promote wound healing in both animal studies and human trials.

In this study, we found superior wound healing scores in the test group. Within a one-week post-operative, the results were certainly comparative. Another study21 investigating the role of vitamin C in wound healing during implant placement was conclusive in terms of superior wound healing in the periodontitis group supplemented with vitamin C. In a literature review22, concluded that the plasma ascorbic acid level is essential, and its deficiency would hinder wound healing.

In the current research, the test group showed a significant reduction in postoperative pain in patients supplemented with vitamin C. This is in contrast to results from a previous study21 which reported no significant effect of vitamin C in the reduction of post-operative pain. Studies23, 24, and literature reviews25, 26 and systematic metanalysis27 have reported the antinociceptive and pain-reducing effects of vitamin C on reducing post-operative pain. Because of its analgesic effects, Vitamin C is also one of the agents for multimodal anaesthesia.

In the present study, the test group showed significant improvement in GI scores, but no significant change in PI scores was observed throughout the study. Two RCTs17,28 demonstrated superior effects in gingival and periodontal indices (GI, SBI, or PD) with the administration of vitamin C alone or with non-surgical therapy. Studies by Staudte et. al. (2005)29, Leggot et. al. (1986)30 and Raghavendra et. al. (2018)31 also reported similar conclusions. They found improvement in GI, SBI scores but no change in PI score. A study by Leggott et. al. (1986)30 reported a rapid increase in the percentage of bleeding sites, concurrent with experimental ascorbate deficiency, and a rapid resolution of bleeding during ascorbate depletion. In contrast, Abou Sulaiman and Shehadeh (2010)32 and Vogel et al. (1986)33 did not find statistical significance in any clinical parameters including GBI when mega-doses of Vitamin C supplementation were given as an adjunct to scaling and root planning.

Vitamin C has an anti-oxidative, neuro-protective and neuro-modulating effect in living organisms, particularly at the intracellular level, and this is thought to decrease the oxidative stress generated in inflammatory conditions. Additionally, vitamin C reduced the cytotoxic and apoptotic activity of Porphyromonas gingivalis in human periodontal ligament cells and human gingival fibroblasts which may have contributed to these effects. However, how ascorbic acid deficiency leads to an increase in positive bleeding sites remains unclear.

The periodontal parameters (PPD and CAL) of the vitamin C supplementation group showed a significant improvement at the end of 6 months. Studies34 reported that vitamin C depletion exhibited more bone loss compared with the non-depleted patients, while other RCTs18, 19 reported an inverse independent relationship between serum vitamin C concentration and CAL –lower the level of serum vitamin C, higher was the periodontal attachment loss. But, contradicting studies35, 19, 28 did not demonstrate improvement and reported an inefficacy of vitamin C administration in improving the pocket depth and attachment level.

There can be several plausible biological explanations regarding how vitamin C could affect periodontal tissues. For example, vitamin C plays a key role in collagen formation36, which decreases the permeability of gingival mucosa37, 38 and increases neutrophil function39. The experimental vitamin C depletion and supplementation showed a direct relationship between gingival inflammation and vitamin C status.30, 40 Also, vitamin C induces the in vitro osteogenic differentiation of periodontal ligament progenitor cells.

Periodontal disease is not a continuous process, but it is characterized by episodes of activity, followed by periods of relative. Significantly higher numbers of inflammatory cells are present in active sites as

compared with inactive sites42, thereby posing an increased demand for ascorbic acid in such tissues. Thus, in periodontal diseases, stability, remission, or quiescence is less frequently accompanied by residual bleeding and inflamed tissues.28,42 Ascorbic acid deficiency is characterized by increased capillary permeability, susceptibility to traumatic hemorrhages, and sluggishness of blood flow.43 Although ascorbic acid deficiency does not cause gingivitis, it does increase the severity of the condition. It is known to aggravate the gingival response to plaque and worsen the edema, enlargement, and bleeding.44 Studies have shown that during wound healing and inflammatory conditions, including gingivitis, periodontitis, and T2DM, ascorbic acid is necessary.45,46 As such, vitamin C remains essential for the whole post-operative condition. Recent studies also have shown that citrus fruits are more effective in increasing plasma vitamin C levels as compared to high dose supplements. As natural sources of citric fruits have no known adverse effects, we propose daily intake of adequate vitamin C, and further added intake during stress, oral inflammatory condition, and surgery.

Within its limitations, this is the first study in our knowledge which compared the role of vitamin C in flap surgery. However, further clinical trials are needed to determine its effect on postoperative wound healing, pain, and other periodontal parameters. Also, its optimal dose of administration and long-term benefits on periodontal parameters are yet to be evaluated.

7. Conclusion

In our study, we conclude that vitamin C plays an elemental role in wound healing. It demonstrated a reduction in post-operative pain, with exceptional changes in gingival and periodontal parameters. With the positive effects that vitamin C has on improving wound healing and post-operative periodontal changes, we recommend that vitamin C supplementation be given to patients to increase plasma vitamin C levels and improve postoperative responses. There are no reported adverse effects associated with the use of vitamin C supplements when used in the recommended dosage range. However, further studies are needed to assess the optimal dosage for any particular condition to improve wound healing in patients undergoing flap surgery.

References

1. Varela-López A, Navarro-Hortal MD, Giampieri F, Bullón P, Battino M, Quiles JL. Nutraceuticals in Periodontal Health: A Systematic Review on the Role of Vitamins in Periodontal Health Maintenance. Molecules. 2018;23(5):1226. Published 2018 May 20. doi:10.3390/molecules23051226
2. Najeeb S, Zafar MS, Khurshid Z, Zohaib S, Almas K. The Role of Nutrition in Periodontal Health: An Update. Nutrients. 2016;8(9):530. Published 2016 Aug 30. doi:10.3390/nu8090530
3. Woolfe, S. N., Kenney,E.B., and Hume, W. R.: Ascorbic acid and periodontal disease: a review of the literature. West Soc Periodontol 28: 44, 1980.
4. Buzina, R., Bzodarec, M., Jusic, M., et al.: Epidemiology of angular stomatitis and bleeding gums. Int J Vitam Nutr Res 43: 401, 1973.
5. El-Ashiry, G. M., Ringsdorf, W. M., and Cheraskin, E.: Local and systemic influences in periodontal disease. II. Effect of prophylaxis and natural versus synthetic vitamin C upon gingivitis. J Periodontol 35: 250, 1964.
6. Frei B, Birlouez-Aragon I, Lykkesfeldt J. Authors' perspective: what is the optimum intake of vitamin C in humans? Crit Rev Food Sci Nutr. 2012; 52:815-829.
7. Schleicher RL, Carroll MD, Ford ES, Lacher DA. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003-2004 National Health and nutrition examination survey (NHANES). Am J Clin Nutr. 2009; 90:1252-1263.
8. Chatterjee, I. B. (2009). The history of vitamin C research in India. Journal of Biosciences, 34(2), 185–194. doi:10.1007/s12038-009-0021-7
9. Irvin TT. Vitamin C requirements in postoperative patients. Int J Vitam Nutr Res Suppl. 1982;23 :277-86.
10. Lykkesfeldt J. Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction methods: reliable reduction with tris[2-carboxyethyl] phosphine hydrochloride. Anal Biochem 2000;282:89–93.
11. Ng LL, Ngkeekwong FC, Quinn PA, Davies JE. Uptake mechanisms for ascorbate and dehydroascorbate in lymphoblasts from diabetic nephropathy and hypertensive patients. Diabetologia 1998;41:435 – 42.
12. Iwasaki, M.; Manz, M.C.; Taylor, G.W.; Yoshihara, A.; Miyazaki, H. Relations of serum ascorbic acid and α-tocopherol to periodontal disease. J. Dent. Res. 2012, 91, 167–172.
13. Brown K, Phillips T (2010) Nutrition and wound healing. Clin Dermatol 28(4): 432–9
14. Demling R (2009) Nutrition, anabolism, and the wound healing process: an overview. Eplasty. http://tinyurl.com/prn3a93 (accessed 25 October 2013).
15. Food Standards Agency (FSA) (2006) FSA Nutrient and Food Based Guidelines for UK Institutions. http://tinyurl.com/qc4tmjk (accessed 25 October 2013).
16. Sculean A, Gruber R, Bosshardt DD. Soft tissue wound healing around teeth and dental implants. J Clin Periodontol. 2014;41(suppl 15): S6-S22.
17. Shimabukuro, Y.; Nakayama, Y.; Ogata, Y.; Tamazawa, K.; Shimauchi, H.; Nishida, T.; Ito, K.; Chikazawa, T.; Kataoka, S.; Murakami, S. Effects of an ascorbic acid-derivative dentifrice in patients with gingivitis: A double-masked, randomized, controlled clinical trial. J. Periodontol. 2015, 86, 27–35.
18. Amaliya, Timmerman, M. F., Abbas, F., Loos, B. G., Van der Weijden, G. A., Van Winkelhoff, A. J., Winkel, E. G., & Van der Velden, U. (2007). Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis. Journal of Clinical Periodontology, 34(4), 299–304. doi:10.1111/j.1600- 051x.2007.01053.x
19. Amarasena, N., Ogawa, H., Yoshihara, A., Hanada, N., & Miyazaki, H. (2005). Serum vitamin C-periodontal relationship in community-dwelling elderly Japanese. Journal of Clinical Periodontology, 32(1), 93–97. doi: 10.1111/j.1600-051x.2004.00643.x
20. Mohammed BM, Fisher BJ, Kraskauskas D, et al. Vitamin C promotes wound healing through novel pleiotropic mechanisms. Int Wound J. 2016;13:572-584
21. Li, X., Tang, L., Lin, Y. F., & Xie, G. F. (2018). Role of vitamin C in wound healing after dental implant surgery in patients treated with bone grafts and patients with chronic periodontitis. Clinical Implant Dentistry and Related Research. doi:10.1111/cid.12647
22. Moores, Jane. (2013). Vitamin C: a wound healing perspective. British journal of community nursing. Suppl. S6, S8-11. 10.12968/bjcn.2013.18.Sup12.S6.
23. Jain, S. K., Dar, M. Y., Kumar, S., Yadav, A., & Kearns, S. R. (2018). Role of anti- oxidant (vitamin-C) in post-operative pain relief in foot and ankle trauma surgery: A prospective randomized trial. Foot and Ankle Surgery. doi:10.1016/j.fas.2018.05.001
24. Carr, A. C., & McCall, C. (2017). The role of vitamin C in the treatment of pain: new insights. Journal of Translational Medicine, 15(1). doi:10.1186/s12967-017-1179-7
25. Chaitanya NC, Muthukrishnan A, Krishnaprasad CMS, Sanjuprasanna G, Pillay P, Mounika B. An Insight and Update on the Analgesic Properties of Vitamin C. J Pharm Bioallied Sci. 2018 Jul-Sep;10(3):119-125. doi: 10.4103/jpbs.JPBS_12_18. PMID: 30237682; PMCID: PMC6142887.
26. Hasanzadeh Kiabi, F., Soleimani, A., Habibi, M. R., & Emami Zeydi, A. (2013). Can Vitamin C Be Used as an Adjuvant for Managing Postoperative Pain? A Short Literature Review. The Korean Journal of Pain, 26(2), 209. doi:10.3344/kjp.2013.26.2.209
27. Chen S, Roffey DM, Dion CA, Arab A, Wai EK. Effect of perioperative vitamin C supplementation on postoperative pain and the incidence of chronic regional pain syndrome: a systematic review and meta-analysis. Clin J Pain. 2016;32:179–85.
28. Gokhale, N. H., Acharya, A. B., Patil, V. S., Trivedi, D. J., & Thakur, S. L. (2013). A Short-Term Evaluation of the Relationship Between Plasma Ascorbic Acid Levels and Periodontal Disease in Systemically Healthy

and Type 2 Diabetes Mellitus Subjects. Journal of Dietary Supplements, 10(2), 93–104. doi:10.3109/19390211.2013.790332

29. Staudte H, Sigusch BW, Glockmann E. Grapefruit consumption improves Vitamin C status in periodontitis patients. Br Dent J 2005;199:213-7
30. Leggott, P. J., Robertson, P. B., Rothman, D. L., Murray, P. A., & Jacob, R. A. (1986). The Effect of Controlled Ascorbic Acid Depletion and Supplementation on Periodontal Health. Journal of Periodontology, 57(8), 480–485. doi:10.1902/jop.1986.57.8.480
31. Raghavendra U, Rao A, Kashyap SR, D'Souza J, Kumar V, Kalal BS, D'Souza N. Vitamin C supplementation as an adjunct to nonsurgical therapy in the treatment of chronic periodontitis: A clinical and biochemical study. J Int Oral Health 2018;10:256- 61.
32. Abou Sulaiman AE, Shehadeh RM. Assessment of total antioxidant capacity and the use of Vitamin C in the treatment of non-smokers with chronic periodontitis. J Periodontol 2010;81:1547-54.
33. Vogel, R. I., Lamster, I. B., Wechsler, S. A., Macedo, B., Hartley, L. J., & Macedo, J. A. (1986). The Effects of Megadoses of Ascorbic Acid on PMN Chemotaxis and Experimental Gingivitis. Journal of Periodontology, 57(8), 472–479. doi:10.1902/jop.1986.57.8.472
34. Kuzmanova, D., Jansen, I. D. C., Schoenmaker, T., Nazmi, K., Teeuw, W. J., Bizzarro, S., van der Velden, U. (2012). Vitamin C in plasma and leucocytes in relation to periodontitis. Journal of Clinical Periodontology, 39(10), 905–912. doi:10.1111/j.1600- 051x.2012.01927.x
35. Leggott, P. J., Robertson, P. B., Jacob, R. A., Zambon, J. J., Walsh, M., & Armitage, GC. (1991). Effects of Ascorbic Acid Depletion and Supplementation on Periodontal Health and Subgingival Microflora in Humans. Journal of Dental Research, 70(12), 1531–1536. doi: 10.1177/00220345910700121101
36. Berg, R. A., Steinmann, B., Rennard, S. I. & Crystal, R. G. (1983) Ascorbate deficiency results in decreased collagen production: unhydroxylation of proline leads to increased intercellular degradation. Archieves of Biochemistry and Biophysiology 226, 681–686.
37. Alfano, M. C., Miller, S. A. & Drummond, J. F. (1975) Effect of ascorbic acid deficiency on the permeability and collagen biosynthesis of oral mucosal epithelium. Annals of New York Academy of Science 258, 253–263.
38. Alvares, O. & Siegel, I. (1981) Permeability of gingival sulcular epithelium in the development of scorbutic gingivitis. Journal of Oral Pathology 10, 40–48.
39. Washko, P., Rotrosen, D. & Levine, M. (1991) Ascorbic acid in human neutrophils. The American Journal of Clinical Nutrition 54 (6 Suppl.), 1221S–1227S.
40. Jacob, R. A., Omaye, S. T., Skala, J. H., Legott, P. J., Rothman, D. L. & Murray, P. A. (1987) Experimental vitamin C depletion and supplementation in young men. Nutrient interactions and dental health effects. Annals of the New York Academy of Science 498, 333–346.
41. Goodson JM, Tanner AC, Haffajee AD, Sornberger GC, Socransky SS. Patterns of progression and regression of advanced destructive periodontal disease. J Clin Periodontol. 1982;9: 472–481.
42. Zappa U, Reinking-Zappa M, Graf H, Case D. Cell populations associated with active probing attachment loss. J Periodontol. 1992;63(9):748–752.
43. Lee RE, Lee NZ. The peripheral vascular system and its reactions in scurvy: an experimental study. Am J Physiol. 1947;149:465–475.
44. Glickman I. Acute vitamin C deficiency and periodontal disease. I. The periodontal tissues of the guinea pig in acute vitamin C deficiency. J Dent Res. 1948;27(1):9–23.
45. Tanzer F, Ozalp I. Leucocyte ascorbic acid concentration and plasma ascorbic acid levels in children with various infections. Mater Med Pol. 1993;25(1):5–8.
46. Cevikel MH, Tuncyurek P, Ceylan F, Meteoglu I, Kozaci D, Boylu S. Eur Surg Res. 2008;40(1):29–33.

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