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Original Article

EFFECTIVENESS OF CHITOSAN NANOPARTICLES LOADED 0.7% TETRACYCLINE ON CLINICAL PARAMETERS AND *INTERLEUKIN-1β* LEVEL IN PERIODONTITIS PATIENT

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ABSTRACT

Objective: Chitosan nanoparticles are natural polymer materials that have several good characteristics, such as biodegradability, a controlled drug delivery system, non-toxicity, anti-bacterial properties, and good biocompatibility. Tetracycline as adjunctive treatment in periodontal therapy showed an improvement in clinical parameters.

Methods: The research sample was first or second molar region in periodontitis patients with pocket depth ≥ 6 mm. The research used a pretest and posttest control group design. The research sample were 32 non-contiguous first or second molar in periodontitis patients aged 18-55 years with pocket depth ≥ 6 mm were divided into three groups (14 each groups), namely: scaling-root planing [SRP] group accompanied by subgingival application of chitosan nanoparticle loaded 0.7% tetracycline; SRP group accompanied by subgingival application of chitosan nanoparticle without tetracycline; and SRP group only. Interleukin-1β levels in gingival crevicular fluid and clinical parameters used are papillary bleeding index (PBI) and pocket depth (PD) were examined before and seven days after treatment.

Results: The results showed reducing in clinical parameters PD and PBI) and IL-1β levels after treatment in all groups ($p<0.05$). The most reduction of clinical parameters and IL-1β level was found in SRP accompanied by subgingival application of chitosan nanoparticle-loaded 0.7% tetracycline group compared with another group ($p<0.05$). Pocket depth reduction has a positive correlation with IL-1β level reduction ($p<0.05$). However, the short seven-day follow-up limits the assessment of long-term clinical outcomes.

Conclusion: Chitosan nanoparticle-loaded 0.7% tetracycline is effective as an adjunctive therapy in periodontal treatment.

Keywords: Chitosan nanoparticle, Interleukin-1β, Papillary bleeding index, Pocket depth, Tetracycline

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INTRODUCTION

Periodontal disease is a chronic inflammatory condition of the gingiva, alveolar bone, cementum and periodontal ligament, which supports the teeth caused by dental biofilm [1]. Clinical signs of periodontitis are characterized by periodontal pockets, clinical attachment loss, papillary bleeding index, tooth mobility, and bone loss [2].

The treatment to remove dental biofilm includes scaling-root planing [SRP]. Scaling-root planing aims to remove dental biofilm, and the effectiveness of this treatment can be assessed by the disappearance of clinical symptoms and the reduction or disappearance of periodontal pathogens [3]. Scaling-root planing has several limitations, such as reaching deep periodontal pockets, so supporting therapy in antibiotics is needed to reduce or stop bacterial development [4].

Local drug of antibiotics as an adjunctive for mechanical therapy provides a better response than systemic. Indications for local use of antibiotics can be used to treat localized infections in periodontal tissue and deep pockets [5]. The advantages of local drug application are that it is minimally invasive, has direct contact with pathogenic bacteria, and minimizes side effects [6].

Tetracycline is a bacteriostatic antibiotic with a broad spectrum, which effective on g-negative and g-positive bacteria, aerobes, and anaerobes. Tetracycline is an antibiotic that has anti-collagenase properties and can increase adhesion and regeneration by increasing fibroblasts and reducing damage to alveolar tissue and bone [7].

Chitosan was obtained through the natural partial deacetylation of chitin. Chitosan can be obtained from crustaceans' exoskeletons, including crabs, shrimps and lobsters, fungi and insects [8]. Chitosan has good biodegradability, a promising drug delivery system, non-toxicity, anti-bacterial, anti-inflammatory, and biocompatibility. Chitosan is a matrix that is useful for spreading and constant drug release [9]. Popa l *et al.*, in their research, explained chitosan as a gel is an adequate system for local drug release in the periodontal pocket, can remain in the pocket, and control the release of antimicrobial agents in the gingival crevicular fluid [10]. Nanoparticular systems were provided and have several advantages compared to microparticles, such as increasing the effectiveness of the drug delivery system, higher anti-bacterial activity, and controlled drug release [11].

Periodontal pathogens in dental biofilm are characterized by a flora dominated by g-negative anaerobic bacteria. Bacteria that penetrate periodontal tissue interact with host responses such as neutrophils and macrophages, secreting proinflammatory cytokines such as interleukin-1β [12].

Macrophages, monocytes, and dendritic cells produce interleukin-1β. Interleukin-1β is a proinflammatory molecule and is a significant component of osteoclast-activating factor that causes bone resorption and can induce the production of proteinase that can damage the periodontal tissue [13]. Biological effects caused by IL-1β include stimulating fibroblast proliferation, stimulation of PGE2 production, and activation of different cells to release MMPs that degrade extracellular matrix proteins. Zheng P *et al.* found that patients with the deeper pocket depth (PD) and papillary bleeding index [PBI], those with severe cases, increased levels of IL-1β in the gingival crevicular fluid [14].

The prior research has shown the clinical effectiveness of a 0.7% tetracycline concentration in periodontal treatment, thereby endorsing its use. As an illustration, Susanto *et al.* (2017) found that subgingival use of 0.7% tetracycline led to considerable decreases in

probing depth and clinical attachment loss, as well as a reduction in microbial counts within periodontal pockets. Tetracycline also has a therapeutic effect in modulating the immune response of the host. Tetracycline decreases the generation of proinflammatory cytokines like interleukin-1β (IL-1β) by preventing the activation of nuclear factor-kappa B (NF-κB) stimulated by bacterial endotoxin. Decreased IL-1β levels correlate with diminished osteoclast activity, lowered production of matrix metalloproteinases (MMPs), and restricted extracellular matrix degradation. These effects on a molecular level ultimately aid in the preservation of periodontal tissues and enhancement of clinical outcomes [15].

Based on this description, the researchers want to evaluate the effectiveness of the subgingival application of chitosan nanoparticles loaded with 0.7% tetracycline on clinical parameters and IL-1 β levels in periodontitis patients.

MATERIALS AND METHODS

This research is a pre and post-test experimental research with a control group design. The preparation of chitosan nanoparticles loaded 0.7% tetracycline was made in the research laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara. Sampling and measurement of clinical parameters were carried out at RSGM, Universitas Sumatera Utara. Examination of IL-1β levels was carried out in the Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara. The ethical feasibility of the research was approved by the Research Ethics Commission of Faculty of Medicine, Universitas Sumatera Utara (N.855/KEPK/USU/2022).

The sample size was determined based on a power analysis to detect significant differences in clinical parameters and IL-1β levels with a confidence level of 95% and power of 80%, resulting in a minimum of 10 samples per group, increased to 14 to account for potential dropouts. The research sample were 32 non-contiguous first or second molar in periodontitis patients aged 18-55 y with pocket depth ≥ 6 mm, without involvement of systemic diseases, not smoking or drinking alcohol, not pregnant or breastfeeding, and not using drugs or having undergone periodontal treatment in the last 3 mo that could impact periodontal health. Samples were divided into three groups (14 each group):

1. Scaling-root planing group accompanied by subgingival application of chitosan nanoparticle loaded 0.7% tetracycline
2. Scaling-root planing group accompanied by subgingival application of chitosan nanoparticles without tetracycline.
3. Scaling-root planing group only.

The research was carried out in the manufacturing stages of chitosan nanoparticles based on the ionotropic gelation method (fig. 1):

1. 2g chitosan was dissolved in 1% [50 ml] acetic acid and stirred for 24 h using magnetic stirring.
2. The pH was adjusted by providing 0.01 N NaOH until pH 5.5
3. Tripolyphosphate (TPP) 0.005 gr was added to the chitosan solution and stirred with magnetic stirring at room temperature.
4. A concentration of 0.7% tetracycline, 0.35 g, was dissolved in water and added to the chitosan nanoparticle gel.

The solution were stirred again for 20 min to obtain the final mixture of 0.7% tetracycline based on chitosan nanoparticles. Particle measurements were using a particle size analyzer, and the average particle size was 10 nm (fig. 2).

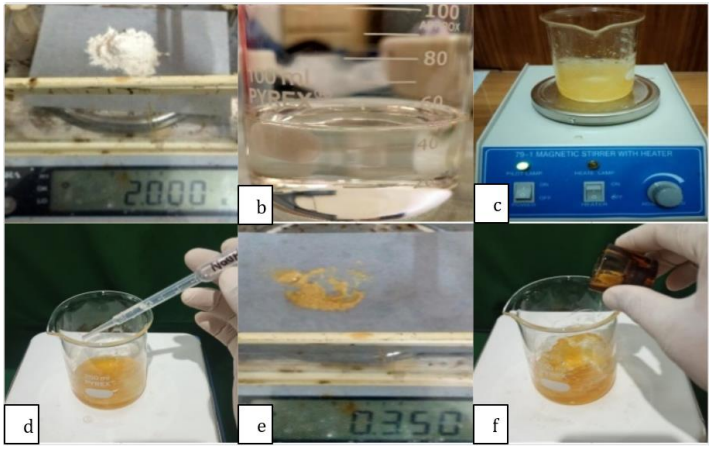

Fig. 1: The preparation of chitosan nanoparticle loaded with 0,7% tetracycline. a) weighing 2g of chitosan, b) dissolution of chitosan with acetic acid, c) stirring the solution with a magnetic stirrer, d) the addition of Tripolyphosphate to chitosan solution, e) weighing 0,35g of chitosan solution, f) the addition of 0.7% tetracycline to chitosan solution

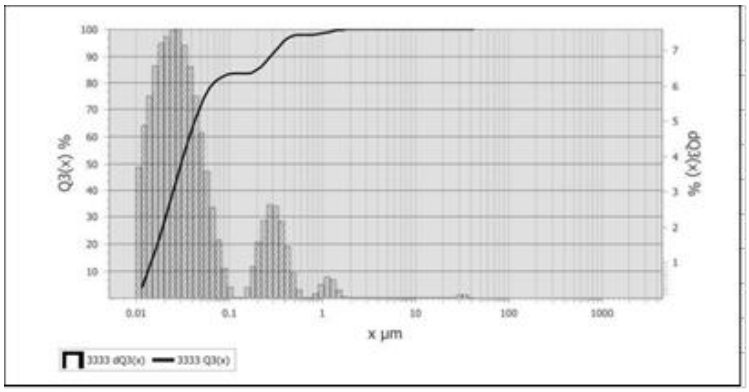

Fig. 2: The graph of particle measurement results using a particle size analyzer

Examination of clinical parameters (PBI, PD) and measurement of IL-1β levels from gingival crevicular fluid was performed on all groups on day 0 before application and day seven after application.

Gingival crevicular fluid is taken using a 1-10 ml microcapillary pipette, which is placed gently using a superficial intracrevicular technique for 5-10 min. During the collection of gingival crevicular fluid, blood should not occur (fig. 3). Then the gingival crevicular fluid in the microcapillary was taken and put into a microcentrifuge tube and stored in a cooling box before being stored in a refrigerator at-80 °C.

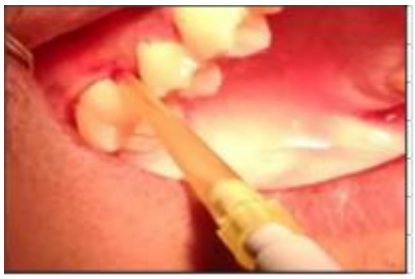

Fig. 3: Retrieval of gingival crevicular fluid

After scaling-root planing, 1 cc of chitosan nanoparticles loaded with 0.7% tetracycline were applied to each samples of the first group and 1 cc chitosan nanoparticles to the second group with a small blunt-tipped syringe. Isolated the teeth and inserted the tip into the pocket with no pressure to prevent the trauma. The gel is applied into the pocket until it reaches the gingival margin and then covered with a periodontal pack (fig. 4).

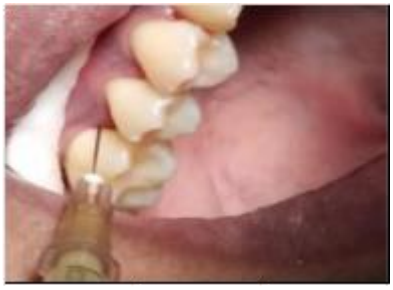

Fig. 4: The application 1 cc of chitosan nanoparticles loaded with 0.7% tetracycline used a small blunt-tipped syringe

Data processing was done by analysis of the average before and seven days after treatment for each group using the Wilcoxon test. Correlation between clinical parameters and IL-1β levels in all groups used the Spearman correlation test.

RESULTS

The clinical parameters assessed in this study include PBI (table 1) and PD (table 2). Assessments were performed before treatment and seven days after treatment.

Table 1: Mean of papillary bleeding index (PBI) before and 7 d after treatment in each group

Treatment group	n	PBI (±SD)		
		Day 0	Day 7	p value (η^2)
SRP+Chitosan-Tetracycline	14	2.57±0.51	1.43±0.41	0.001*
SRP+Chitosan	14	2.64±0.49	1.79±0.42	0.001*
SRP	14	2.43±0.51	1.79±0.42	0.003*

*Wilcoxon test, significance p<0.05
PBI= papillary bleeding index; SD= standard deviation; SRP= scaling root planing

Table 2: Mean of pocket depth (PD) before and seven days after treatment in each group

Treatment group	n	PD (±SD)		
		Day 0	Day 7	p value (η^2)
SRP+Chitosan-Tetracycline	14	6.86±0.66	5.57±0.64	0.001*
SRP+Chitosan	14	6.43±0.51	5.86±0.66	0.005*
SRP	14	6.07±0.26	5.79±0.57	0.046*

*Wilcoxon test, significance p<0.05
PD= pocket depth; SD= standard deviation; SRP= scaling root planing

Tables 1 and 2 show a significant decrease in the value of PBI and PD (p<0.05) for all groups. However, SRP+application of chitosan nanoparticle gel loaded 0.7% tetracycline group showed the most significant decrease after 7 d.

Table 3 shows significantly reduced levels of IL-1β (p<0.05) in two groups: SRP+Chitosan nanoparticles loaded 0.7% tetracycline and SRP+Chitosan nanoparticles. The largest decrease was shown in the SRP+Chitosan nanoparticles loaded in the 0.7% tetracycline group.

The correlation test between reduction level IL-1β with clinical parameters used *Spearman* test showed that the clinical parameter that correlated significantly (p<0.05) with IL-1β levels was PD, with a strength of relationship/coefficient correlation (r-value) of 0.430, including in the moderate relationship category, with a positive relationship direction which means that the more significant the difference in PD reduction values, the greater the difference in IL-1β expression reduction values in all groups (table 4).

Table 3: Mean of IL-1β levels before and seven days after treatment in each group

Treatment group	n	IL-1β ±SD (pg/ml)		
		Day 0	Day 7	p value (η^2)
SRP+Chitosan-Tetracycline	14	1500.74±433.92	1061.30±233.92	0.001*
SRP+Chitosan	14	1348.13±366.38	986.07±278.50	0.001*
SRP	14	1491.54±713.94	1255.09±384.65	0.198

*Wilcoxon test, significance p<0.05
IL-1β= Interleukin 1β; SD= standard deviation; SRP = scaling root planing

Table 4: Correlation between clinical parameters and IL – 1β level in all groups

ELISA	Clinical parameters	p value (η^2)	r
IL-1β	Pocket depth [PD]	0.004*	0.430
	Papillary bleeding index [PBI]	0.572	0.090

**Spearman correlation test*, significance p<0.05
IL–1β = Interleukin 1β; PD= pocket depth; PBI= papillary bleeding index

DISCUSSION

This research showed that chitosan nanoparticles loaded with 0.7% tetracycline have better effectiveness than other groups in reducing clinical parameters and IL-1β levels. This is related to the efficacy and characteristics of chitosan and tetracycline [15]. Tetracycline reaches higher concentrations in the crevicular fluid than in the serum by binding to calcium-loaded substances. Tetracycline can bind calcium ions and Zn ions, which are located on the active site of the collagenase enzyme, and inhibit the collagenase enzyme, producing an antiproteolytic effect that can inhibit bone resorption [16].

Sharma *et al.* showed a significant improvement in all clinical parameters, such as PD and level of attachment loss, and a substantial reduction in bacterial colonies compared to the control group when using tetracycline as a local drug delivery system in chronic periodontitis [17]. Nadig *et al.* in clinical research showed that local use of tetracycline combined with SRP in chronic periodontitis can reduce gingival index, plaque index and pocket depth. Chitosan has several characteristics, including anti-bacterial, anti-inflammatory, healing and drug delivery systems [18].

Chitosan has several characteristics, including anti-bacterial, anti-inflammatory, healing, and drug delivery systems. Chitosan as a drug delivery system has three main beneficial properties: mucoadhesive properties, the ability to open dense epithelium temporarily and biodegradability [19]. The mucoadhesive ability occurs through different interactions (electrostatic attraction, hydrogen bonding and hydrophobic effect) between chitosan and mucosa [20].

Chitosan can be combined with other drugs because chitosan is a good cross-linking and drug-releasing agent. Susanto *et al.* in their research showed that the tetracycline 0.7% based on chitosan gel has the most potent anti-bacterial activity and can be used as a local drug delivery system [21]. Silvia in experimental animal studies, showed that there was an improvement in clinical parameters and a fibroblast growth factor-2 using the subgingival application of chitosan nanoparticle-loaded 0.7% tetracycline [22].

Physical modification of chitosan includes changing the size of the chitosan particles to a smaller size so that nanoparticle size. Due to their small size, nanoparticles can penetrate areas that may be inaccessible, such as deep periodontal pockets [23]. Chitosan nanoparticles have several advantages, such as slower drug release, better drug solubility and stability, and non-toxicity. Luis et al. showed that smaller particle size gives more significant surface area of the particles, increasing the ability of chitosan as an absorbent [24].

Treatment of periodontal disease with SRP only has demonstrated significant long-term success in periodontal treatment. Scaling-root planing aims to restore gingival health by removing sources of inflammation, such as biofilm, calculus, and bacterial endotoxin from the tooth surface, but SRP has limitations and difficulty in reaching deep pockets, tortuous pockets, or furcation involvement and the inability to eliminate bacteria from the tubules, dentin, and soft tissue [25].

Interleukin-1β levels in gingival crevicular fluid reduced after seven days of treatment was seen in two groups. The decrease in IL-1β levels is because tetracycline has a better absorption, protein binding ability, and diffusion into tissue structures, helping to increase tissue repair or regeneration by increasing fibroblast activity and inhibiting collagenase activity, which plays a role in the destruction of connective tissue and reducing levels of inflammatory mediators [16]. Xu *et al.* reported that topical application of tetracycline can reduce the inflammatory response in the healing phase. Oringer *et al.*'s research, saw a decrease in IL-1β levels within one month after the topical application of tetracycline [26].

Using chitosan as an anti-inflammatory by modulating IL-1β, reducing levels of IL-1β, an essential inflammatory mediator involved in tissue damage in periodontitis [27]. Chitosan involves nuclear factor [NF] as an anti-inflammatory because it controls the expression pro-inflammatory genes such as cytokines, adhesion molecules, and enzymes producing cytotoxic molecules. Davydova *et al.* stated that chitosan is considered an immunomodulator for stimulating the synthesis of several cytokines that cause stimulation or inhibition of immunity [28].

Based on the results of this research, only PD was significantly correlated [p<0.05] with IL-1β levels, with a relationship strength/coefficient correlation [r value] of 0.430, including in the moderate relationship category with a positive relationship direction. Pocket Depth is measured at sampling sites containing various cellular and biochemical mediators, showing the metabolic status of periodontal tissue-carrying molecules involved in the destructive process and associated with the healing phase [29]. The observed anti-inflammatory effects in the SRP plus chitosan group may stem from the inherent antibacterial and immunomodulatory properties of chitosan. Bacterial membranes are disrupted and microbial colonization is reduced by chitosan, which can lead to decreased immune stimulation and lower IL-1β production [19, 27]. Moreover, it moderates host responses by inhibiting the NF-κB pathway, which leads to a decrease in proinflammatory cytokine expression [28]. The decrease in pocket depth is probably associated with a reduction in bacterial load and inflammation, thereby aiding tissue repair and stabilization [16]. This is consistent with earlier results demonstrating a correlation between reduced IL-1β levels and enhanced periodontal healing [26, 29].

Mombelli *et al.*, in their research, showed a positive correlation between IL-1β levels and PD after SRP accompanied by tetracycline-impregnated collagen fiber, while the clinical parameters of PBI did not correlate significantly due to changes in the activity of pro-inflammatory and anti-inflammatory cytokines when periodontal inflammation occurred, which affects the intensity and duration of inflammation [25].

This study has some limitations that need to be recognized. First, the lack of microbiological data, including bacterial colony counts that's restricts the ability to directly link clinical and biochemical improvements to the antibacterial effects of the chitosan-tetracycline formulation. Although changes in clinical and inflammatory markers indicate antimicrobial activity, future studies that include microbiological analysis are necessary to verify this mechanism. Secondly, the seven-day follow-up period limits the ability to evaluate long-term periodontal stability and tissue regeneration. To establish the lasting impact of the treatment on clinical outcomes and inflammatory markers, extended observation is essential. In addition, although particle size was measured using a particle size analyzer, validation using Transmission Electron Microscopy (TEM) was not performed. Future research should include TEM characterization to confirm particle morphology and size distribution.

CONCLUSION

The use of chitosan nanoparticle gel loaded 0.7% tetracycline as adjunctive therapy has better effectiveness in reducing clinical parameters of periodontal disease [PD, PBI] and reducing IL-1β levels in gingival crevicular fluid after seven days of treatment are related to the effectiveness of tetracycline in eliminating periodontal bacteria and has anti-collagenase. Chitosan serves as an excellent drug delivery system and anti-bacterial. Although these findings from the short term should be viewed cautiously, since they might not directly forecast long-term clinical outcomes. Future research

should incorporate microbiological evaluations and lengthen follow-up durations to a minimum of 30 d in order to assess the durability and stability of periodontal enhancements.

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AUTHORS CONTRIBUTIONS

Irma Ervina-Conception, writing original draft preparation and revision manuscript, data design and analysis, performed the experiments; Aini Hariyani Nasution-Supervision and visualization, revision of manuscript; R. Tri Rizky Ananda-Revision manuscript, data design and performed the experiments; Harry Agusnar-Supervision and visualization, revision of manuscript; R. Lia Kusumawati-Visualization and revision of manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest in the publication of this paper

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