

# Efficacy of Minocycline Hydrochloride Ointment with Metronidazole in the Treatment of Chronic Periodontitis and its Impact on Periodontal Microecology

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## Sun *et al.*: Minocycline Hydrochloride and Metronidazole in Treating Chronic Periodontitis

Examining the efficacy of the combined application of minocycline hydrochloride ointment and metronidazole for treating chronic periodontitis and studying its impact on periodontal microecology. The research involved dividing 235 chronic periodontitis patients into an observation group and a control group for further analysis. Both sets of participants underwent fundamental periodontal therapy, with the control cohort undergoing metronidazole treatment, and the observation cohort undergoing combined minocycline hydrochloride ointment and metronidazole treatment. A month into the treatment, the treatment effectiveness was appraised, and an evaluation of the periodontal indices, such as the gingival index, plaque index and probing depth, was carried out. Collection of gingival crevicular fluid samples for assessment of inflammatory factors and immune cell levels was conducted, alongside the documentation of bacterial colony counts in the periodontal microecology. The overall effectiveness rate in the observation group stood at 96.00 %, a significant increase compared to the 84.00 % noted in the control group. Decreases in the gingival index, plaque index and probing depth indices were observed in the observation group, and these reductions were greater than those in the control group. Furthermore, the reduction in inflammatory factor levels in the gingival crevicular fluid was more notable in the observation group than in the control group. Greater declines in the levels of  $T_h17$  and  $T_{reg}$  cells were observed in the observation group relative to the control group. Additionally, the observation group saw a reduction in the bacterial colony count of harmful bacteria in periodontal microecology, paired with an increase in beneficial bacteria. The use of minocycline hydrochloride ointment in conjunction with metronidazole in chronic periodontitis patients leads to a notable improvement in periodontal indices, a reduction in inflammatory responses, the modulation of  $T_h17$  and  $T_{reg}$  cell levels, and the preservation of periodontal microecology.

**Key words:** Chronic periodontitis, minocycline hydrochloride, metronidazole, efficacy, microecology

Originating from oral microbial infection, chronic periodontitis is typified by persistent inflammation of the periodontal tissues. Although it can emerge at any age, its prevalence is more prominent in adults, with a marked spike in incidence post the age of 35 y<sup>[1,2]</sup>. Inadequate and untimely treatment may lead to the degradation of the periodontal tissues, impaired tooth stability, and the potential for tooth loss<sup>[3]</sup>. Managing chronic periodontitis effectively involves mitigating inflammation and preventing infections within the periodontal tissues<sup>[4]</sup>.

Mechanical removal of dental plaque remains the primary approach in addressing chronic periodontitis<sup>[5]</sup>. However, dependence solely on mechanical cleaning methods can present challenges in fully eradicating periodontal pathogens, and patients may encounter difficulties in performing these procedures. Hence, the search for locally administered treatment options for chronic periodontitis is of great importance.

Minocycline hydrochloride ointment is extensively utilized in oral disease treatment regimens<sup>[6,7]</sup>.

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Scientific investigations have demonstrated minocycline hydrochloride's antibacterial, anti-inflammatory, and anti-fibrinolytic qualities, providing noteworthy efficacy in periodontitis management<sup>[8,9]</sup>. In contrast, metronidazole serves as an antimicrobial therapy, effectively inhibiting periodontal pathogens and alleviating the symptoms of chronic periodontitis<sup>[10]</sup>. The combined administration of these two treatments may generate a synergistic influence, enhancing treatment efficacy.

Conversely, periodontal microecology stands as a critical factor in upholding periodontal well-being<sup>[11]</sup>. Establishing and maintaining a balanced and healthy periodontal microecology is imperative in forestalling the emergence and advancement of periodontitis<sup>[12]</sup>. Nevertheless, at present, there is a dearth of extensive and methodical studies regarding the influence of concurrently using minocycline hydrochloride ointment and metronidazole on periodontal microecology in addressing chronic periodontitis.

As a result, this study sought to assess the effectiveness of combining minocycline hydrochloride ointment with metronidazole in managing chronic periodontitis, and explore its influence on periodontal microecology. Our objective is to contribute to the enhancement of individualized and effective treatment alternatives for chronic periodontitis and to make a valuable contribution to sustaining oral health by obtaining a more thorough grasp of the treatment effectiveness and its impact on periodontal microecology through this combined therapy.

## MATERIALS AND METHODS

### General information:

235 patients diagnosed with chronic periodontitis, who were admitted to our hospital from June 2019 to October 2023, were chosen for the study under the endorsement of the Medical Ethics Committee.

**Inclusion criteria:** Meeting the diagnostic criteria for chronic periodontitis; having >20 remaining teeth, and periodontal pocket depth  $\geq 4$  mm; no history of periodontal treatment or antibacterial drug intake in the 3 mo prior to the start of the study; absence of occlusal trauma and poor restorations, and patients provided informed consent.

**Exclusion criteria:** Severe cardiac, hepatic, or renal

dysfunction; periodontal tissue trauma; concomitant respiratory or digestive system infections and cognitive impairment were excluded from this study. The patients were divided into an observation group (n=120) and a control group (n=115) using a random number table.

### Methods:

Both patient groups underwent fundamental periodontal treatment, which encompassed supragingival scaling, subgingival curettage, and plaque control. Notably, the control group was administered metronidazole, where metronidazole tablets (from Wudang Hubei Kuihua Pharmaceutical Group Co., Ltd., National Drug Approval Number H20045869, specification of 0.2 g) were orally administered at a dose of 0.4 g, three times a day. Minocycline hydrochloride ointment combined with metronidazole treatment was administered to the observation group, following the same protocol for metronidazole as the control group. Subsequent to routine essential care, periodontal pockets were weekly filled with minocycline hydrochloride ointment (from Japan Shin-Etsu Chemical Co., Ltd., approval number: H20100244, specification: 0.5 g) until an overflow occurred. The treatment was continuously upheld for both groups over a period of 1 mo.

### Observation indices:

**Clinical efficacy:** Following 1 mo of treatment, an extensive assessment of treatment effectiveness was performed for both patient cohorts. A significant reduction in gingival swelling, absence of pus in the periodontal pockets, and a reduction in periodontal pocket depth of >2 mm, along with a marked improvement in tooth mobility, were considered as cured. A mild improvement in gingival swelling, absence of pus in the periodontal pockets, a reduction in periodontal pocket depth of >1 mm, and slight improvement in tooth mobility were considered as improvement. Failure to achieve the above criteria after treatment was classified as ineffectiveness.

Total effective rate=(Number of cured cases+number of improvement cases)/total number of cases $\times 100$  %

**Periodontal index:** Both groups of patients had their Gingival Index (GI), Plaque Index (PLI), and Probing Depth (PD) checked before and after 1 mo of treatment. GI is appraised on a scale from 0 to 3, accounting for the patients' gingival color, bleeding

situation, and texture modifications, wherein a higher score indicates heightened gingival inflammation. PD is the depth from the gingival margin to the bottom of the periodontal pocket, measured using a periodontal probe. PLI is leveraged to gauge dental plaque thickness, with scoring ranging from 0 to 3, where greater scores indicate more pronounced levels of plaque.

**Gingival crevicular fluid inflammatory factor index:** Gingival crevicular fluid samples from both groups of patients were obtained before and after 1 mo of treatment, and the levels of Monocyte Chemotactic Protein-1 (MCP-1), Matrix Metalloproteinase-8 (MMP-8), and Interleukin-6 (IL-6) were determined using enzyme-linked immunosorbent assay.

**T<sub>h</sub>17 and T<sub>reg</sub> cell levels:** Both patient groups provided fasting venous blood samples (2 ml per sample) before and after 1 mo treatment, and the levels of T<sub>h</sub>17 and T<sub>reg</sub> cells were quantified using a FACSCalibur™ flow cytometer fabricated by BD in the United States.

**Paradental micro ecological indicators:** Prior to and following the treatment, patients were advised to gargle with mouthwash, followed by using a sterilized cotton ball to dry their mouths. Then, a sterilized paper point was placed in the periodontal pocket and crown-blind pocket for 30 s. The next step involved submerging the paper point in 1 ml of anaerobic transport fluid

for analysis. Subsequently, the bacterial colony counts of *Porphyromonas gingivalis* (*P. gingivalis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Streptococcus mutans* (*S. mutans*), and facultative anaerobic streptococci were logged.

### Statistical analysis:

Utilizing the Statistical Package for the Social Sciences (SPSS) 25.0, the analysis was performed, comparing the measurement data, presented as mean±standard deviation, between the two groups via an independent sample t-test. Utilizing the Chi-square ( $\chi^2$ ) test, the count data were analyzed. The criteria for establishing statistical significance were defined at a level of  $p < 0.05$ .

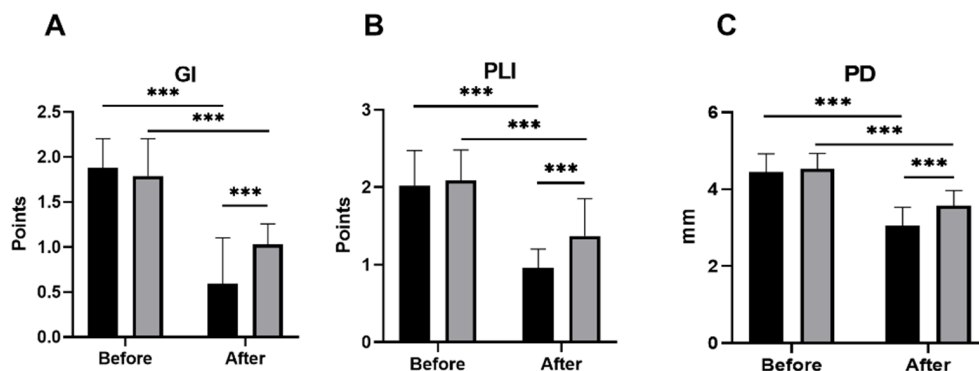
## RESULTS AND DISCUSSION

The total treatment effectiveness rate in the observation group stood at 96.00 %, surpassing the 84.00 % in the control group, with a marked difference ( $p < 0.05$ ) as shown in Table 1.

Prior to treatment, there were no remarkable variances ( $p > 0.05$ ) between the two groups concerning GI, PLI, and PD. However, after 1 mo of treatment, the levels of these four indices in both groups depleted, with the observation group levels being inferior to those of the control group, and all contrasts were notable ( $p < 0.05$ ) as shown in fig. 1.

**TABLE 1: CURATIVE EFFECT**

Group (n)	Cured	Improvement	Ineffectiveness	Overall effective rate
Observation (120)	63 (52.50)	53 (44.17)	4 (3.33)	116 (96.67)
Control (115)	48 (41.74)	49 (42.61)	18 (15.65)	97 (84.35)
$\chi^2$				10.502
P				0.001



**Fig. 1: Periodontal index, (A): GI; (B): PLI and (C): PD**  
Note: \*\*\* $p < 0.001$ , (■): Observation (120) and (■): Control (115)

Prior to treatment, there were no meaningful divergences ( $p > 0.05$ ) between the two groups regarding gingival crevicular fluid MCP-1, MMP-8, and IL-6 levels. However, after 1 mo of treatment, the levels of these five indices in both groups decreased, with the observation group levels being lower than those of the control group, and all differences were statistically substantial ( $p < 0.05$ ) as shown in Table 2.

Prior to treatment, no notable disparities ( $p > 0.05$ ) were observed between the two groups in relation to  $T_H17$  and  $T_{reg}$  cell levels. Nonetheless, after 1 mo of treatment, there was a decline in the levels of these two indices in both groups, with the observation group levels being lower than those of the control group, and all differences were remarkably significant ( $p < 0.05$ ) as shown in Table 3.

Prior to treatment, differences in terms of the bacterial colony counts of *P. gingivalis*, *A. actinomycetemcomitans*, *S. mutans*, and facultative anaerobic streptococci did not display statistical significance ( $p > 0.05$ ) between the two groups. Nevertheless, following a month of treatment, the quantity of *P. gingivalis*, *A. actinomycetemcomitans*, and *S. mutans* experienced a reduction in both groups, with the observation group demonstrating lower counts than the control group, while the quantity of facultative anaerobic streptococci increased, and the observation group count surpassed that of the control group. These disparities were all statistically notable

( $p < 0.05$ ) as shown in Table 4

Food impaction, inadequate restorations, and plaque are local stimuli that contribute to gingival inflammation. If not promptly removed, these factors could lead to the spread of inflammation to deeper tissues, resulting in collagen loss, epithelial growth towards the root, periodontal pocket formation, alveolar bone resorption, and the onset of chronic periodontitis<sup>[13,14]</sup>. Metronidazole, commonly prescribed for periodontitis, possesses antimicrobial and anti-amoebic properties and is categorized as a nitroimidazole drug. However, it often falls short in terms of short-term treatment efficacy, frequently requiring long-term medication, thereby elevating the risk of drug resistance and unsatisfactory treatment outcomes<sup>[15,16]</sup>. Containing aminomethyl propanol copolymer, concentrated glycerol, and other components, minocycline hydrochloride ointment effectively blocks bacterial protein synthesis, possessing a broad antibacterial range and demonstrating strong antibacterial effects against *S. mutans*, anaerobic bacteria, *Actinomyces*, *Clostridium*, and other strains. With its potent local sustained release antibacterial impact, fast onset, and extended duration, it yields exemplary overall results<sup>[17]</sup>. The research observed that the observation group, employing a combination of minocycline hydrochloride ointment and metronidazole, significantly outperformed the control group in terms of total treatment effectiveness.

**TABLE 2: GINGIVAL CREVICULAR FLUID INFLAMMATORY FACTOR INDEX ( $\bar{x} \pm s$ , POINTS)**

Group (n)	MCP-1 (pg/ml)		MMP-8 (pg/ml)		IL-6 (pg/ml)	
	Before	After	Before	After	Before	After
Observation (120)	135.46 $\pm$ 21.25	61.73 $\pm$ 9.91*	580.66 $\pm$ 51.48	343.60 $\pm$ 46.30*	64.21 $\pm$ 6.09	21.47 $\pm$ 3.69*
Control (115)	136.36 $\pm$ 20.45	88.23 $\pm$ 10.44*	580.28 $\pm$ 52.53	430.74 $\pm$ 44.27*	65.06 $\pm$ 6.08	34.84 $\pm$ 3.89*
t	0.332	5.932	-0.056	8.293	1.068	11.738
p	0.740	0.000	0.955	0.000	0.286	0.000

Note: (\*) indicates noteworthy difference following treatment compared with prior to treatment

**TABLE 3:  $T_H17$  AND  $T_{reg}$  CELL LEVELS ( $\bar{x} \pm s$ , POINTS)**

Group (n)	Proportion of $T_H17$ to $CD4^+$ T cells (%)		Proportion of $T_{reg}$ in $CD4^+$ T cells (%)	
	Before	After	Before	After
Observation (120)	2.67 $\pm$ 0.52	1.31 $\pm$ 0.28*	5.34 $\pm$ 0.92	4.23 $\pm$ 0.80*
Control (115)	2.65 $\pm$ 0.50	1.73 $\pm$ 0.34*	5.37 $\pm$ 0.90	4.87 $\pm$ 0.94*
t	0.252	10.387	0.256	5.594
p	0.801	0.000	0.798	0.000

Note: (\*) indicates noteworthy difference following treatment compared with prior to treatment

**TABLE 4: PERIDENTAL MICROECOLOGICAL INDICATORS ( $\bar{x}\pm s$ , CFU/ml)**

Group (n)	Anaerobic bacillus melanin genes		<i>Fusobacterium nucleatum</i>		<i>Peptostreptococcus</i>		<i>Streptococcus facultative aerobes</i>	
	Before	After	Before	After	Before	After	Before	After
Observation (120)	5.53±0.77	0.98±0.18*	3.84±0.53	0.55±0.52*	2.71±0.46	0.93±0.26*	9.58±1.01	33.52±3.65*
Control (115)	5.52±0.78	1.65±0.48*	3.91±0.41	0.97±0.18*	2.76±0.43	1.20±0.40*	9.57±1.04	22.04±2.76*
t	-0.115	14.271	1.146	8.145	0.831	6.222	-0.136	8.371
p	0.908	0.000	0.253	0.000	0.407	0.000	0.892	0.000

Note: (\*) indicates noteworthy difference following treatment compared with prior to treatment

GI, PLI, and PD are utilized as clinical indicators to assess the effectiveness of treating chronic periodontitis. Minocycline hydrochloride ointment not only displays antibacterial properties but also impedes bone resorption, encouraging the regeneration of periodontal tissues. Furthermore, it encourages the proliferation of fibroblasts in periodontal tissues, thus effectively bolstering gingival health and backing the recovery of periodontal tissues. Following 1 mo of treatment in this study, the GI, PLI, and PD indices in the observation group notably decreased and were lower than those of the control group. This finding illustrates that the combination of minocycline hydrochloride ointment and metronidazole can effectively ameliorate the symptoms of periodontitis, thereby facilitating the restoration of periodontal tissues.

Inflammation contributes to the development and progression of chronic periodontitis. MCP-1 and IL-6 serve as inflammation indicators reflecting periodontal inflammation; an increase in their levels signifies the further development of inflammation and worsening of periodontitis<sup>[18,19]</sup>. Elevated MMP-8 expression, as a MMP, can worsen periodontal inflammation. This research found that the observation group experienced a notably more substantial reduction in the levels of gingival crevicular fluid MCP-1, MMP-8, and IL-6 compared to the control group, suggesting that the combination of minocycline hydrochloride ointment and metronidazole effectively suppresses inflammation and lowers the levels of inflammatory mediators.

Capable of synthesizing IL-17,  $T_H17$ , a subset of Clusters of Differentiation 4 ( $CD4^+$ ) T cells, releases inflammatory factors and collaborates to induce a series of cascading inflammatory reactions, causing a deterioration of periodontal tissues.  $T_{reg}$

cells can release anti-inflammatory factors, hinder the proliferation and differentiation of T cells, control the immune response of the body, and produce anti-inflammatory effects<sup>[20]</sup>. Following the treatment, the investigation revealed that the observation group displayed a more pronounced decline in  $T_H17$  and  $T_{reg}$  cell levels compared to the control group. These results imply that the use of minocycline hydrochloride ointment combined with metronidazole might enhance chronic periodontitis by regulating the immune response and balancing the  $T_H17/T_{reg}$  ratio.

Mixed anaerobic bacterial infections are the main cause of the occurrence and development of chronic periodontitis. Anaerobic bacteria such as *P. gingivalis*, *A. actinomycetemcomitans*, *S. mutans*, and facultative anaerobic streptococci are the main pathogenic bacteria. Excessive anaerobic bacterial colonies present in the periodontal microecology impede the restoration of normal flora and ultimately deteriorate the condition of periodontitis. Minocycline hydrochloride ointment has good antibacterial effects, effectively killing anaerobic bacteria, and maintaining a balanced periodontal microecology. Following a month of treatment, the observation group presented a marked decrease in the bacterial colony counts of *P. gingivalis*, *A. actinomycetemcomitans*, and *S. mutans*, resulting in counts lower than those of the control group. An escalation in the bacterial colony count of facultative anaerobic streptococci was noted, with the observation group exceeding the control group, underscoring the ability of minocycline hydrochloride ointment in combination with metronidazole to regulate the periodontal microecology by reducing the level of harmful bacteria and augmenting that of beneficial bacteria.

In conclusion, our research results indicate that the utilization of minocycline hydrochloride ointment in conjunction with metronidazole for chronic periodontitis treatment is more effective in terms of efficacy and its impact on periodontal microecology in comparison to using metronidazole alone. This might be because of its antimicrobial, anti-inflammatory, and immunoregulatory qualities, and its capacity to regulate periodontal microecology. Nonetheless, our study is constrained by limitations such as a short research period and a small sample size. More comprehensive evaluations of the effectiveness and safety of this combination therapy would benefit from further studies with larger sample sizes and longer follow-up periods.

### Conflict of interests:

The authors declared no conflict of interests.

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