



Research Article

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Nature's Anti-inflammatory agent and its use as a Local drug delivery agent for treatment of chronic periodontitis: A clinical, microbiological and biochemical study

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ABSTRACT

Periodontal disease can lead to progressive loss of tooth-supporting tissues and alveolar bone. Due to the clinical limitations of scaling and root planing and recolonization of bacteria, the use of systemic and local administration of antimicrobial agents as adjuncts seems beneficial. In recent years, herbal and ayurvedic remedies are being researched to treat common infections and inflammatory conditions. Here an attempt was made to evaluate the effect of curcumin 10mg (Curenex) (CU) used as an adjunct to non-surgical periodontal therapy. A total of 10-15 sites in 14 patients with probing pocket depth 5-7mm were included. In experimental group, sites were treated with SRP+CU and in control group sites were treated with SRP alone. Plaque index, gingival bleeding index, gingival index, probing pocket depth and clinical attachment level were assessed at baseline, 21st day, 30th day, and 90th day. Subgingival plaque samples were collected to assess periodontal pathogens like *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* by anaerobic culture. GCF samples were collected to assess lactate dehydrogenase at baseline and 21st day. Results showed significant reduction in clinical parameters (PI, GBI, GI, PPD and CAL) and high statistically significant reduction in periodontal pathogens and lactate dehydrogenase in both the treatment groups, significant difference was seen in SRP+CU group. To conclude, the adjunctive use of curcumin 10mg (curenex[®]) as an anti-inflammatory, antimicrobial, and antiplaque agent along with routine mechanical debridement is definitely a promising therapy that would add to the potential benefits of the periodontal treatment.

Keywords: Curcumin, Chronic Periodontitis, Scaling and root planing, Herbal dentistry.

INTRODUCTION

Periodontal disease is defined as an infectious pathology affecting the tissues surrounding the teeth, which if not properly treated, causes progressive loss of tissue attachment and alveolar bone. One of the main etiological factor is the accumulation of bacterial biofilm [1]. The initiation and progression of the disease is influenced by a complex interplay between the host immune response and the pathogenic microorganisms. The primary aim of periodontal therapy is to remove the bacterial biofilm and all factors that favor its accumulation. Reducing the quantity of dental biofilm has been the main stay of periodontal therapy towards plaque induced diseases [2].

The initial treatment of periodontitis involves scaling and root planing (SRP), mechanical debridement of surfaces and oral hygiene instructions to the patients. Various studies have reported that scaling and root planing alone doesn't eradicate the subgingival bacteria and have a limited effect on some pathogenic species. This could be explained by the ability of these bacteria to reside in soft tissues, dentinal tubules, or in root surface irregularities, thereby contributing to the failure of the treatment [3].

To overcome these drawbacks, various antimicrobials can be given systemically or delivered locally along with the non-surgical therapy. But the disadvantage of adjunctive use of systemic antibiotics is the distribution of the drug throughout the body which could result in various side effects and toxicity [4].

On the other hand antimicrobials like Tetracycline, Doxycycline, Metronidazole, Chlorhexidine and Minocycline can be locally-delivered into the tissues as well. These drugs can inhibit or eliminate the pathogenic periodontal microorganisms, or they can modulate the inflammatory tissue response [1]. The concentration of the antimicrobial agent attained in sub-gingival sites is 100 fold higher in local route of drug delivery when compared to systemic administration [5]. The administration of LDD brings about less systemic side effects, reduction in drug resistance and deeper penetration of the drug into the disease-site

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causing elimination of destructive pathogenic microorganisms [6].

Use of medicinal plants as traditional treatment agents for human diseases has been a common practice in several regions of the world. It is the primary source of medicine in certain rural parts of developing countries. Due to the frequent use and misuse of the available antimicrobials, resistant strains of common pathogens has increased. Along with that there is an increased chance of any adverse effects occurring with their use. Therefore, it becomes necessary to search for natural alternatives which are safe to use [7].

Turmeric (or *Curcuma longa*) is an Indian spice derived from the rhizomes, a perennial member of the Zingiberaceae family. Its primary constituent is Curcumin, which is responsible for its activity and also its intense yellow color. Lampe and Milobedzka were the first ones to identify Curcumin in 1910. It is routinely used as a dietary spice, coloring agent in food and textile industry and as a home remedy for treatment of various ailments. It exhibits anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-viral and antimicrobial properties which attributes to its use in traditional medicine. It has shown to down regulate the activity of Cyclooxygenase-2 and Lipoxygenase and thereby modulate the inflammatory response by inhibiting the production of the inflammatory cytokines [8].

Curenex is an Ayurvedic proprietary medicine in gel form which contains 10mg of Curcuma longa extract. [Fig. 1] It a commercially available gel manufactured by Abbott healthcare.



Figure 1: Curcumin 10mg (Curenex gel)

This study is designed to evaluate Curenex as a Local Drug Delivery agent for treatment of Chronic Periodontitis and assess its effects on clinical parameters, biochemical markers and its efficacy against periodontal microorganisms.

MATERIALS AND METHODS

A Double blind, split-mouth design clinical trial was conducted to assess the effectiveness of Curenex (10mg Curcumin) as a Local Drug delivery agent along with Scaling and Root planing (SRP) on clinical, biochemical and microbiological parameters in periodontitis patients.

14 systemically healthy patients diagnosed with Chronic Periodontitis (according to AAP International Workshop for Classification of Periodontal Diseases, 1999) [9] were selected from Out-patient department of Periodontics, College of Dental sciences, Davangere, Karnataka. The study protocol was approved by the Institutional Ethical committee of College of Dental sciences, Davangere, Karnataka. A written and verbal informed consent was acquired from all the participants.

Patient selection Criteria

Patients having mild to moderate periodontal pockets (5-7mm) clinically and radiographic evidence of bone loss were included for the study.

Exclusion criteria:

- Patients with any known systemic diseases
- Patients who underwent any periodontal surgical or non-surgical therapy in past 6 months
- Patients on any chemotherapeutic mouth rinses or oral irrigation for past one month
- Pregnant or lactating females
- Patients who were smokers or alcoholics

Study Design

Patients' mouth were divided using split mouth protocol into Control and test group. Control group consisted of SRP alone in the upper arch whereas Test group consisted of SRP + Curenex gel in the lower arch.

Clinical parameters that were recorded are

- Plaque Index (Silness & Loe, 1964).
- Gingival index (Loe H. and Silness P., 1963).
- Gingival Bleeding Index (Ainamo & Bay, 1975).
- Probing pocket depth (PPD).
- Clinical attachment level (CAL).

Clinical parameters were recorded at baseline, 21st day, 30th day and 90th day. Hu-Friedy UNC-15 probe was used for the measurements.

Gingival crevicular fluid (GCF) sample and subgingival pooled plaque samples were collected from selected sites at baseline and on 21st day and transferred to phosphate buffered saline (PBS) and reduced transport fluid (RTF). The microbiologist and statistician were also blinded regarding coding of the plaque samples.

Study Procedure

After fulfilling the inclusion and exclusion criteria, clinical examination was performed with detailed case history for each patient. Clinical measurements were obtained.

GCF COLLECTION

GCF samples were procured from at least 8-10 sites of 5-7 mm pocket depth. The site was isolated with sterile cotton rolls. GCF collection was done prior to sub-gingival plaque collection in order to avoid any blood contamination of GCF sample. A sterile ISO 30 endodontic paper point was introduced into sulcus till a resistance was felt and kept in position for 30 seconds. After that the paper point was dispensed in separate vials containing phosphate buffered saline (PBS) transport media and tightly sealed to prevent contamination. Processing of the samples was done within 2 days of collection.

PLAQUE SAMPLE COLLECTION

After collection of GCF, Subgingival pooled plaque samples were obtained from the selected sites. Plaque was obtained by introducing a

sterile curette into the base of the pocket. The curette with the collected plaque was dispensed into separate vials containing reduced transport fluid (RTF) transport media and tightly sealed to prevent any contamination. Processing of the samples was done within 2 days of collection.

PROCEDURE FOR PERIODONTAL THERAPY

SRP was performed using Ultrasonic (Cavitron- BOBCAT PRO, DENTSPLY; Power-240AC 50/60Hz 80VA) and hand instruments (Universal Gracey Curettes, 2R/2L and 4R/4L Hufriedy-USA). Once SRP was completed, Curcumin 10mg (Curenex) was dispensed subgingivally to the base of pocket by means of a disposable syringe in the test sites. [Figure 2] The area was packed using Coe-Pak in order to retain the material.



Figure 2: Local Drug delivery of Curenex gel

Patients were instructed to brush two times in a day for minimum 2 minutes using the 'Roll-on technique'. Patients were asked to report back on 7th day for removal of periodontal pack and subsequent follow ups were done on 21st, 30th and 90th day. Mouthwash and interdental aids were not advised. Patient was asked to report if any adverse effects to Curenex was noted.

Biochemical Analysis

LDH enzyme activity was calculated and analyzed for the GCF volume in each paper point. All the vials containing GCF samples were put in the spectrophotometric automatic apparatus. LDH total unit activity = GCF volume x volume activity x L/10⁶ µl was calculated.

Microbiological Analysis

Determination Of Minimal Inhibitory Concentration (Mic) Of The Product Curenex (Curcumin 10 Mg)

9 dilutions of each drug was done with Thioglycollate broth to determine the MIC. In the initial tube 20µL of drug was added into the 380µL of Thioglycollate broth. For dilutions, 200µL of Thioglycollate broth was dispensed into the next 9 tubes separately. Then from the initial tube 200µL was transferred to the first tube containing 200µL of Thioglycollate broth. This was considered as 10⁻¹ dilution. From 10⁻¹ diluted tube 200µL was transferred to second tube to make 10⁻² dilution. The serial dilution was repeated up to 10⁻⁹ dilution for each drug. From the maintained stock cultures of required organisms, 5µL

was taken and added into 2mL of Thioglycollate broth. In each serially diluted tube 200µL of above culture suspension was added. The tubes were incubated for 48-72 hours in an anaerobic jar at 37°C and was observed for turbidity.

Microbiological Procedure

Detection and quantification of the three periodontal pathogens, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* was done. Once received in the laboratory, the samples of plaque were mixed thoroughly and 5µL each were inoculated using sterile loop onto the following mediums:

- 1) Kanamycin blood agar [KBA] (for *Porphyromonas gingivalis*).
- 2) Kanamycin, vancomycin blood agar [KVBA] (for *Prevotella intermedia*).
- 3) Crystal violet Erythromycin agar [CVE] (for *Fusobacterium nucleatum*).

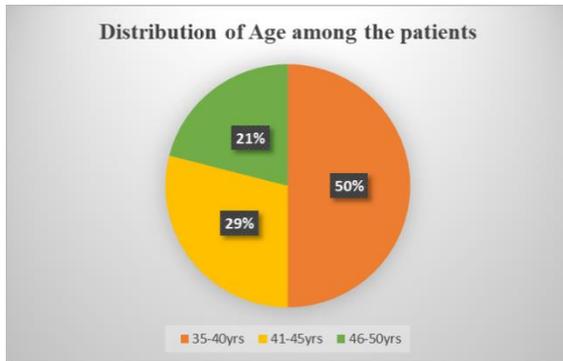
The plates were kept in the anaerobic jar and the anaerobic atmosphere was created by making use of the combination of sodium borohydrite, citric acid and sodium bicarbonate in the presence of palladium catalyst. The remnants of the oxygen from the jar were removed with help of a suction apparatus. The conditions of the anaerobic atmosphere were continuously monitored by the pressure gauge attached to the anaerobic jar. The inoculated plates were kept for a minimum of 72 hours. Later the plates were taken out of the jar, the colony characteristics such as size, shape, hemolysis and pigmentation were recorded and the numbers of each colony types were counted. The count was multiplied by 200 (dilution factor) to express Colony Forming Unit (CFU) per ml. Further identification of the organisms was done by making use of Gram's stain and key biochemical reactions as per the standard protocol.

Statistical analysis

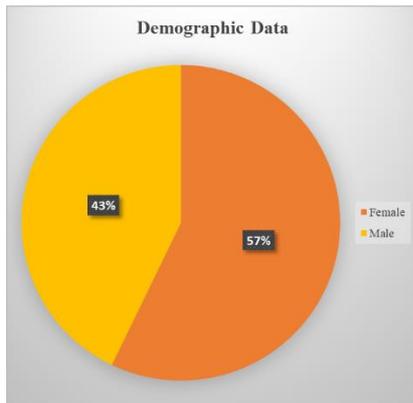
Statistical analysis was performed with SPSS (version 20) USA. Comparison of data was done by applying specific statistical tests to find out the statistical significance of the results. Since the data obtained was of continuous type, parametric tests were used for analysis. Calculation of the Mean and Standard Deviation (SD) was done. Statistical tests employed in this study were: Repeated measure Analysis Of Variance (ANOVA) test for intra group comparisons at different time intervals followed by unpaired 't' test for inter group comparisons. For Gingival bleeding index (GBI), Chi square test was used as it was measured in percentage. For microbiological parameters and biochemical parameters, repeated measures ANOVA test for intra group comparison and unpaired 't' test for intergroup comparison were performed. For all the tests a P-value of 0.05 or less was considered to be statistically significant.

RESULTS

A total number of 14 patients who were suffering from generalized chronic periodontitis were included for this study and were divided into experimental and control group of 7 patients each. The patients belonged to the age group of 35-50 years and enrolled 6 males and 8 females. (Graph 1 and 2)



Graph 1: Distribution of Age among the patients



Graph 2: Demographic Data

On comparison, all clinical, microbiological and biochemical parameters recorded at baseline in both groups were statistically non-significant. (Table 1 and 2)

Table 1: Baseline values of Clinical Parameters

GROUP	PI	GI	GBI	PD	CAL
SRP	2.31±0.55	2.37±0.51	89.47±28.88	6.36±1.35	5.96±1.56
SRP+CU	2.43±0.50	2.54±0.41	95.08±12.85	6.65±1.08	5.50±1.18
P-VALUE	0.55 NS	0.33 NS	0.38 NS	0.53 NS	0.38 NS

SRP: Scaling and Root Planing; CU: Curenext Gel; PI: Plaque Index; GI: Gingival Index; GBI: Gingival Bleeding Index; PD: pocket depth; CAL: Clinical Attachment Level

Table 2: Baseline values of Microbiological and Biochemical Parameters

GROUP	Pg	Pi	Fn	LDH
SRP	126.42±33.65	145±20.28	126.78±14.75	126.22±20.57
SRP+CU	126.42±33.65	145±20.28	126.78±14.75	142.97±23.02
P VALUE	1.00 NS	1.00 NS	1.00 NS	0.053 NS

SRP: Scaling and Root Planing; CU: Curenext Gel; Pg: Porphyromonas gingivalis; Pi: Prevotella intermedia; Fn: Fusobacterium nucleatum

On intragroup comparison and intergroup comparison of control group and experimental group, clinical parameters showed statistically high significance from baseline to 90th day. In both the SRP group and SRP + CU group, the PI, GI, GBI, PPD, and CAL showed statistically significant reduction within the groups after 90 days. (Table 3)

Table 3: Intragroup & Inter Group Comparison For Clinical Parameters

GROUP	BASELINE	21 DAYS	30 DAYS	90 DAYS	Mean reduction	P value
Plaque Index						
SRP	2.31±0.55	1.09±0.11	1.04±0.06	1.06±0.08	1.24±0.54	0.000 (HS)
SRP+CU	2.43±0.50	1.10±0.10	1.04±0.04	1.12±0.28	1.31±0.60	0.000 (HS)
P VALUE	0.55 (NS)	0.10	0.009	0.045 (S)		
Gingival Index						
SRP	2.37±0.51	1.20±0.28	0.97±0.35	0.47±0.38	1.89±0.72	0.000 (HS)
SRP+CU	2.54±0.41	1.34±0.44	0.88±0.43	0.27±0.37	2.26±0.59	0.000 (HS)
P VALUE	0.33 (NS)	0.2	0.006	0.05 (S)		
Gingival bleeding index						
SRP	89.47±28.88	36.65±13.62	15.75±5.86	6.54±5.17	89.32±14.39	0.000 (HS)
SRP+CU	95.08±12.85	28.39±14.47	10.77±7.28	4.24±4.64	90.84±12.94	0.000 (HS)
P VALUE	0.38 (NS)	0.19	0.005(S)	0.001 (HS)		
Probing Pocket Depth						
SRP	6.36±1.35	5.55±0.87	4.69±0.89	3.81±0.76	2.55±1.42	0.000 (HS)
SRP+CU	6.65±1.08	5.57±0.86	4.88±0.80	3.62±0.57	3.03±1.03	0.000 (HS)
P VALUE	0.53 (NS)	0.34	0.010	0.05 (S)		
Clinical attachment level						
SRP	5.96±1.56	5.62±1.01	5.06±0.78	5.04±0.70	0.91±1.72	0.000 (HS)
SRP+CU	5.50±1.18	5.68±1.50	5.27±0.94	5.36±0.58	0.14±0.86	0.000 (HS)
P VALUE	0.38 (NS)	0.25	0.007	0.045 (S)		

SRP: Scaling and Root Planing; CU: Curenext Gel; PI: Plaque Index; GI: Gingival Index; GBI: Gingival Bleeding Index; PD: pocket depth; CAL: Clinical Attachment Level; S: Significant; HS: Highly Significant; NS: Non significant

The intergroup and intragroup comparison for clinical and biochemical parameters is depicted in Table 4 and 5. At the end of 21st day, microbial count reduction of Pg, Pi and Fn was statistically highly

significant ($p < 0.000$) and also biochemical level reduction of lactate dehydrogenase was statistically significant ($p < 0.045$).

Table 4: Intergroup And Intragroup Comparison Of Microbiological Parameters

GROUP	BASELINE	21 DAYS	Mean reduction	P value
Porphyromonas gingivalis				
SRP	126.42±33.65	40.35±4.98	86.07±33.17	0.000 (HS)
SRP+CU	126.42±33.65	17.21±7.75	109.21±32.63	0.000 (HS)
P VALUE	1.00 (NS)	0.000 (HS)		
Prevotella intermedia				
SRP	145±20.28	38.14±12.55	106.85±25.14	0.000 (HS)
SRP+CU	145±20.28	10.14±4.12	134.85±19.79	0.000 (HS)
P VALUE	1.00 (NS)	0.000 (HS)		
Fusobacterium Nucleatum				
SRP	126.78±14.75	44.64±8.19	82.14±19.87	0.000 (HS)
SRP+CU	126.78±14.75	9.85±3.52	116.92±14.45	0.000 (HS)
P VALUE	1.00 (NS)	0.000 (HS)		

SRP: Scaling and Root Planing; CU: Curenext Gel; S: Significant; HS: Highly Significant; NS: Non significant

Table 5: Intergroup And Intragroup Comparison Of Biochemical Parameters

GROUPS	BASELINE	21 DAYS	Mean reduction	P value
SRP	126.22±20.57	54.85±30.64	71.37±28.83	0.000 (HS)
SRP+CU	142.97±23.02	30.73±14.47	112.24±28.69	0.000 (HS)
P VALUE	0.053 (NS)	0.004(S)		

SRP: Scaling and Root Planing; CU: Curenext Gel; S: Significant; HS: Highly Significant; NS: Non significant

DISCUSSION

Periodontitis is a chronic inflammatory disease with an infectious etiology which destroys the tooth-supporting apparatus and also commonly lead to tooth loss in adults. The microbes and inflammatory response both play an important role in the causation of the disease.^[10] For the microbes to initiate periodontal disease, it is necessary for them to colonize the subgingival pockets along with production of virulence factors that damage the tissues of the host. Thus, the major goal of nonsurgical periodontal therapy is to minimize the pathogenic subgingival micro flora and decrease or eliminate the associated inflammatory lesion.

The gold standard of non-surgical periodontal therapy is Scaling and root planing. There is reduction seen in the levels of periodontopathogenic bacteria following a single round of SRP which can be appreciated by improvement in the clinical conditions.

But mechanical debridement alone is unable to remove bacteria present, thereby requiring use of chemical agents to eliminate them. Locally applied antimicrobials such as Chlorhexidine, tetracyclines or other systemic antibiotics like metronidazole could be used as adjuncts to SRP^[11]. However use of systemic antibiotics has multiple drawbacks such as hypersensitivity reactions, gastrointestinal intolerance and the development of bacterial resistance and also the inability of the active drug to reach an adequate concentration and retain in the local site for

a sufficient amount of time. On contrary, LDD administration is associated with greater levels of drug at the diseased site along with minimal systemic side effects and reduced chance of drug resistance^[12].

Currently there is an increase in use of herbal products in dentistry as they are easily available, low in cost and rarely cause any side effects^[13]. One of these herbal product is turmeric (*Curcuma longa*) or Nature's anti-inflammatory agent. Components of tumeric are named curcuminoids, which mainly includes curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin^[14]. Commercial available preparations of "curcumin" contain approximately 77% curcumin, 17% demethoxycurcumin and 3% bisdemethoxycurcumin.

In Ayurvedic medicine, Curcumin is being used extensively due to its non-toxicity and easy availability. Along with that over 3000 studies claim that it has anti-oxidant, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, anti-proliferative, pro-apoptotic and anti-atherosclerotic effects. Therefore the present study aimed at evaluating the clinical, microbiological and biochemical outcomes of application of 10mg Curcumin, extract of curcuma longa (Curenext) as a local drug delivery agent in treatment of chronic periodontitis patients.

In this study, the clinical parameters were recorded and evaluated at baseline, 21st day, 30th day and 90th day whereas microbiological and biochemical parameters at baseline and 21st day. This is similar to study performed by Paolantonio M *et al.* in 2009, where they evaluated the Clinical, microbiological and biochemical effects of Xanthan-based Chlorhexidine gel in treatment of periodontitis and the parameters were recorded 0, 60 and 180th day^[15].

It was found that both experimental and control groups showed significant reductions in PI, GI, GBI, PD. The SRP + CU group demonstrated significant reduction of PI, GI, and PD as compared to the SRP alone group. Highly significant reduction was demonstrated with respect to GBI in SRP+CU than SRP group. This is in agreement

with the studies conducted by Anuradha *et al.* and Gottumukkala *et al.* [16, 17].

Microbial analysis for *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* was done in this study. Inter-group mean CFU reduction of Pg, Pi, and Fn was maximum for CU + SRP treatment followed by SRP. The SRP group also showed reduction, although statistically highly significant but was less compared to experimental groups. Bhatia M *et al.*, 2014 evaluated in a split mouth study design group the efficacy of locally delivered 1% Curcumin gel with SRP on clinical and microbiological parameters in patients with chronic periodontitis. They showed significant reductions in the counts of microorganisms such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *capnocytophaga* after 6 months in test group when compared to the control group. They also claimed that Curcumin possesses anti-bacterial properties and there is decreased antibiotic resistance as it acts by suppression of NF- κ B activation [18].

For the biochemical analysis LDH was used and when compared to SRP group, SRP+CU group showed statistically highly significant reduction in LDH level from baseline to 21st day on intra group and intergroup comparison. There are no comparative studies as this is first biochemical study evaluating Lactate dehydrogenase reduction with curcumin.

Although a slight increase in plaque scores and bleeding on probing was noted in both groups after 90 days. This could be due to the reduction in the concentration of Curcumin, therefore its inability to cause any long-term effects. None of the patient reported with any side effects and no adverse reactions was observed by the clinician during the current study.

Overall, the use of curcumin 10mg (curenex[®]) led to statistically significant reduction of plaque, gingival inflammation, gingival bleeding, probing pocket depth and gain in clinical attachment level. The microbial reduction of Pg, Pi and Fn along with reduction in LDH level showed statistically high significance and was in line with the clinical parameters changes.

CONCLUSION

The adjunctive use of curcumin 10mg (curenex[®]) as an anti-inflammatory, antimicrobial agent, and antiplaque agent along with routine mechanical debridement is definitely a promising therapy that would likely add to the potential benefits of the periodontal treatment. Further research on this product with frequent applications and larger sample size need to be done to detect better outcomes and for use in regular clinical practice.

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