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RESEARCH ARTICLE

EFFICACY OF NAOCL GEL IN ADJUNCT TOMINIMAL INVASIVE NON-SURGICAL TREATMENT OF TYPE 2 DIABETIC PATIENTS WITH PERIODONTITIS

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Abstract

Background: Subgingival biofilm removal represent the most importa nt step in periodontitis treatment. However, its mechanical removal bec omes difficult and frequently incomplete in deep periodontal pockets.
Aim: To evaluate the clinical and microbiological effect of amino acid buffered sodium hypochlorite (NaOCl) gel application in minimally invasive non-surgical therapy for management of in type 2diabetic patients with periodontitis stage II grade B.
Methods: The study was carried out on a total number of forty subjects of both genders, with age ranged between 25 to 45 years old that divided into two equal groups. Group I (Diabetic group) and Group II (Non diabetic group).Subgingival plaque samples were collected at baseline, 6 and 12 weeks using sterile paper points and transported in thioglycolate broth. Samples were processed within 2 hours for quantitative culture on modified blood agar under anaerobic conditions, Colony-forming units (CFU) and the total bacterial count were calculated. Identification of P. gingivalis and P. intermedia was confirmed by species-specific PCR and gel electrophoresis (404 bp and 575 bp, respectively)

Results: Clinical parameters (GI),(PI), (CAL),(PPD) in study sides in groups I and II demonstrated a highly significant decrease following (SRP + application of sodium hypochlorite NaOCl gel) after 6 and 12 weeks in

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comparison to baseline values. Additionally, MINST+NaOCl compared to MINST alone,there were statistically significant differences between the right and left sides in terms of PPD and CAL (P=0.04 and P=0.04, respectively).

Conclusion: MINST and standard scaling and root planing (SRP) resulted in significant decreases in the number of sites with PD ≥ 5 mm, GI and other clinical parameters, as well as P. gingivalis, P. intermedia. Thus stay the most important step to successful periodontal therapy in patients with stage II grade B periodontitis.

Introduction:-

Diabetes mellitus is a metabolic disorder of multiple etiology. It is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes mellitus is a public health problem due to the rising number of overweight and obese individuals, and the current projection is over 640 million people with diabetes by 2040. [1]DM is a risk factor of periodontitis, but the relationship is bidirectional because periodontitis is not only an early sign of DM, but, also a risk factor of several systemic diseases, including (macro-vascular conditions) such as coronary heart disease, stroke and peripheral arterial disease, and (micro-vascular conditions),such as diabetic kidney disease, retinopathy, nephropathy and peripheral neuropathy. Another argument is that periodontal treatment has effects in reducing glycated hemoglobin in DM. [2]Periodontitis is a multi-factorial disease that initiated from an aberrant host response to subgingival anaerobic Gram-negative bacteria such as Porphyromonas gingivalis and Prevotella intermedia, and characterized by periodontal pocket formation, gingival recession, and loss of supporting tissues.[3]

Periodontitis affects approximately 10% of adults worldwide and is the sixth most common condition in the world in its severe form. [3]Severe periodontitis affects around 10% of adults worldwide and is the sixth most prevalent chronic condition.[3] Chronic periodontal infection may worsen systemic insulin resistance, and persistence of P. gingivalis, particularly type II fimbriae clones, has been associated with impaired glycemic control in diabetic patients.[4]Scaling and root planing (SRP) is a non-surgical periodontal therapy, aiming to remove subgingival biofilms, calculus, and bacterial endotoxins to promote periodontal healing. However, mechanical debridement alone may not fully eliminate pathogens, particularly in deep periodontal pockets.[5]Minimally invasive non-surgical therapy (MINST) has been proposed to address this limitation by using delicate ultrasonic tips, micro-instruments, and enhanced visualization to optimize debridement while minimizing tissue trauma.[6]The Aim of the study is to assess the clinical and microbiological effect of amino acid buffered sodium hypochlorite (NaOCl) gel application in minimally invasive non-surgical therapy for management of in type 2diabetic patients with periodontitis stage II grade B.

Patients and Methods:-

This study was conducted on a total number of forty subjects of both genders; ranged between 25–45 years, diagnosed with periodontitis stage II grade B according to the classification of the American Academy of Periodontology 2017. Participants were recruited from Oral Medicine and Periodontology Clinic, Faculty of Dentistry, Mansoura University.They were divided into two groups:

Group 1: Twenty diabetic patients (type II) who were clinically diagnosed as having periodontitisstage II grade B.

Group 2: Twenty non-diabetic patients (systemically healthy) diagnosed with periodontitis stage II grade B.

The study was approved by the Research Ethics Committee of Faculty of Dentistry, Mansoura University, By code number (A17020822). All the participant clearly understood the purpose of study and agreed to enroll in the study and informed consent were obtained from patient.

Inclusion Criteria:

Diabetic and non-diabetic patients diagnosed with periodontitis stage 2 grade B, patients between the age 25 and 45 years, Patients with10 teeth per arch, presence at least of two teeth with PD < 5 mm in both sides and Co-operative patients who were motivated for maintaining oral hygiene instructions.

Exclusion Criteria:

Patients were excluded if they were pregnant or lactating, smokers or tobacco chewers, had received periodontal therapy within the last 6 months, or had a history of prolonged antibiotic or anti-inflammatory therapy during the same period. Other systemic conditions influencing periodontitis progression or healing (except type 2 diabetes) were excluded, as with furcation involvement, acute abscesses, and third molars.

Split mouth design was used in this study. One side in both groups were treated with minimally invasive non-surgical treatment (MINST) alone and the other side in both groups was treated with MINST and NaOCl gel application.

Clinical procedure:
Baseline periodontal evaluation included PI, GI, PPD, and CAL .[7-9]MINST alone was performed in left side of the two groups. MINST with gel application was performed in right side of the two groups. MINST was performed with ultrasonic scalers and Gracey micro-curettes, followed by polishing. Patients rinsed twice daily with 0.12% chlorhexidine for 2 weeks; no systemic antibiotics were given. At test sites, after anesthesia, amino acid–buffered NaOCl gel was applied via syringe into pockets for 30 seconds without rinsing.

Microbiological investigations:
P. intermedia and P. gingivalis were isolated from clinical specimens using a quantitative culture method. Concurrently, colony identification was carried out based on culture characteristics and conventional PCR at baseline and again 6 and 12 weeks after treatment.

Sample collection:
[1] Subgingival plaque samples were collected at baseline, 6 weeks, and 12 weeks from two deep pocket sites per patient. Tooth was isolated with cotton rolls and dried with an air syringe to avoid contamination with saliva, and supragingival plaque was removed with a sterile curette to avoid contamination between supragingival and subgingival plaque. Sterile endodontic paper points were inserted into the pocket for 15 seconds.
[2] Samples were transferred to thioglycolate broth[10] and processed at the Microbiology Diagnostics and Infection Control Unit (MDICU), Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University.

Sample processing:
The collected samples were processed within two hours of collection to prevent alterations in microbial counts over time. For quantitative analysis, each specimen was immersed in 1 ml of sterile saline for 15 seconds and vortexed, followed by serial dilution in Mueller Hinton Broth (MHB). Aliquots from each dilution were plated on modified blood agar and incubated anaerobically at 37 °C for 72 hours. Colony-forming units (CFU) were counted, and the total bacterial count per sample was calculated by multiplying the colony count by the dilution factor.[11-13]

Identification of isolated colonies:
P. gingivalis and P. intermedia were identified through the detection of species-specific genes. Chromosomal DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, EU), and the purified DNA was stored at −80 °C until further analysis. For gene amplification, each PCR reaction contained 2 µl of DNA template in a final volume of 25 µl. The reaction mixture (23 µl) comprised 12.5 µl of 2× PCR Master Mix (i-Taq, iNtRON Biotechnology,EU), 1.5 µl of each forward and reverse primers (Table 1), and 7.5 µl of RNase-free water. [14-16]PCR amplification was performed with an initial denaturation at 94 °C for 3 minutes, followed by 36 cycles of denaturation at 94 °C for 45 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds. A final extension step was carried out at 72 °C for 10 minutes. The amplified products were analyzed by gel electrophoresis using a 100–1500 bp DNA ladder as a molecular marker. Identification of P. gingivalis was confirmed by the presence of a 404 bp band, whereas P. intermedia was verified by a 575 bp band.[17]

Table (1): Forward and reverse primers of P. gingivalis and P. intermedia genes.

Primer	Sequence (5/ - 3/)	Amplicon size
pgn-F	AGGCAGCTTGCCATACTGCG	404
pgn-R	ACTGTTAGCAACTACCGATGT	
pim-F	TTTGTTGGGGAGTAAAGCGGG	575
pim-R	TCAACATCTCTGTATCCTGCGT	

Microbiological sampling:
Subgingival plaque samples were collected at baseline, 6 weeks, and 12 weeks from two deep pocket sites per patient. Tooth was isolated with cotton rolls and dried with an air syringe to avoid contamination with saliva, and supragingival plaque was removed with a sterile curette to avoid contamination between supragingival and subgingival plaque.

Sterile endodontic paper points were inserted into the pocket for 15 seconds. Samples were transferred to thioglycolate broth and processed at the Department of Microbiology and Immunology, Faculty of Medicine, Mansoura University. Culturing was performed to detect P. gingivalis, P. intermedia, and total bacterial counts.
Following the sampling of plaque, any substantial calculus deposits, if they existed, were eliminated to prevent interference with the probe insertion. Subsequently, the PPD and CAL measurements were taken for all groups.

Statistical analysis and data interpretation:
Data were analyzed with SPSS v26. Qualitative data were presented as frequency/percentage, and quantitative data as mean ± SD or median (range) after Shapiro–Wilk testing. Between-group comparisons used t-test or Mann–Whitney U; intragroup analyses used Wilcoxon, Friedman, or repeated-measures ANOVA. Significance was set at $p < 0.05$.

Results:-

Forty patients were selected from the Out Patient Clinic, Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University. These participants were divided into two groups. Group Iincluded 20 diabetic patient's with periodontitis stage II gradeB. While Group II included 20 non-diabetic patient'swith periodontitis stage II grade B. A split mouth design was used in each group. The test side (right side) was treated scaling and root planingwith minimally invasive non-surgical treatment (MINST) and NaOCl gel application. The control side(left side) was treated scaling and root planingwith minimally invasive non-surgical treatment (MINST) alone.A sterile paper point size no.35 was inserted into pocket's base until resistance was felt and left in place for 30 seconds. Subsequently, these points were meticulously deposited into securely sealed sterile tubes designed for transporting samples to a specialized laboratory in order to measure Porphyromonas gingivalis, Provotella intermedia, and the total bacteria count.

(I)Demographic characteristicof the study groups:-
Group I (Diabetic group) included 11 males (55%) and 9 females(45 %) with mean age; 39.4±3.42. Group II (Non diabetic group) included 10 males (50%) and 10 females (50 %) with mean age 38.75±4.34. There were no statistical significant differences in age and sex between the studied groups, (P=0.602 & P=0.751, respectively).

(II) Periodontal assessment:
Regarding plaque index, there were no statistically significant differencebetween right and left side in groupI at three points (baseline, 6 weeks&12 weeks) (P=0.537, P=0.115& P=1.0, respectively). Also, there were no statistically significant differencebetween right and left side in group II (atbaseline, 6 weeks&12 weeks after treatment) (P=0.355, P=0.35& P=1.0, respectively). Among right side in diabetic group I plaque index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.55±0.21, 1.40±0.23 & 0.375±0.0, respectively).Pairwise comparison demonstratesstatistically significant difference between each of studied pairs (P value <0.001).Among left side in diabetic groupI Plaque index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.51±0.20, 1.52±0.26 & 0.375±0.0, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among right side in non-diabetic group IIplaque index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.51±0.20, 1.50±0.25 & 0.375±0.0, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).

Among left side for non-diabetic group IIplaque index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.57±0.20, 1.58±0.24 & 0.375±0.0, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).When comparing both right sides (test sides), plaque index mean values in between group I versus group II (the diabetic patients versus non-diabetic patients), the analysis revealed non-significant difference between all tested time periods (baseline, after 6 weeks and after 12 weeks of treatment). Similar results regarding both left sides (control sides) betweengroup I versus group II where non significant difference were also found regarding plaque index mean values at the three tested periods as illustrated in table (1) .Regarding gingival index, there were no statistically significant differencebetween right and left sides in groupI at three tested time points (baseline, 6 weeks&12 weeks after treatment) (P=0.216, P=0.119, &P=1.0).Also, there were no statistically significant differencebetween right and left sides in group IIat (baseline, 6 weeks&12 weeks after treatment) (P= 0.06, P= P=0.531& P= 1.0, respectively).

Among right side in groupIdiabetic patients gingival index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.28±0.04, 1.60±0.13 & 0.91±0.00, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Amongleft side in groupIdiabetic patients gingival index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.29±0.04, 1.66±0.12 & 0.91±0.0, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among right side in non-diabetic group II,gingival index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.28±0.04, 1.63±0.12 & 0.910±0.0, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among left side in non-diabetic group IIgingival index shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (2.31±0.04, 1.66±0.12 & 0.910±0.0, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001)

When comparing both right sides (test sides) gingival index mean values in betweengroup I versus group II (the diabetic patients versus non-diabetic patients), the analysis revealed non-significant difference between all tested time periods (baseline, after 6 weeks and after 12 weeks of treatment). Similar results regarding both left sides (control sides) between group I versus group II in the form of non significant were also found at the three tested periods as illustrated in table (2).Regarding periodontal probing depth, there were no statistically significant difference between right and left sides in groupI at three tested time points (baseline, 6 weeks&12 weeks after treatment) (P=0.235,P=0.435 &P=0.095). Also, there were no significant differencebetween right and left sides in group II at baseline, 6 weeks after treatment (P= 0.274, P= 0.112 respectively), but, there was statistically significant difference between right and left side of the same group II at 12 weeks (P=0.04).Among right side in diabetic group Ipatients,periodontal probing depth shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.35±0.27, 3.55±0.30 & 3.07±0.22, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).

Among left side in diabetic group I patients, periodontal probing depth shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.44±0.19, 3.63±0.34& 3.19±0.23, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among right side in non-diabetic group IIpatients, periodontal probing depth shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.31±0.31, 3.28±0.21 & 3.0±0.0,respectively). Pairwise comparisondemonstrates statistically significant difference between each of studied pairs (P value <0.001). Amongleft side in non-diabetic groupIIpatients, periodontal probing depth shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.40±0.23, 3.39±0.25 & 3.05±0.09, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).When comparing both right sidesin group Iversus group II regarding the PPD mean values , significant difference was only found at 6 weeks after treatment (Pa=0.002), meanwhile, regarding comparison of both left sides in group I versus group II, significant differences were found in PPD mean values at 6 weeks and at 12 weeks after treatment (Pb=0.02 &Pb=0.015), as shown in table (3).

Regarding clinical attachment loss, there were no statistically significant difference between right and left sides in groupI at three tested time points (baseline, 6 weeks&12 weeks) (P=0.235,P=0.435 &P=0.095).Also, there were no statistically significant differencebetween right and left sides in group II at baseline and 6 weeks after treatment (P= 0.274, P= 0.112, respectively), but there was statistically significant difference in the same group between right and left side at 12 weeks after treatment (P=0.04).Among right side in diabetic groupI CAL mean values show statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.35±0.27, 3.55±0.30 & 3.07±0.22, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among left side for diabetic group I, CAL mean values show statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.44±0.19, 3.63±0.34& 3.19±0.23, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among right side for non-diabetic group II CAL mean values show statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.31±0.31, 3.28±0.21 & 3.0±0.0, respectively).

Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among left side for non-diabetic group IICAL mean values show statistically significant decrease during

follow up from baseline to 6 weeks and to 12 weeks (4.40±0.23, 3.39±0.25 & 3.05±0.09, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value<0.001).When comparing both right sidesin group Iversus group II regarding the PPD mean values , significant difference was only found at 6 weeks after treatment (Pa=0.002), meanwhile, regarding comparison of both left sides in group Iversus group II, significant differences were found in PPD mean values at 6 weeks and at 12 weeks after treatment (Pb=0.02 &Pb=0.015), as shown in table (4).

(III) Microbiological assessment:

P. gingivalis:-

Regarding P. gingivaliscount, there were no statistically significant difference between right and left side in groupIat two time interval (baseline& 6 weeks) (P=0.725 &P=0.552). Also, there were no statistically significant difference in group II between right and left side at (baseline, 6 weeks) (P= 0.636, P= 0.481, respectively), but there was statistically significant difference between right and left side in each group I & group IIat 12 weeks (P=0.004 &P=0.02), respectively.Among right side in diabetic group Imedian P. gingivalis count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (9.5, 37.96 & 0.463, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Amongleft side in diabetic group I median P. gingivalis count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (9.37, 7.52 & 4.3, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).

Among right side in non-diabetic group II median P. gingivalis count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.8 , 3.85 & 0.927, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Amongleft side in non-diabetic group II median P. gingivalis count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (4.88, 4 & 1.93, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001). When comparing both right sidesin group I versus group II regarding the P. gingivalis count mean values, significant difference were found only at baseline and 6 weeks after treatment (Pa=0.001 &Pa=0.002), respectively. Additionally, regarding comparison of both left sides in group I versus group II, significant differences were found in the P. gingivalis count mean values, at baseline, 6 weeks and at 12 weeks after treatment(Pb=0.001), for all as shown in table (5) and figure (1 & 2).

P. Intermedia:-

Regarding P. intermedia count, there were no statistically significant difference between right and left side in groupI at two time points tested (baseline & 6 weeks) (P=0.808, & P=0.256,). At 12 weeks there was statistically significant difference between right and left side in group I (P=0.002). There were no statistically significant difference in group II between right and left side at (baseline, 6 weeks, and 12 weeks) (P=0.394, P=0.402 and P= 0.860 respectively).Among right side in diabetic group Imedian value of P. intermedia count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (13.97, 8.51 & 3.62, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Among left side for diabetic groupImedian value of P. intermedia count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (13.27, 9.93 & 6.03, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).

Among right side in non-diabetic group IImedian value of P. intermedia count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (7.65, 5.56 & 2.25, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).Amongleft side in non-diabetic group IImedian value of P. intermedia count shows statistically significant decrease during follow up from baseline to 6 weeks and to 12 weeks (6.12, 4.18 & 2.03, respectively). Pairwise comparison demonstrates statistically significant difference between each of studied pairs (P value <0.001).When comparing both right sidesin group I versus group II regarding theP. intermedias count mean values, highly significant difference were found at each tested time interval at baseline, 6 weeks and 12 weeks after treatment (Pa=0.001, (Pa=0.001 & Pa=0.001), respectively. Additionally, regarding comparison of both left sides in group I versus group II, significant differences were found in the P. Intermedia count mean values, at baseline, 6 weeks and at 12 weeks after treatment (Pb=0.001, Pb=0.001 & Pb=0.0001), respectively, as shown in table (6) and figure (3 &4).

Table (1): Intra-group and Inter-group comparison of plaque index of studied groups.

Plaque index	Diabetic group		Test of significance	Non diabetic group		Test of significance
	Right side	Left side		Right side	Left side	
Baseline	2.55±0.21	2.51±0.20	t=0.623 p=0.537	2.51±0.20	2.57±0.20	t=0.936 p=0.355 P^a =0.537 P= 0.355
6 weeks	1.40±0.23	1.52±0.26	t=1.61 p=0.115	1.50±0.25	1.58±0.24	t=0.946 p=0.350 P^a =0.206 P^b=0.531
12 weeks	0.375±0.0	0.375±0.0	t=0.0 p=1.0	0.375±0.0	0.375±0.0	t=0.0 p=1.0 P^a=1.0 P^b=1.0
Repeated Measures ANOVA test	F=2247.0 p=0.001*	F=2255.3 p=0.001*		F=225.37 P=0.001*	F=2311.7 P=0.001*	
Within group significance	p1<0.001* p2<0.001* p3<0.001*	p1<0.001* p2<0.001* p3<0.001*		p1<0.001* p2<0.001* p3<0.001*	p1<0.001* p2<0.001* p3<0.001*	

P : comparison between right and left side in each group.
P^a : comparison between diabetic versus non diabetic for right side.
P^b: comparison between diabetic versus non diabetic for left side
P^1: difference between baseline versus 6 weeks after treatment
P^2: difference between baseline versus 12 weeks
P^3: difference between 6 versus 12 weeks.
T: student test

Table (2): Intra-group and Inter-group comparison of gingival index of studied groups.

Gingival index	Diabetic group		Test of significance	Non diabetic group		Test of significance
	Right side	Left side		Right side	Left side	
Baseline	2.28±0.04	2.29±0.04	t=1.26 p=0.216	2.28±0.04	2.31±0.04	t=1.94 p=0.06 P^a=1.0 P^b=0.520
6 weeks	1.60±0.13	1.66±0.12	t=1.59 p=0.119	1.63±0.12	1.66±0.12	t=0.632 p=0.531 P^a=0.355

						P^b=1.0
12 weeks	0.91±0.0	0.91±0.0	t=0.0 p=1.0	0.910±0.0	0.910±0.0	t=0.0 p=1.0 P^a=1.0 P^b= 1.0
Repeated Measures ANOVA test	F=1602.78 p=0.001*	F=1890.67 p=0001*		F=1459 p=0.001*	F=1897 p=0.001*	
Within group significance	p1<0.001* p2<0.001* p3<0.001*	p1<0.001* p2<0.001* p3<0.001*		p1<0.001* p2<0.001* p3<0.001*	p1<0.001* p2<0.001* p3<0.001*	

P: comparison between right and left side in each group.
P^a: comparison between diabetic versus non diabetic for right side ,
P^b: comparison between diabetic versus non diabetic for left side.
P^1: difference between baseline versus 6 weeks after treatment ,
p^2: difference between baseline versus 12 weeks,
p^3: difference between 6 versus 12 weeks.
T: student test

Table (3): Intra-group and inter-group comparison of periodontal probing depth of studied groups.

PPD index	**Diabetic group**		**Test of significance**	**Non diabetic group**		**Test of significance**
	Right side	Left side		Right side	Left side	
Baseline	4.35±0.27	4.44±0.19	t=1.21 p=0.235	4.31±0.31	4.40±0.23	t=1.11 p=0.274 P a=0.626 Pb=0.557
6 weeks	3.55±0.30	3.63±0.34	t=0.789 p=0.435	3.28±0.21	3.39±0.25	t=1.63 p=0.112 P a=0.002* Pb=0.02*
12 weeks	3.07±0.22	3.19±0.23	t=1.71 p=0.095	3.0±0.0	3.05±0.09	t=2.13 p=0.04* P a=0.186 Pb=0.015*
Repeated Measures ANOVA test	F=316.02 P=0.001*	F=437.02 P=0.001*		F=365.72 P=0.001*	F=628.83 P=0.001*	
Within group significance	P1<0.001* P2<0.001* P3<0.001*	P1<0.001* P2<0.001* P3<0.001*		P1<0.001* P2<0.001* P3<0.001*	P1<0.001* P2<0.001* P3<0.001*	

P : comparison between right and left side in each group.
P^a: comparison between diabetic versus non diabetic for right side ,
p^b: comparison between diabetic versus non diabetic for left side
P1: difference between baseline versus 6 weeks after treatment ,

p2: difference between baseline versus 12 weeks,
p3: difference between 6 versus 12 weeks.
T: student test

Table (4): Intra-group and inter-group comparison of clinical attachment loss of studied groups.

CAL index	Diabetic group		Test of significance	Non diabetic group		Test of significance
	Right side	Left side		Right side	Left side	
Baseline	4.35±0.27	4.44±0.19	t=1.21 p=0.235	4.31±0.31	4.40±0.23	t=1.11 p=0.274 P a=0.626 Pb=0.557
6 weeks	3.55±0.30	3.63±0.34	t=0.789 p=0.435	3.28±0.21	3.39±0.25	t=1.63 p=0.112 P a=0.002* Pb=0.02*
12 weeks	3.07±0.22	3.18±0.22	t=1.71 p=0.095	3.0±0.0	3.05±0.09	t=2.13 p=0.04* P a=0.186 Pb=0.015*
Repeated Measures ANOVA test	F=149.10 P=0.001*	F=181.8 P=0.001*		F=191.69 P=0.001*	F=258.19 P=0.001*	
Within group significance	P1<0.001* P2<0.001* P3<0.001*	P1<0.001* P2<0.001* P3<0.001*		P1<0.001* P2<0.001* P3<0.001*	P1<0.001* P2<0.001* P3<0.001*	

P : comparison between right and left side in each group.
P [a] : comparison between diabetic versus non diabetic for right side ,
P[b]: comparison between diabetic versus non diabetic for left side
P[1]: difference between baseline versus 6 weeks after treatment ,
P[2]: difference between baseline versus 12 weeks,
P[3]: difference between 6 versus 12 weeks
T: student test

Table (5): Intra-group and Inter-group comparison of P. gingivalis of studied groups.

P. gingivalis	Diabetic group		Test of significance	Non diabetic group		Test of significance	Mann Whitney U test
	Right side	Left side		Right side	Left side		
Baseline	9.50 (5.5-13.17)	9.37 (6.17-13.07)	z=0.352 p=0.725	4.8 (3.1-7.1)	4.88 (2.9-6.63)	z=0.473 p=0.636	P [a]=0.001* P[b]=0.001*
6 weeks	7.96 (0.22-10.67)	7.52 (4.2-11.57)	z=0.595 p=0.552	3.85(1.77-5.63)	4(1.8-5.67)	z=0.704 p=0.481	P [a]=0.002* P[b]=0.001*
12 weeks	0.463 (0.18-10.07)	4.3 (0.27-8.43)	z=2.89 p=0.004*	0.927 (0.05-4)	1.93 (0.35-3.3)	z=2.30 p=0.02*	P [a]=0.181 P[b]=0.001*
Friedmann test	Fr=36.4 p=0.001*	Fr=40 p=0.001*		Fr=36.4 p=0.001*	Fr=40 p=0.001*		

Within group significance	p1<0.001* p2<0.001* p3<0.001*	p1<0.001* p2<0.001* p3<0.001*		p1<0.001* p2<0.001* p3<0.001*	p1<0.001* p2<0.001* p3<0.001*		

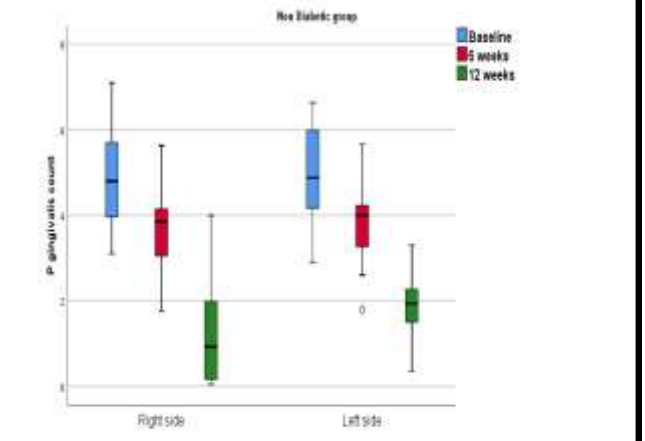

Figure (1 &2) P gingivalis count among right and left side among diabetic and non-diabetic group

Discussion:-

Periodontitis represents a chronic, multi-factorial inflammatory pathology affecting the supportive structures of the dentition and characterized by the progressive degradation of the periodontal attachment andalveolar boneending in loss of teeth.[18]Biofilm associated with plaque is acknowledged as a key causative factor in the development of periodontal disease, which subsequently undergoes a process of mineralization to form calculus, thereby establishing a micro-environment that favors the accumulation of non-calcified plaque, consequently offering optimal niches for the colonization and metabolic activities of microorganisms. The resultant tissue damage is fundamentally contingent upon the dynamic interaction between the virulent microbial factors & the host's immunological responses. [19, 20]DM is a recognized risk factor for systemic complications , including coronary heart disease, stroke, peripheral arterial disease, diabetic kidney disease, retinopathy, nephropathy and peripheral neuropathy.Also, Diabetes mellitus is a risk factor of periodontitis, but the diabetes mellitus – periodontitis interrelationship is bidirectional.[21]

DM increases the risk of periodontitis, by a presumably increase of salivary glucose levels, favouring bacterial growth in the gingival biofilm, and host immune cells function changes.[11]The pro-inflammatory response in periodontitis patients with DM could leads to the increased periodontitis susceptibility and progression. On the other direction, another argument is that periodontal treatment has effects in reducing glycated hemoglobin in Diabetes mellitus.[2]The aim of non-surgical treatment is to reduce bacterial load, control infection, resolve inflammation, and achieve pocket reduction.[22] The cornerstone of the therapy is the mechanical debridement including the mechanical removal of the supra- & subgingival biofilm and mineralized deposits, to facilitate the resolution of the inflammatory response and the restoration of the tissue's adhesion. [23]Significant evidence suggests that during supportive periodontal therapy (SPT), periodontitis can be effectively managed and treated through comprehensive mechanical plaque removal performed by the patient, in conjunction with supra- and subgingival debridement conducted by the therapist, with or without the application of local antimicrobials.[24]One potential method to enhance

the results of SRP could involve the use of sodium hypochlorite. Its extensive antimicrobial properties, rapid bactericidal effect, and safety at the concentration used for application have been recognized for many years.[25]
The main benefit of the sodium hypochlorite is whenthe activation of sodium hypochlorite with amino acids and its application to biofilm, even in difficult-to-access areas, modifies the matrix of the biofilm and aids in the mechanical removal of bacteria.[26]

A sodium hypochlorite (NaOCl) gel formulation was presented as a supplementary method for the mechanical removal of biofilm. Results from an in-vitro study demonstrated that the novel NaOCl gel exhibits antimicrobial properties, particularly against Gram-negative species linked to periodontitis. [26]Although the NaOCl gel did not succeed in eradicating a multi-species biofilm, it significantly reduced biofilm vitality and altered the composition of the biofilm matrix, indicating its considerable potential as an adjunct in the mechanical treatment of periodontal diseases.[27]The aim of this clinical and microbiological investigation was to assess the clinical impact of locally applied amino acid buffered sodium hypochlorite (NaOCl) gel in conjunction with a minimally invasive non-surgical therapy protocol for type 2 diabetic patients suffering from periodontitis stage II grade B. Additionally, the study sought to evaluate the antimicrobial efficacy of the amino acid buffered sodium hypochlorite (NaOCl) gel against periodontitis-related bacteria, particularly Porphyromonas gingivalis, Prevotella intermedia, and the overall bacterial count following periodontal treatment.In the current study, gender and age distribution of participants were comparable in the two groups. Their age ranged between twenty-five and forty-five years which are the common ages of periodontitis and type2 diabetes mellitus.

All participants from both groups were diagnosed with periodontitis stage II grade B, according to the 2017 Classification of Periodontal and Peri-Implant Diseases and Conditions, jointly presented by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP).A comprehensive medical history was collected from all participants involved in the study. Additionally, fasting blood glucose levels and glycosylated hemoglobin (Hb A1C) tests were conducted on all patients to ensure that diabetes was maintained at a controlled level for those with diabetes and at a normal level for non-diabetic individuals. According to the analysis of sample size calculation, this research was conducted on forty patients who remained until the conclusion of the study, with replacements made for those who did not complete their follow-up. Throughout this investigation, no adverse effects were reported, and patients did not experience any post-operative pain or discomfort.[28]Patients with known systemic conditions other than DM and those with uncontrolled diabetes mellitus were omitted from this research. Given that healing encompasses a multifaceted series of events characterized by cellular and molecular interactions among various subsets of cells, growth factors, and cytokines, any medical condition that disrupts one of these elements may hinder and adversely impact the healing process.[29]

Additionally, there are many known risk factors for periodontal disease, such as smoking. Smokers were excluded from the present study,[23] as smoking is a well-documented modifier of periodontal disease progression and healing, since it has adverse effects on vascularity, immune response, and fibroblast function.[30]Moreover, pregnant and lactating females were excluded. Pregnancy is a period characterized of physiological and physical disturbance, accompanied with increase in sex hormones which alter the gingival tissue condition and causing gingival inflammation even under reasonable oral hygiene control.[31]Consequently, to reduce the potential confounding effects, individuals who smoke, those with systemic illnesses, and pregnant women were excluded from this study.
There were no statistically significant differences regarding age and sex between diabetic and non-diabetic participants. This homogeneity in demographic characteristics at baseline is important to ensure that differences in treatment outcomes can be attributed to the intervention rather than demographic confounders.

A Split mouth design was used in the study. The left side in both groups was treated with minimally invasive non-surgical treatment (MINST) alone, and the right side in both groups was treated with MINST and NaOCl gel application. Split mouth design decreases the inter-subject variability by having both the study and control sides in the same condition, oral environment, oral hygiene and tissue response, thereby increasing the study's power. However split mouth design may increase the risk of antiseptic transfer from assigned quadrant to other. To address this issue in the current investigation, the concerned quadrants were isolated from one another. In this study, hand instruments with a thinner profile and/or longer shanks and ultrasonic tips were used to allow easier access into the depth of pockets and furcations [32] during MINST described by Nibali et al. [33] This approach emphasizes minimal tissue trauma, preservation of soft tissue architecture, and improved patient comfort while achieving effective biofilm removal, particularly in deep pockets and complex anatomical sites such as furcations.

In this current study, at baseline inter-group comparison revealed no statistically significant differences in all tested clinical parameters (PI, GI, PPD, and CAL) between the right and left sides among the two studied groups (group I and group II) ensuring comparable treatment outcomes during follow-up, and make the differences in treatment outcomes can be attributed to the intervention.Regarding plaque index, intra-group comparison in both left and right side showed significant improvement revealing a notable decrease 6 weeks and 12 weeks post-treatment and , while the inter-group comparison revealed no statistical significant difference. However, it is crucial to remember that these findings align with the observation that all participants in the study demonstrated a high standard of oral hygiene and underwent stringent periodontal maintenance, which included oral hygiene education and supragingival tooth cleaning conducted monthly throughout the entire duration of the 3-month study. This result coincide with Luca Ramaglia et al, results indicating a notable decrease in plaque index following the mechanical disruption and removal of the subgingival biofilm [34].

This result coincide with Lobene et al.[35], Iorio-Siciliano et al.[34]findings indicating a notable decrease in the gingival index following non-surgical periodontal treatment.Concerning gingival index, The assessment of the GI was intended to clinically evaluate the condition of the gingiva. In both groups, intra-group comparisonsof both left and right sides showed significant reduction in gingival index mean values revealing a notable decrease in gingival inflammation during follow up from baseline to 6 weeks and to 12 weeks. While, inter-group comparison between both groups revealed no statistically significant difference. These clinical results may be attributed to the effective impact of phase I therapy in reducing inflammation.[32]This result coincides with Luca Ramaglia and Lobene et al. results that reported significant reduction in gingival index after non-surgical periodontal therapy. [34-36] However, between the two groups, no statistically significant differences were found in terms of gingival index at baseline and at the 6 as well as at 12-weeks follow-up. [34-36]

All pockets in the present study displayed periodontal probing depths (PPD) ranging from 4 to 5 mm at baseline. A markedly significant decrease in PPD was noted across all groups (group I and group II) and on both treatment sides from baseline to the 6 and 12-week marks. Nevertheless, at the 12-week post-treatment interval in the non-diabetic cohort, a statistically significant enhancement was recorded on the NaOCl treated side (right side) compared to the control side (left side).The significant reduction in PPD when comparing the left side in both groups (diabetic & non-diabetic patients) noted after 6 & 12 weeks of treatment could be attributed to the successful therapeutic effect of the local debridement by SRP confirming that SRP still represent the gold standard in periodontitis therapy.

These results coincide with Ramanauskaite et al who conducted a comparison of clinical outcomes achieved through mechanical subgingival debridement combined with a sodium hypochlorite and amino acids gel versus mechanical debridement used in isolation. They reported that both treatment modalities led to statistically significant enhancements across all assessed clinical parameters. Furthermore, the additional subgingival application of sodium hypochlorite and amino acids during scaling and root planing (SRP) resulted in statistically significant improvements when compared to SRP performed alone.[37]Regarding CAL, a significant CAL gain was observed within all groups and on both treatment sides from baseline to 6 and to 12 weeks after treatment. However, at 12 weeks follow up in non-diabetic group, a statistically significant improvement in NaOCl treated side (right side) than control side (left side) was observed.

While to date, few work has been done to evaluate the clinical impact of amino acid buffered sodium hypochlorite (NaOCl) gel in patients with periodontitis and type 2 diabetes mellitus. However, a detailed examination of the findings indicates that the remarkable clinical results concerning probing pocket depth (PPD) and clinical attachment level (CAL) can be attributed to multiple factors, including the well-managed health condition of the diabetic patient and the implementation of MINST, which comprises scaling and root planing (SRP) along with consistent patient motivation to maintain good oral hygiene. This approach facilitates comprehensive biofilm removal from the root surfaces and periodontal pockets, thereby minimizing soft tissue inflammation and allowing the treatment to positively influence the periodontal healing process.[34]This result coincides with Ribeiro et al.,[38] and Nibali et al,[33]who evaluated the clinical effect of MINST for achieving satisfactory results in the clinical parameters after therapy of periodontal pockets. Also it coincides in non-diabetic (control) group withJurczyk et al,[26]and Schmidlin et al.,[36]who reported higher significant clinical improvement when NaOCl gel was applied. These clinical findings seem to corroborate the evidence regarding the antibacterial properties of this innovative NaOCl formulation, as well as its beneficial impact on the survival, adhesion, and proliferation of periodontal ligament cells.

The clinical response of diabetic patients to both treatments paralleled that of non-diabetic patients over 12 weeks. This suggests that with rigorous professional intervention and reinforced oral hygiene, favourable periodontal healing can be achieved despite the systemic challenges of diabetes. This aligns with the meta-analysis conducted by Engebretson & Kocher, which discovered that diabetic patients with good control can respond to non-surgical periodontal treatment similarly to non-diabetic individuals.[39]In the present study, we focused on P. gingivalis and P. intermedia to assess the effect of NaOCl gel combined with MINST on the healing of periodontal tissues in both diabetic and non-diabetic patients. Previous study indicated that P. gingivalis plays a dominate role and frequently presents at higher level in site exhibiting signs of active disease.[35]P. intermedia is usually the common cause of periodontal and endodontic infections. [26]The selection of these bacteria was founded on a significant correlation between their elevated presence in GCF and their influence on clinical indicators of periodontitis.[26]There was a great reduction in Porphyromonas gingivalis, Prevotella intermedia and total bacterial count in all groups and on both treatment sides from baseline to 6 weeks and to 12 weeks after treatment. These outcomes align with the findings of ElMobadder et al., who revealed a significant reduction of bacterial count whether the treatment was SRP alone or SRP+NaOCl+laser.[40]

At baseline, P. gingivalis counts were significantly higher in diabetic patients compared to non-diabetic individuals on both the right and left sides. This finding aligns with Salvi et al. who found that diabetic individuals had higher proportions of red-complex pathogens, including P. gingivalis, compared to systemically healthy controls.[41]Similarly, Takahashi et al.[42] and Campus et al.[43] demonstrated that hyperglycemia may favor colonization by P. gingivalis due to alterations in host immune function and gingival crevicular fluid composition, suggesting that diabetes mellitus creates a subgingival environment favoring the growth of pathogenic species such as P. gingivalis, likely due to impaired immune response, increased gingival crevicular fluid glucose content, and altered host–bacteria interactions.These findings didn't agree with Mirnić et al, [44] who demonstrated that the majority of diabetics and non-diabetic patients harboured P. gingivalis, T. forsythia and P. intermedia, with a similar prevalence of these pathogens across the groups.However, the intergroup differences persisted at 6 weeks, with diabetic patients maintaining higher counts. Interestingly, at 12 weeks, the right-side comparison between diabetics and non-diabetics was no longer statistically significant, while the left side remained significantly different. This may suggest that the microbiological benefits of therapy were achieved more slowly reflecting delayed response in diabetic patients, with partial microbial convergence at least on certain sites.

Similar to P. gingivalis, P. intermedia counts at baseline were significantly higher in diabetic patients than non-diabetics on both sides. While therapy led to significant intra-group reductions over time in both groups, intergroup differences persisted at all follow-up intervals. Even at 12 weeks, diabetic patients exhibited higher P. intermedia counts ($Pa = 0.001$, $Pb = 0.0001$) despite the overall microbial count reduction.The persistence of intergroup differences for P. intermedia longer than for P. gingivalis might be due to differences in ecological adaptability. P. intermedia is known for its ability to thrive under inflammatory conditions and may be more resistant to suppression in a hyperglycemic environment.This is supported by microbial profiling study showing higher abundances of Prevotella species in diabetic periodontitis patients, particularly in those with poorer glycemic control, suggesting enhanced growth potential in hyperglycemic environments.[45]Thus, the management of periodontal diseases emerges as a critical factor in slowing and controlling the progression of DM and improving the overall outcomes of this metabolic disease. Therefore, it would not only share in prevention of the occurrence of complications, but also it improves the quality of life of diabetic patients for whom successful easily achieved.

Conclusion:-

MINST, along with standard scaling and root planing (SRP), led to notable reductions in the number of sites exhibiting periodontal disease (PD) of 5 mm or greater, as well as improvements in gingival index (GI) and other clinical metrics. Consequently, these interventions remain the crucial components for effective periodontal treatment in patients diagnosed with periodontitis stage II grade B.MINST and standard SRP combined with sodium hypochlorite (NaOCl) gel treatment have a more prominent microbial shift effect on both diabetic and non-diabetic with periodontitis, lowering the pathogenicity of microbiota in the oral environment, but still there are no clear clinical and microbial difference when compare it with treatment by MINST and SRP alone.

Recommendation:-

We recommended that future research should include multi-center studies to validate our findings.

Conflict of interest: no conflicts of interest.

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