Original Article

Evaluation of Lipid Profile in Type 2 Diabetes Mellitus Patients after Nonsurgical Periodontal Therapy

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ABSTRACT

Background: Improvement in systemic / haematological components has been reported after non-surgical periodontal therapy (NSPT). Few studies have reported reduction in total cholesterol following intensive periodontal therapy consisting of SRP with adjunctive local delivery of minocycline-HCl after 2 months. Present study aimed to determine the effect of NSPT on lipid profile of type 2 diabetes mellitus with chronic periodontitis (T2DM-CP) patients

Objective: To determine the effect of non-surgical periodontal therapy (NSPT) on lipid profile of type 2 diabetes mellitus patients with chronic periodontitis (T2DM-CP).

Materials and Methods: Forty-five subjects were divided into; experimental group I (T2DM-CP): which include chronic periodontitis (Stage II or III or IV/ Grade B or C periodontitis) with type 2 diabetes mellitus and random blood sugar ≥ 140 mg/dl), and control group II (NDM-CP): which include chronic periodontitis (Stage II or III or IV/ Grade A periodontitis) without type 2 diabetes mellitus and random blood sugar ≤ 140) for the study. All subjects underwent complete scaling root planing (SRP) and subgingival debridement. Systemic parameters [Glycemic control (HbA1c) and lipid profile [total cholesterol (TC), total triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL) and TC/ HDL] were measured at baseline, 1 month and 4 months after NSPT.

Results: Thirty eight (19 in each group) subjects completed the study uneventfully. Both at 1 and 4 months, mean RBS and HbA_{1c} levels in experimental group I (T2DM-CP) were significantly higher as compared to that in control group II (NDM-CP) ($p<0.05$). For other parameters, no statistically significant difference was observed between two groups ($p>0.05$).

Conclusion: NSPT results in significant improvement in RBS, glycemic level (HbA1c) and lipid profile in both T2DM-CP (experimental group-I) and NDM-CP (control group-II). The improvement was more in experimental group-I (T2DM-CP) as compared to control group-II (NDM-CP) after NSPT.

Keywords: Chronic periodontitis, scaling root planing, type 2 diabetes mellitus

INTRODUCTION

Periodontitis, a chronic inflammatory periodontal disease, results in progressive destruction of tooth-supporting tissues and the formation of periodontal pockets between the tooth and surrounding gingival tissues.[1] It is characterised by a non-resolving inflammation, generated in response to virulence factors elaborated by periodontal pathogens, and which is ineffective at controlling the infection.[2] The inflammatory and immune responses in periodontitis are a continuum of the normal host response to gram-negative infection that eventually becomes the pathology when homeostasis is lost.[3] Exaggerated inflammatory/ immune response to the microbial plaque that accumulates around gingival margin occurs in a predisposed group of population resulted in inadvertent or collateral host tissue damage.[4]

Periodontitis is the sixth complication of diabetes, and its incidence and severity have been reported to be doubled in type 2 diabetic patients,[5] and presence of periodontitis may

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exert a converse, negative impact upon cardio-metabolic risk status in type 2 diabetes patients.[1] Current paradigm has revealed that periodontitis may produce any number of alterations in systemic health, and significant associations between periodontitis and acute cerebral infarction/ stroke, failure of joint/ organ transplant, kidney dialysis, coronary heart disease, preterm low birth weight, aspiration pneumonia and diabetes, is now an emerging concept.[6]

Recent investigations have indicated that serum lipid profile may also be associated with inflammatory host response.[7] Further, significant associations between periodontitis and increased serum cholesterol have been reported by many cross-sectional studies.[8,9] Biologically plausible effect of periodontal infections on the metabolic state of an individual has been validated through series of investigations.[9]

Improvement in systemic / haematological components has been reported after non-surgical periodontal therapy (NSPT).[10,11] Although few studies have reported reduction in total cholesterol following intensive periodontal therapy consisting of SRP with adjunctive local delivery of minocycline-HCl after 2 months.[9] Present study aimed to determine the effect of NSPT on lipid profile of type 2 diabetes mellitus with chronic periodontitis (T2DM-CP) patients.

MATERIALS AND METHODS

Study Design: Present study was conducted in the Department of Periodontology, Saraswati Dental College and Hospital, Lucknow, India. This was secondary analysis of the data which was primarily collected to evaluate the effects of impact of scaling and root planing (SRP) on the C-reactive protein (CRP) levels of gingival crevicular fluid (GCF) and serum in chronic periodontitis patients with type 2 diabetes mellitus (T2DM-CP) or without type 2 diabetes mellitus (NDM-CP).[11] Study protocol was approved by the institutional ethical committee and all the participants were explained about the study protocol, and gave written informed consent before entering the study. Forty-five subjects (23 males and 22 females, between 30 to 65 years) with generalised chronic periodontitis (Stage II or III or IV/ Grade A or B or C) agreed to participate voluntarily.

Non-alcoholic, non-smoker subjects with having at least 20 remaining teeth in the oral cavity, probing pocket depth (PPD) of 5 mm or more and clinical attachment level (CAL) of 3 mm or more, and demonstrated radiographic bone loss, at more than 30% of the sites involved, were included for the present study. Patients with a history of any systemic disease other than type-2 diabetes mellitus (T2DM); on any medication other than hypoglycaemic agents; undergone periodontal treatment over the preceding 6 month and needing prophylactic antibiotic in association with periodontal probing or having tissue necrosis and trauma were excluded. Careful anamneses and records were analysed to retrieve the data regarding diabetic state and general health status of the patients. Only those T2DM patients who were treated by either dietary intervention or oral hypoglycaemic agents were included for the study. For subjects with T2DM, no change in the oral hypoglycaemic medications or physical exercise of the patients was encouraged during the course of the study.

Patients fulfilling the selection criteria were initially screened for vitals (blood pressure, temperature, respiratory rate and pulse rate), and random blood sugar (RBS) using automated cell counter (Cell-Tech Junior Hematology Analyzer, Logotech Pvt. Ltd., F.I.E, Patparganj, Delhi, India). Based on initial screening**,** subjects were divided into; experimental group I (T2DM-CP): which include chronic periodontitis (currently, Stage II or III or IV/ Grade B or C periodontitis) with type 2 diabetes mellitus and random blood sugar ≥ 140 mg/dl, and control group II (NDM-CP): which include chronic periodontitis (currently, Stage II or III or IV/ Grade A periodontitis) without type 2 diabetes mellitus (and random blood sugar ≤ 140) for the study.

Sample Collection: To evaluate systemic parameters [Glycemic control (HbA1c) and Lipid profile], blood samples were obtained from venous puncture of anticubital vein. Verbal confirmation of fasting status was obtained from participants before clinical sample collection. A tourniquet was tied around the arm of the patient from which the blood had to be drawn. Two

venous blood samples (5 ml total volume) were collected from the anticubital vein by venipuncture, using a 20-gauge needle with a 5-ml syringe.[12] One blood sample (3ml volume) was allowed to clot for 1 hour at room temperature and then centrifuged for 10 min, and serum was extracted. This serum sample was placed in plastic vials and sent to the laboratory for the estimation of lipid profile. Other blood sample (2 ml volume) was placed in plastic vial containing EDTA and was sent to the laboratory for the estimation of HbA1c.[13] Collected serum and blood samples were immediately transferred to airtight plastic vials and was placed in a thermocol box containing reusable gel refrigerants and was transported to the laboratory and stored at -20^{0}C until further processing.

Clinical Procedure: At baseline, plaque index (PI) by Silness and Loe,[14] and gingival index (GI) by Loe and Silness[15] were recorded. Subsequently, all forty-five patients were recruited for NSPT within 6 hours.[10] Complete saling root planing (SRP) and subgingival debridement were performed within 6 hours using magnetostrictive ultrasonic scalers and tips No.TFI 3 and 10 (Cavitron, Dentsply), and Gracey curettes (Hu-Fridey Instruments, Chicago, IL, USA). Local anesthesia in the form of infiltration and spray were used as and when required.

Clinical measurements, probing pocket depth (PPD) and clinical attachment level (CAL), were made by the same investigator. Examination included duplicate measurements using a UNC-PCP 15 (Hu-Fridey) probe; measurements were rounded to the nearest millimetres. Patients were given oral hygiene instructions and were prescribed 0.2% chlorhexidine mouthwash for 2 weeks. Patients were recalled at 1 month and 4 months. All clinical (PI, GI, PPD and CAL) parameters were recorded and blood samples were collected and sent to the laboratory for the estimation of HbA1c and lipid profile at subsequent visits.

Estimation of HbA1c: Nephlometry method (Agappe Diagnostics, Ernakulum, Kerala, India) was used for HbA1c estimation that utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total haemoglobin and HbA1c have the same nonspecific absorption rate to latex particle. When mouse antihuman HbA1c monoclonal antibodies is added (R2), latex HbA1c- mouse antihuman HbA1cantibody complex is formed. Agglutination occurs when goat antimouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed onto the surface of latex particles. Reagents and their composition used were: HbA1c R1**,** contains Latex (0.13 w/v) and glycine buffer; HbA1c R2, contains glycine buffer, mouse anti-human HbA1c, monoclonal antibody, goat anti-mouse IgG, polyclonal antibody and stabilizers; and HbA1c R3, contains haemolysis reagent.

The test procedure and the calibration data is provided in the smart card along with the kit. Smart card is inserted to card reader slot and display will prompt to add R1 and sample. Pipette 180µl R1 and 5µl sample to cuvette and place the cuvette into cuvette holder. After incubation display will prompt to add R2. Pipette 60µl R2 using attached sensor pipette to the cuvette. The results will show in the display and take print out.[16]

Estimation of Lipid Profile: Total cholesterol (TC), total triglyceride (TG), high density lipoprotein (HDL), were measured using blood sample collected from each subject after a minimum of eight hour fasting period. Serum triglyceride (TG) and cholesterol (TC) levels were measured by Trinder method-endpoint using triglyceride and cholesterol kit (FAR Sri, Verona, Italy). Serum HDL levels were measured by Accelerator Selective Detergent method using Direct HDL-Cholesterol test kit (Span Diagnostics Ltd, Surat, India). Low density lipoprotein (LDL) was calculated using the Friedewald formula (LDL-C = Total cholesterol – (TG/5 + HDL), and very low density lipoprotein (VLDL) was calculated using the formula:[12] VLDL-C = Triglyceride (TG)/5

Statistical Analysis: Data was analyzed using statistical package for social sciences (SPSS) version 15.0. The sample size for each group was less than 30; hence distributions were assessed for normality (assessment of normality). Between

group comparisons were made using Mann Whitney U test while within group change was studied using Wilcoxon signed rank test. The confidence level of the study was kept at 95%, hence a "p" value less than 0.05 indicated statistically significant association.

RESULTS

A total of 45 patients in the age group of 30 to 65 years initially enrolled for the study. Out of these, only 38 patients (n=19 per group, 23 males and 15 females) completed the study uneventfully, and seven female subjects were excluded after baseline procedures (4 subjects did not report after 1 month and 3 subjects never came back after baseline). Majority of subjects, irrespective of the group were males. Experimental (T2DM-CP) group subjects had significantly lower PI, and significantly higher CAL values as compared to control group ($p<0.05$). For other parameters, statistically no significant difference was observed between two groups ($p>0.05$). With respect to anthropometric and vital parameters, except for systolic BP which was found to be significantly higher in experimental group (T2DM-CP) as compared to control group (NDM-CP) ($p=0.020$), for none of the other parameters, a significant difference was observed between two groups ($p>0.05$) (Table 1).

Table 1: Anthropometric and periodontal characteristics of subjects with Type-2 Diabetes Mellitus Chronic Periodontitis (T2DM-CP) and Non Diabetes Mellitus Chronic Periodontitis (NDM-CP)

Parameter	T2DM-CP (n=19)	NDM-CP (n=19)	Significance of difference	
	Mean±SD	Mean±SD	z	p*
Age (years)	45.74±10.28	36.89±6.38	3.043	0.002
Gender (Male: Female)	10 (52.6%): 9(47.4%)	13 (68.4%): 6 (31.6%)	χ^2=0.991; p=0.319 (NS)	
Weight (kg)	63.68±9.67	63.74±6.55	0.161	0.885
Height(m)	1.55±0.14	1.60±0.03	1.351	0.191
Body mass index (BMI)(kg/m$^{2)}$	28.04±7.26	24.88±2.53	1.315	0.191
Systolic BP(mmHg)	129.53±7.89	124.74±3.84	2.360	**0.020**
Diastolic BP(mmHg)	80.26±3.98	80.95±1.68	0.247	0.817
Temperature (^{0}F)	97.94±0.28	97.98±0.34	0.330	0.751
Pulse Rate (beats/min)	84.79±4.32	85.79±2.02	1.056	0.311
Respiratory Rate (breath/min)	16.16±0.69	16.37±0.60	0.934	0.435
Plaque index (PI)	1.68±0.19	1.82±0.13	2.428	**0.014**
Gingival index (GI)	1.71±0.20	1.81±0.12	1.653	0.103
Probing pocket depth PPD (mm)	4.79±0.37	4.59±0.38	1.621	0.109
Clinical attachment level (CAL)(mm)	4.18±0.54	3.53±0.31	3.479	**<0.001**

***Mann-Whitney U test**

Table 2: Mean Values of glycemic and Lipid parameters in Chronic Periodontitis with Type-2 Diabetes Mellitus (T2DM-CP) or without Diabetes (NDM-CP)

Time intervals	Parameters	T2DM-CP	NDM-CP	z	p*
Baseline	Random Blood Sugar (mg/dl)	258.92±58.07	98.97±3.17	5.322	<0.001
	Glycated Haemoglobin HbA_{1c} (%)	7.98±1.28	5.56±0.47	4.803	<0.001
	Total Cholesterol [TC] (mg/dl)	201.08±45.32	178.92±39.02	1.679	0.096
	Serum triglyceride [TG] (mg/dl)	162.21±86.24	156.49±60.35	0.277	0.795
	High Density Lipoprotein [HDL] (mg/dl)	37.50±4.82	40.94±3.76	2.430	0.014
	Low density lipoprotein [LDL] (mg/dl)	127.75±38.46	106.68±30.22	1.693	0.091
	Very low density lipoprotein [VLDL] (mg/dl)	32.44±17.25	31.30±12.07	0.277	0.795
	Total Cholesterol/High Density Lipoprotein [TC/HDL]	5.31±1.88	4.37±1.15	1.752	0.080
1 month	Random Blood Sugar (mg/dl)	215.41±52.54	99.32±4.43	5.290	<0.001
	Glycated Haemoglobin HbA_{1c} (%)	7.56±1.35	5.52±0.40	4.225	<0.001
	Total Cholesterol [TC] (mg/dl)	185.43±35.76	184.19±41.29	0.292	0.773
	Serum triglyceride [TG] (mg/dl)	143.25±73.21	162.99±50.80	1.811	0.070
	High Density Lipoprotein [HDL] (mg/dl)	39.38±4.30	39.63±2.09	0.917	0.370
	Low density lipoprotein [LDL] (mg/dl)	113.63±27.50	111.97±35.02	0.511	0.624
	Very low density lipoprotein [VLDL] (mg/dl)	29.41±14.98	32.60±10.16	1.533	0.130
	Total Cholesterol/High Density Lipoprotein [TC/HDL]	4.74±1.41	4.67±1.12	0.321	0.751
3 months	Random Blood Sugar (mg/dl)	181.68±30.52	98.42±3.95	5.279	<0.001
	Glycated Haemoglobin HbA_{1c} (%)	7.29±1.03	5.35±0.41	4.886	<0.001
	Total Cholesterol [TC] (mg/dl)	178.42±31.61	167.26±26.62	1.316	0.191
	Serum triglyceride [TG] (mg/dl)	142.96±83.83	144.32±47.40	0.964	0.339
	High Density Lipoprotein [HDL] (mg/dl)	41.53±4.25	43.16±2.71	1.493	0.146
	Low density lipoprotein [LDL] (mg/dl)	104.20±23.43	97.45±19.91	0.993	0.325
	Very low density lipoprotein [VLDL] (mg/dl)	28.59±16.77	28.86±9.48	0.964	0.339
	Total Cholesterol/High Density Lipoprotein [TC/HDL]	4.37±1.06	3.88±0.67	1.563	0.123

Table 3: Mean value changes in glycaemic and lipid parametersT2DM-CP at different time intervals.

Markers	Between Baseline and 1 month			Between Baseline and 4 months			Between 1 month and 4 months		
	Mean±SD	z	p**	Mean±SD	z	p**	Mean±SD	z	p**
RBS (mg/dl)	-43.52±65.34	-3.061	0.002	-77.24±71.21	-3.099	0.002	-33.72±40.85	-3.563	<0.001
HbA_{1c} (%)	-0.42±0.71	-2.234	0.026	-0.68±0.79	-3.018	0.003	-0.26±0.61	-1.751	0.080
TC (mg/dl)	-15.66±22.59	-3.060	0.002	-22.66±26.83	-3.825	<0.001	-7.01±9.62	-2.705	0.007
TG (mg/dl)	-18.96±32.89	-2.484	0.013	-19.25±33.62	-3.462	<0.001	-0.29±21.19	-1.631	0.103
HDL (mg/dl)	1.88±2.84	-2.379	0.017	4.02±3.71	-3.268	0.001	2.15± 1.64	-3.481	<0.001
LDL (mg/dl)	-14.12±20.86	-2.817	0.005	-23.56±23.02	-3.823	<0.001	-9.43±9.21	-3.261	0.001
VLDL (mg/dl)	-3.03±5.58	-2.484	0.013	-3.85±6.72	-3.462	<0.001	-0.82±5.93	-1.712	0.087
TC/HDL	-0.57±0.89	-2.435	0.015	-0.94±1.16	-2.918	0.004	-0.37±0.61	-3.019	0.003

*** Wilcoxon signed rank test**

Table 4: Mean value changes in glycaemic and lipid parameters (NDM-CP) at different time intervals

Markers	Between Baseline and 1 month			Between Baseline and 4 months			Between 1 month and 4 months		
	Mean±SD	z	p**	Mean±SD	z	p**	Mean±SD	z	p**
RBS (mg/dl)	0.35±2.67	-0.844	0.399	-0.55±3.74	-0.784	0.433	-0.89±3.11	-1.236	0.216
HbA_{1c} (%)	-0.05±0.36	-0.342	0.732	-0.21±0.28	-2.810	0.005	-0.16±0.20	-2.902	0.004
TC (mg/dl)	5.28±28.18	-1.330	0.184	-11.65±15.58	-3.659	<0.001	-16.93±27.89	-3.423	<0.001
TG (mg/dl)	6.50±34.95	-1.268	0.205	-12.17±16.37	-3.824	<0.001	-18.67±30.09	-3.829	<0.001
HDL (mg/dl)	-1.31±2.91	-2.077	0.038	2.22±2.49	-3.101	0.002	3.53±2.12	-3.752	<0.001
LDL (mg/dl)	5.29±23.16	-1.328	0.184	-9.23±18.44	-3.099	0.002	-14.52±26.40	-2.958	0.003
VLDL (mg/dl)	1.30±6.99	-1.268	0.205	-2.43±3.27	-3.824	<0.001	-3.73±6.02	-3.829	<0.001
TC/HDL	0.30±0.90	-1.650	0.099	-0.49±0.61	-3.260	0.001	-0.79±0.82	-3.824	<0.001

*** Wilcoxon signed rank test**

Intergroup comparison: At baseline, mean RBS and HbA_{1c} levels in experimental group-I (T2DM-CP) were significantly higher and mean HDL levels were significantly lower as compared to that in control (NDM—CP) group ($p<0.05$). For other parameters, no statistically significant difference was observed between two groups ($p>0.05$). Both at 1 and 4 months, mean RBS and HbA_{1c} levels in experimental group I (T2DM-CP) were significantly higher as compared to that in group II(NDM-CP) ($p<0.05$). For other parameters, no statistically significant difference was observed between two groups ($p>0.05$) (Table 2).

Intra-group comparison: A significant decrease in all the parameters was observed between baseline and 1 month follow up ($p<0.05$) in experimental group-I (T2DM-CP). For all the parameters, a significant change was observed between baseline to 4 months ($p<0.05$). In all the parameters except HDL, decrease was observed. For HDL, an increase was observed between baseline and 4 months. A decrease in all the parameters except HDL was observed, during 1 to 4 months period. For HDL, an increase in mean levels was observed during the period. All the changes except change in HbA_{1c}, TG and VLDL were statistically significant too (Table 3).

Statistically no significant change in any of the parameters except for a significant decrease in HDL level was observed between baseline and 1 month interval in control group (NDM-CP). For all the parameters except RBS and HDL, a significant decrease was observed during the period ($p<0.05$). For RBS though a decrease was observed yet it was not significant statistically ($p=0.433$). For HDL a significant increase during the period was observed ($p=0.002$). A significant decrease in all the parameters except RBS and HDL was observed ($p<0.05$) between 1 and 4 months in control group. For RBS though a decrease was observed but it was not significant statistically ($p=0.216$). For HDL, a significant increase was observed ($p<0.001$) (Table 4).

DISCUSSION

Primarily this study was performed to evaluate the effects of NSPT on the lipid profile of T2DM-CP patients. Our data informed that NSPT results in significant improvement in periodontal health parameters (PI, GI, PPD and CAL), glycemic level (HbA1c) and lipid profile (TC, TG, HDL, LDL, VLDL and TC/HDL) in both T2DM-CP (experimental group-I) and NDM-CP (control group-II) after 4 months. There was non-significant increase in RBS, TC, TG, LDL, VLDL and TC/HDL, and significant decrease in

HDL in control group I (NDM-CP) at 1 month. First time our results clued-up that the mean improvement in periodontal health parameters, HbA1c and lipid profile (TC, TG, HDL, LDL,VLDL, TC/HDL) were more in experimental group-I (T2DM-CP) as compared to control group-II (NDM-CP) after NSPT.

Positive association between periodontal pocket with higher cholesterol and LDL- cholesterol blood levels in men have been reported.[8] Also, available literature has reported that patients with type2 diabetes mellitus (T2DM) and chronic periodontitis exhibit clinical improvement after NSPT. D'Auto *et al.*[9] reported significant improvement in CRP levels and insignificant lipid marker changes after standard periodontal therapy in chronic periodontitis patients.

While comparing anthropometric and vitals between experimental group I (T2DM-CP) and control group II (NDM-CP) at baseline, non-significant difference was observed in weight, height, body mass index (BMI), diastolic BP, temperature (Temp), pulse rate (PR) and between the two groups, except for systolic BP which was found to be significantly higher in experimental group I (T2DM-CP) as compared to control group II (NDM-CP). Kamath *et al.*,[17] stated that association of diabetes with central obesity is stronger than the association with general fat. Waist circumference and waist/hip ratio have been used as a measure of general obesity. Studies have indicated that central obesity might be more important in the Indian population. Central obesity has been associated with decreased glucose tolerance, alterations in glucose insulin homeostasis, reduced metabolic clearance of insulin and decreased insulin stimulated glucose disposal. In contrast, present study evaluated BMI using formula that include general obesity instead of central obesity.[17] Studies have also reported that the prevalence of hypertension is twice in diabetic patients as compared to non diabetic patients.[18] Concurrent to present study, D'Aiuto *et al*.[19] addressed the relationship between blood pressure (BP) and periodontal disease and stated that a decrease in systolic BP in 2 months that correlated with the degree of reduction in gingival bleeding, a sensitive clinical marker of periodontal inflammation and infection. Studies have revealed that BMI increases with age, and BMI of diabetic subjects is positively correlated with age more than that in non-diabetic subjects.[20] Ramachandran *et al.,*[21] showed that diabetes has a positive and independent association with age and BMI.

Mean values for PI was significantly higher in control group II (NDM-CP) as compared to experimental group I (T2DM-CP) indicating that in spite of having poor plaque control habits, control group II (NDM-CP) patients showed less PPD and CAL, as compared to experimental group I (T2DM-CP). This further indicates exaggerated destructive response to dental plaque microorganisms in chronic periodontitis patients with T2DM.[1,22] In accordance with present study, Kardesler *et al*.,[23] also demonstrated that after NSPT there is significant reduction in periodontal health parameters (PI, GI, PPD) at 1 and 3 months. CAL levels were significantly decreased at 1 month compared to the baseline values in the well-controlled diabetic patients while in poorly controlled diabetic patients, there was a slight but non-significant decrease in CAL measurements at 3 months. Koromantzos *et al.*,[24] demonstrated improvement in periodontal health parameters (BOP, GI, PPD, CAL) after 6 months of NSPT in the T2DM patients with moderate to severe periodontal disease. Navarro-Sanchez *et al.*,[25] stated that both diabetic and non-diabetic subjects showed a significant improvement in their periodontal status by the end of the study, confirming the widely documented clinical improvement in periodontal status after non-surgical treatment. They further stated that diabetic and non-diabetic subjects do not differ in periodontal healing over the short term after non-surgical periodontal treatment. In their study design, any potential difference in periodontal healing between the groups could be attributable to baseline differences in levels of disease, because the periodontal examination at the first visit showed a significantly higher percentage of periodontal pocket and deeper probing depth in the diabetic patients than in the non-diabetic controls.[25] However, in the present interventional trial it was observed that in spite of maintaining good plaque control during the study in experimental group-I (T2DM-CP), there was more reduction in periodontal destructive

changes in control group II (NDM-CP) as compared to experimental group I (T2DM-CP) that signifies exaggerated destructive changes in diabetic patients, and the cumulative effects of altered cellular response to local factors, impaired tissue integrity, and altered collagen metabolism undoubtedly play a significant role in the susceptibility of diabetic patients to infections and destructive periodontal disease.[26]

There was gradual decrease in mean RBS and HbA1c values of experimental group I (T2DM-CP) from baseline to 4 months, whereas control group II (NDM-CP) showed increase in mean RBS value at 1 month and then decrease after 4 months, but gradual decrease in HbA1c values from baseline to 4 months. The fasting glucose and casual glucose tests provide "snapshots" of the blood glucose concentration at the time the blood was drawn; HbA1c assay has been shown by a large interventional study to provide an accurate measure of the average blood glucose concentrations over the preceding 2 to 3 months.[27] Allen *et al*.[1] in comparative cross-sectional study reported higher values for blood glucose level and glycated haemoglobin levels in T2DM with chronic periodontitis patients as compared to non-diabetic periodontitis patients, which is similar to our study. In accordance with Navaro-Sanchez *et al*.,[25] O'Connell *et al*.,[28] Kiran *et al*.,[29] and Montoya- Carralero *et al*.,[30] in present study also periodontal treatment was accompanied by a significant reduction in HbA1c levels in the T2DM subjects at 4 months.

The mean values for lipid profile were significantly higher for all in experimental group -I (T2DM-CP), except for HDL which was recorded higher in control group-II (NDM-CP). Mean decrease in lipid profile (TC, TG, LDL, VLDL and TC/HDL) of experimental group-I (T2DM-CP) was higher as compared to control group-II (NDM-CP) from baseline to 1 month, and from baseline to 4 months, however, HDL value increased significantly in experimental group-I (T2DM-CP) as compared to control group II (NDM-CP), both at 1 month and 4 months. Reddy *et al*.,[31] compared anthropometric variables and lipid profile in diabetic and non-diabetic patients and stated that diabetic patients had higher TG and VLDL and lower HDL values as compared to non-diabetic patients, and attributed this variation between two groups to diabetic dyslipidemia which is in accordance with the present study. Kandula and Shegokar,[32] stated that in diabetic patients there is significant elevation of TC, TG and LDL. HDL was significantly lower in diabetic group as compared to non-diabetic group. In contrast to the present study, Allen *et al.*,[1] in a cross-sectional study reported lower levels of total and LDL cholesterol in diabetic group with periodontitis as compared with non-diabetic group with periodontitis, and they attributed this reduction to the statin medications taken exclusively by the diabetic groups. In present study, any patient who was taking any medication other than oral hypoglycaemic drugs was excluded, and this may have resulted in higher baseline values. Further, lowest HDL and highest triglyceride levels in type 2 diabetic patients with periodontitis were observed as compared to non-diabetes periodontitis patient levels,[1] which is in accordance with present study. Statins have modest effects on HDL and triglyceride levels, and they suggested that statin medication did not completely negate the possible dyslipidaemic effect of periodontal inflammation in the type2 diabetic patients.[1]

Similar to the present study, two randomised controlled trials (RCTs)[10,19] examined a sample of otherwise healthy individuals affected by severe generalized periodontitis and showed that NSPT caused reductions in TC and LDL levels at 2 and 6 months follow- up. In another RCT, Oz *et al*.[33] performed periodontal treatment in 50 individuals also suffering from hypercholesterolemia, and evaluated their serum lipid concentrations 3 months after the treatment. There was a substantial decline in TC and LDL profiles in the treatment group. In contrast, other authors have reported contradictory results to the present study.[34-36]

Another important finding in the present study was higher TC/HDL in experimental group as compared to control group representing higher insulin resistance in experimental group as compared to control group. This is in accordance with the study by Jeppesen *et al.*,[37] who reported that individuals with high ratio of TC/HDL are insulin resistant.. Periodontal disease is considered a predisposing factor for the

development of insulin resistance in type 2 diabetes mellitus (T2DM) because of the release of inflammatory mediators (TNF-α and IL-6). Besides acting locally, these mediators diffuse to the systemic circulation. TNF-α alters the insulin signal by reducing the tyrosine phosphorylation of the insulin receptor and its substrates. Moreover, TNF-α induces IRS-1 phosphorylation on serine, which makes this molecule inhibitory to the signalling capacity of the insulin receptor.[38] Increasing evidence also suggests that severe chronic periodontitis represents a sub-clinical septicemic state and TNF-α is closely linked to insulin resistance (IR), which, further, plays a role in the regulation of CRP expression.[39]

In addition, the visceral fat drains directly into the portal circulation and has been linked to morbidities, such as cardiovascular disease and T2DM. Adipose tissues modulate energy balance by regulating both food intake and energy expenditure. They also have a considerable effect on glucose balance, which is mediated by endocrine (mainly through the synthesis and release of peptide hormones, the so-called 'adipokines') and non-endocrine mechanisms. According to the current view, intracellular lipids may contribute to insulin resistance. Other factors tend to raise blood glucose includes resistin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and retinol-binding protein 4 (RBP 4). TNF-α is produced in macrophages and reduces insulin action. IL-6 is produced by adipocytes, and has insulin-resistance-promoting effects as well. Such 'adipocytokines' can induce insulin resistance through several mechanisms, including c-Jun N-terminal kinase 1 (JNK1)-mediated serine phosphorylation of insulin receptor substrate-1 (IRS-1), I κB kinase- (IKK-β)-mediated nuclear factor-κB (NF-κB) activation, induction of suppressor of cytokine signalling 3 (SOCS3) and production of reactive oxygen species (ROS).[39]

Further**,** C-reactive protein, mainly produced by the liver, but also by adipocytes and vascular smooth muscle cells, and recently demonstrated in gingival tissues,[40] in response to a rise in interleukin (IL)-6 and tissue necrosis factor-alpha (TNF-a).[41] Measurements of CRP in serum or GCF might help identify a subset of patients who are at higher risk of destructive disease (eg T2DM), or those who are undergoing a process of periodontal breakdown (chronic periodontitis), and the rapid rise of CRP in serum following exposure to interleukin-1, which is a potent bone resorber also found in GCF, made the search reasonable.[13]

Although, there was non-significant increase in RBS, TC, TG, LDL, VLDL and TC/HDL, and significant decrease in HDL in control group I (NDM-CP), nevertheless, suggestive increase of CRP level due to NSPT at 1 month reported by D'Auto *et al*.,[14] who suggested that periodontal therapy itself results in short lived systemic inflammation, and their relationship with RBS and lipid profile could not be ruled out. In our study it was observe that out of 19 patients in control group II (NDM-CP), one patient showed higher than normal values mean values for TC (280 mg/dl), TG (366 mg/dl), LDL (169 mg/dl), HDL (37mg/dl), and TC/ HDL ratio of 7.52 that may resulting in non-significant values and signify some underlying systemic factor which was not reported at the baseline by the patient.

NSPT reduces the inflammatory mediators (TNF-a and IL-6) concentration by reducing inflammatory response of the host to local factors, thus improving insulin sensitivity, ultimately leading to better glycemic control (HbA1c),[43] lipid profile due to reduction in serum crp level in patients with T2DM-CP,[44] as shown in present study.

It is well established that type 2 diabetes patients have a significantly elevated risk of periodontitis, the presence of periodontitis may exert a converse, negative impact upon cardio-metabolic risk status in type 2 diabetic patients. In chronic periodontitis patients with diabetes mellitus, periodontal destruction occurs due to exaggerated inflammatory (hyperactive neutrophils due to ROS production) response, because of increased oxidative stress and increased insulin resistance in T2DM patients.[45-49] Studies have shown that insulin resistance is increased in diabetic patient under the influence of local factors present, and hence decreases the healing response of tissue. Hence, it can be hypothesized that after non-surgical periodontal therapy (SRP), local irritant factors were removed that ultimately leads to

decrease in insulin resistance with decrease in pro-inflammatory cytokines (e.g. TNF-α, IL-6) associated with local factors that promote better wound healing capacity of tissue in T2DM patients. Further, there was significant improvement in both the groups but better improvement was observed in diabetic chronic periodontitis patients. As low HDL levels, elevated TC/HDL ratio and elevated CRP levels are known cardiovascular risk factors, the impact of periodontitis on oxidative stress/inflammatory pathways, insulin production and cardiovascular health needs to be further explored in T2DM.[1] Nevertheless, the consistent reductions in CRP serum levels following periodontal therapy reported by several investigators pose in favour of a possible role of periodontitis in causing a state of systemic inflammation, and potentially affecting a variety of chronic disorders including CVD and diabetes.[50]

Similar to Allen *et al*.[1] our study also had low sample size; however, in contrast to their study which was cross-sectional study, present study was short term interventional follow up trial. Absence of control group like non-diabetic periodontally healthy and diabetic periodontally healthy, and within group comparison (controlled, moderately controlled and uncontrolled diabetic patients), and gender and age variations due to attrition of the patients, were the limitations of the present study. Nevertheless, as Garde et al.[51] quoted in their meta-analysis that current literature "stimulate the interest in further exploring the benefits of good oral health for the prevention of diabetes complications and especially to setup well designed clinical trials with lipid profiles as the primary outcome", present study may provide preliminary data. Further multicentric long term randomised controlled clinical trials are thus suggested that concentrate on degree of CVDs prevention that can be associated with periodontal therapy and maintenance of meticulous periodontal health to be focused not only on the systemic effects of periodontal treatment.[52]

CONCLUSION

Within the limitation of the present study it can be concluded that NSPT results in significant improvement in RBS, glycemic level (HbA1c) and lipid profile in both T2DM-CP (experimental group-I) and NDM-CP (control group-II). The improvement was more in experimental group-I (T2DM-CP) as compared to control group-II (NDM-CP) after NSPT. Although, there was non-significant increase in RBS, TC, TG, LDL, VLDL and TC/HDL, and significant decrease in HDL in control group I (NDM-CP) at 1 month, nevertheless, this change may be attributed to increase CRP level due to NSPT.

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