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Effect of Scaling and Root Planing on Micronutrients (Zinc, Magnesium, Iron and Copper) levels and on the Glycemic status in Healthy and Type 2 Diabetes patients with generalised Chronic Periodontitis - A Clinical and Biochemical study

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Abstract

Background: Periodontal disease are microbial induced chronic inflammatory conditions that can leads to alteration in systemic conditions such as Diabetes and imbalance in micronutrients levels leading to increased susceptibility to oxidative damage of tissues. However, only few studies have evaluated the possible relationship between Micronutrient levels, Diabetes and Periodontitis.

Aim: To evaluate the efficacy of non-surgical periodontal therapy on improvement of micronutrient levels in Periodontitis patients as well in Diabetic patients.

Materials and methods: 90 Patients were divided equally into three groups. Group 1 consists of 30 patients with Diabetes Mellitus type 2 and Chronic Periodontitis, Group 2 with 30 Systemically Healthy Patients with Chronic Periodontitis and Group 3 with 30 Systemically Healthy Patients with Healthy Periodontium. Estimation of Fasting and Post Prandial Blood Sugar levels were done using Glucose oxidase method and HbA1c levels by Nycocard Reader. For estimation of Zinc, Magnesium, Iron and Copper levels were done by Biochemical kit on fully Automated Biochemical Analyzer.

Results: Statistically significant difference were observed with periodontal parameters and Glycemic

status between and within all the groups. Statistically significant difference was observed in mean scores of serum Copper, Zinc and Iron levels between baseline and follow-up period for group A and B.

Conclusion: Nonsurgical periodontal therapy had improved the micronutrients levels by decreased in serum Iron and Copper levels and increased in serum Zinc and Magnesium levels leading to an improvement in periodontal parameters and Glycemic status.

Keywords: Nutrients, Periodontitis, Micronutrients, Zinc, Magnesium, Iron, Copper, Diabetes, HbA1c

Introduction

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by microorganisms which results in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both."1This disease is considered to be due to the result of the interaction between pathogenic bacteria and the host's immune response.[2,3]

Diabetes and periodontal diseases are both chronic inflammatory disorders having a major impact on the health and well-being of millions of individuals worldwide. Diabetes and Periodontal disease share a bidirectional relationship.4 Any Periodontal treatment which decreases the periodontal inflammation may play a positive role in restoring insulin sensitivity thereby improving glycemic control.[5]

Nutrition has significant effects on the inflammatory processes as well on the cellular and humoral immune mechanism. The interaction between nutritional status and the immune response to the bacterial challenge can be an underlying factor in the progression of periodontal disease.6 Nutrients can be categorized as either macronutrients or micronutrients. Micronutrients are dietary compounds that do not provide energy but are required by living organisms and are essential for optimal health, proper growth, and metabolism.[7,8] They are also necessary for functioning of many enzyme systems like deoxyribonucleic acid (DNA) polymerase, ribonucleic acid (RNA) polymerase, superoxide dismutase, catalases, alkaline phosphatase.[7]

The vitality of the periodontal tissues in both health and disease depends on the adequate source of essential nutrients being available to the host.6Periodontitis can be attributed to the variation and concentration of plasma micronutrients. Similarly, numerous studies have found alterations in the micronutrient status of patients with diabetes.[7] This imbalance in micronutrients levels in the body can predispose an individual to risk of Periodontitis and various Diabetic complications due to decreased regenerative capacity and impaired immune response with development of oxidative stress.[7]

However, there are only few studies that have evaluated the possible relationship between Micronutrient levels, Diabetes and Periodontitis. Hence, we conducted a study to evaluate the efficacy of non-surgical periodontal therapy on improvement of micronutrient levels in Periodontitis patients as well in Diabetic patients.

Aims and Objectives

The aim of the study was to evaluate the effect of Non-surgical Periodontal Therapy (Scaling and Root Planing) on the Micronutrients (Zinc, Magnesium, Iron and Copper) Levels and Glycemic Status of Healthy and Type 2 Diabetes Patients with Chronic Periodontitis.

Materials and Methods

Clinical Protocol

The present study was carried out in the Department of Periodontics, Nair Hospital Dental College, Mumbai, registered under CTRI with registration no-CTRI/ 2018/ 05/ 13730 and was approved by the Institutional Ethical Committee (EC/PG-07/PERIO/2017).

Patient Selection

A total of 90 Patients were selected and were divided equally into three groups of 30 each. Consent was taken prior to the study and if the patient was willing to discontinue the treatment procedure during the study, he or she was allowed to do so. A thorough medical, dental history and a detailed clinical examination were done from each patient.

Inclusion Criteria

Group A

1. Patients with Type 2 Diabetes who were on oral hypoglycaemic drugs for at least 6 months, and with Chronic Periodontitis.

Patients were categorized into Type 2 Diabetes as

• Fasting Blood Glucose level ≥ 126 mg/dl and

• Post Prandial Blood Glucose level ≥ 180 mg/dl

• Glycosylated Haemoglobin (HbA1c) level -7% or less.

2. Probing depth or clinical attachment level of 4mm or more in more than 30% of sites.

3. Age group between 20-60 years with minimum complement of 20 natural teeth.

Group B

Systemically Healthy Patients with Chronic Periodontitis with no underlying medical conditions.

1. Age group between 20-60 years with minimum complement of 20 natural teeth.

2. Probing depth of about 4mm or more in more than 30% of sites.

Group C

Systemically Healthy Patients with Healthy Periodontium with no underlying medical conditions, Age group between 20-60 years with minimum complement of 20 natural teeth.

Exclusion Criteria

Patients who had undergone Periodontal treatment in the past 6 months, intake of Antibiotics or Anti-inflammatory drugs within 6 months prior to the study, <20 remaining natural teeth, Pregnant or Lactating mothers, on Vitamin supplements, history of Smoking / Tobacco chewers, any Systemic disease other than Diabetes Type 2

Study Design

A total of 90 Patients were selected from Outpatients of Department of Periodontics and were divided equally into 3 groups.

Group–30 Patients with Type 2 Diabetes with Chronic Periodontitis.

Group B – 30 Patients who were systemically healthy with Chronic Periodontitis.

Group C - 30 Patients who were systemically healthy with Healthy Periodontium.

Clinical Parameters

The following clinical parameters recorded at baseline (day zero), and at the end of 3 months post Scaling and Root Planing in group A, B and C were:

1. Plaque Index (P.I.) (Turskey-Gilmore-Glickman Modification of Quigley Hein, 1970)

2. Gingival Index (G.I.) (Loe &Silness, 1963)

3. Probing Pocket Depth (PPD)

4. Clinical Attachment Level (CAL)

Probing depth and clinical attachment level were measured with a University of North Carolina-15 (UNC-15) probe.

Non-Surgical Procedure

Following initial examination Plaque index, gingival index, Probing Pocket Depth and Clinical Attachment Level were recorded. All the subjects in group A, B and C underwent thorough ultrasonic Scaling and Root Planing (SRP) at baseline and at the end of 3 months. Detailed instructions regarding self-performed plaque control measures were given.

Armamentarium

Mouth mirror, UNC-15 probe, Tweezer, Ultrasonic scaler, Currettes, Disclosing agent (Plaksee TM). For blood collection-Tourniquet cuff, Disposable Syringes and needles, Vacuum blood collection tube - EDTA vial, Fluorides bulb vial, Plain vial, Cottons, Surgical gloves.

Sample Collection and Investigations

Subject venous blood samples were collected at baseline and at the end of 3 months. Blood samples were drawn from the median cubital vein through a disposable syringe and needle and transferred it to vacuum blood collection tube.

Sample collection and Biochemical Analysis for Blood Sugar Level

2ml of Subjects Venous blood samples were collected in Fluoride bulb vial in the morning after an overnight fast for estimation of Fasting Blood Sugar Level and 2 hours post lunch for estimation of Post Prandial Blood Sugar Level.

The estimation was done using glucose Reagent by Glucose oxidase method. 2ml of blood samples was collected in an EDTA vial for analysis of HbA1c levels and were estimated in Nycocard Reader by using Nycocard reagent (24x0.2mL.

Sample collection and Biochemical Analysis for Serum (Zinc, Magnesium, Iron, Copper)

5ml blood samples were collected in plain vial and blood was allowed to clot and centrifuged at 2000 rpm for 10 min to separate the plasma for estimation of Zn, Mg, Fe and Cu Levels by Biochemical Reagent. The Reagents were mixed with the plasma and incubated for 5 minutes at 37 C and analysed with fully Automated Biochemical Analyzer.

Follow Up

Recall appointments was made at 3 months in which patients were re-evaluated and assessed clinically for plaque index, gingival index, probing pocket depth and clinical attachment level and at each visit, oral hygiene instructions were reinforced. 3 months post Scaling and Root Planing (SRP) blood samples were again withdrawn from the patient to estimate serum Zinc, Magnesium, Iron and Copper Levels and Glycemic Status for Group A, B and C.

Statistical Analysis

The study was carried out by using the Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA). Student t tests (two tailed, paired and unpaired) were used to find the significance of study parameters on continuous scale between two groups (Intragroup and Intergroup analysis). Analysis of variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis).

Observation and Results

Intragroup analysis

Group A: Type 2 Diabetes Patients with Chronic Periodontitis

Mean scores of Plaques, Gingival index, Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) at baseline were 2.720 ± 0.483, 2.529 ± 0.243, 4.111 ± 0.390 and 5.310 ± 0.502 respectively. While at 3 months after non-surgical therapy were 0.877 ± 0.400, 0.425 ± 0.125, 3.237 ± 0.252 and 4.436 ± 0.427 respectively. Highly statistical significant difference was found in mean scores of Plaque, Gingival index, PPD and CAL between baseline and follow-up period. (As shown in Table 1)

Table 1: Comparison of different variables in terms of {Mean (SD)} at different time intervals using paired t test

Groups	Plaque index	Gingival Index	Probing Pocket Depth (PPD)	Clinical Attachment Level (CAL)
Group A				
Mean value at baseline	2.720 ± 0.483	2.529 ± 0.243	4.111 ± 0.390	5.310 ± 0.502
Mean value at 3 months	0.877 ± 0.400	0.425 ± 0.125	3.237 ± 0.252	4.436 ± 0.427
P value	**<0.001****	**<0.001****	**<0.001****	**<0.001****
Group B				
Mean value at baseline	3.079 ± 0.445	2.552 ± 0.314	3.812 ± 0.480	5.219 ± 0.617
Mean value at 3 months	1.212 ± 0.408	0.394 ± 0.122	3.112 ± 0.298	4.385 ± 0.499
P value	**<0.001****	**<0.001****	**<0.001****	**<0.001****
Group C				
Mean value at baseline	1.122 ± 0.428	0.384 ± 0.345	2.453 ± 0.242	2.473 ± 0.263
Mean value at 3 months	1.138 ± 0.453	0.365 ± 0.346	2.330 ± 0.490	2.451 ± 0.257
P value	0.622	**0.042***	0.187	0.124

($p < 0.05$ - Significant*, $p < 0.001$ - Highly significant**) Mean score of Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS) and Glycosylated Haemoglobin (HbA1c) level at baseline were 156.583 ± 35.600, 211.772 ± 55.829 and 7.890 ± 1.644 respectively while at 3 months after non-surgical therapy was 136.824 ± 41.315, 174.146 ± 56.468 and 6.900 ±1.159 respectively. Highly statistically significant difference and statistically significant difference was found in mean scores of HbA1c, FBS and PPBS levels between baseline and follow-up period respectively. (As shown in Table 2)

Table 2: Comparison of different variables in terms of {Mean (SD)} at different time intervals using paired t test.

Variables	Baseline (mean ±SD)			At 3 months(mean ±SD)			P Value
	Group A	Group B	Group C	Group A	Group B	Group C	
Fasting Blood sugar (mg/dl)	156.583 ± 35.600	102.977 ± 15.208	94.883 ± 6.525	136.824 ± 41.315	102.586 ± 13.906	93.980 ± 7.343	**Group A=0.012*, Group B=0.847, Group C=0.251**
Postprandial Blood sugar (mg/dl)	211.772 ± 55.829	115.670 ± 26.473	103.436 ± 12.367	174.146 ± 56.468	109.031 ± 14.009	100.736 ± 8.855	**Group A=0.011*, Group B=0.093, Group C=0.335**
HbA1c (%)	7.890 ± 1.644	5.726 ± 0.801	5.623 ± 0.500	6.900 ±1.159	5.636 ± 0.418	5.526 ± 0.506	**Group A=<0.001** Group B=0.489, Group C=0.4604**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**) Mean score of Serum Zinc, Magnesium, Iron and Copper levels at baseline were 70.655 ± 45.451, 2.250 ± 0.493, 119.179 ± 46.643 and 117.025 ± 29.304 respectively while at 3 months after non-surgical therapy were 98.925 ± 41.594, 2.367 ± 0.554, 96.534 ± 37.135 and 96.773 ±17.323 respectively. Highly statistically significant difference and statistically significant difference was found in mean scores of serum Copper, Zinc and Iron levels respectively, However, no statistical significant difference was found in mean scores of serum Magnesium levels between baseline and follow-up period. (As shown in Table 3).

Table 3: Comparison of different variables in terms of {Mean (SD)} at different time intervals using paired t test

Groups	Zinc (µg/dL)	Magnesium (mg/dL)	Iron (µg/dL)	Copper (µg/dL)
Group A				
Mean value at baseline	70.655 ± 45.451	2.250 ± 0.493	119.179 ± 46.643	117.025 ± 29.304
Mean value at 3 months	98.925 ± 41.594	2.367 ± 0.554	96.534 ± 37.135	96.773 ±17.323
P value	**0.007***	**0.293**	**0.018***	**<0.001****
Group B				
Mean value at baseline	71.963 ± 31.612	2.268 ± 0.537	125.633 ± 60.202	98.885 ± 18.863
Mean value at 3 months	88.444 ± 21.089	2.484 ± 0.407	100.534 ± 43.247	88.008 ± 24.021
P value	**0.005***	**0.089**	**0.003***	**0.023***
Group C				
Mean value at baseline	72.756 ± 38.716	2.354 ± 0.448	118.252 ± 64.318	97.086 ± 24.420
Mean value at 3 months	72.867 ± 38.586	2.205 ± 0.362	118.582 ± 62.192 0.926	96.387 ± 23.688
P value	0.769	0.1623		0.665

(p < 0.05 - Significant*, p < 0.001 - Highly significant**) **Group B: Systemically Healthy Patients with Chronic Periodontitis**

Mean score of Plaque, Gingival index, PPD and CAL at baseline were 3.079 ± 0.445, 2.552 ± 0.314, 3.812 ± 0.480 and 5.219 ± 0.617 respectively while at 3 months after non-surgical periodontal therapy it were 1.212 ± 0.408, 0.394 ± 0.122, 3.112 ± 0.298 and 4.385 ± 0.499 respectively.

Highly statistical significant difference was found in mean scores of Plaque, Gingival index, PPD and CAL between baseline and follow-up period. (As shown in Table 1)

Mean score of Fasting Blood Sugar (FBS), PPBS and HbA1c levels at baseline were 102.977 ± 15.208, 115.670± 26.473 and 5.726 ± 0.801 respectively while at 3 months after non –surgical periodontal therapy were 102.586 ± 13.906,109.031 ± 14.009 and 5.636 ± 0.418 respectively.

No statistically significant difference was found in mean scores of FBS, PPBS and HbA1c levels between baseline and follow-up period. (As shown in Table 2)

Mean score of Serum Zinc, Magnesium, Iron and Copper levels at baseline were 71.963 ± 31.612, 2.268 ± 0.537, 125.633 ± 60.202 and 98.885 ± 18.863 respectively, while at 3 months after non-surgical periodontal therapy were 88.444 ± 21.089, 2.484 ± 0.407, 100.534 ± 43.247 and 88.008 ± 24.021 respectively.

Statistically significant difference was found in mean scores of serum Zinc, Iron and Copper levels while no statistically significant difference found in mean scores of serum Magnesium level between baseline and follow-up period. (As shown in Table 3)

Group C: Systemically Healthy patients with Healthy Periodontium

Mean score of Plaque and Gingival Index, PPD, CAL at baseline were 1.122 ± 0.428, 0.384 ± 0.345, 2.453 ± 0.242 and 2.473 ± 0.263 respectively while at 3 months after non- surgical periodontal therapy was 1.138 ± 0.453, 0.365 ± 0.346, 2.330 ± 0.490 and 2.451 ± 0.257 respectively.

(As shown in Table 1) Statistically significant difference found in mean scores of Gingival indexes, However, no statistically significant difference was found in mean scores of Plaque index, PPD, CAL between baseline and follow-up period.

Mean score of FBS, PPBS and HbA1c levels at baseline were 94.883 ± 6.525, 103.436 ± 12.367 and 5.623 ± 0.500 respectively while at 3 months after non-surgical periodontal therapy was 93.980 ± 7.343, 100.736 ± 8.855 and 5.526 ± 0.506 respectively. No statistically significant difference found in mean scores of FBS, PPBS and HbA1c levels between baseline and follow-up period. (As shown in Table 2)

Mean score of Serum Zinc, Magnesium, Iron and Copper levels at baseline were 72.756 ± 38.716, 2.354 ± 0.448, 118.252 ± 64.318 and 97.086 ± 24.420 respectively, while at 3 months after non-surgical periodontal therapy was 72.867 ± 38.586, 2.205 ± 0.362, 118.582 ± 62.192 and 96.387 ± 23.688 respectively. (As shown in Table 3)

No statistically significant difference found in mean scores of serum Zinc, Magnesium, Iron and Copper levels between baseline and follow-up period.

Intergroup analysis (As shown in Table 4,5 and 6)

At baseline

Mean scores of Plaque and Gingival index, PPD and CAL, FBS, PPBS, HbA1c levels showed highly statistically significant difference between all the three groups.

Mean scores of Zinc, Magnesium and Iron levels showed no statistical significant difference, However, Mean score of Copper levels showed statistically significant difference between the groups.

At 3 months

Mean scores of Plaque index, PPD, CAL, FBS, PPBS and HbA1c levels showed highly statistically significant while no statistically significant difference was observed in Gingival index between all the three groups. Mean score of Zinc levels showed statistically significant difference However, Mean scores of Magnesium, Iron and Copper levels showed no statistical significant difference between the groups.

Table 4: Comparison of clinical parameters in terms of {Mean (SD)} among all the 3 groups at baseline and 3 months using ANOVA test.

Variables	Groups	Baseline				At 3 months			
		Mean	Std. Deviation	F value	P value	Mean	Std. Deviation	F value	P value
Plaque Index	Group A	2.7207	0.48326	158.570	<0.001**	0.8770	0.40085	5.241	0.007*
	Group B	3.0790	0.44541			1.2123	0.40881		
	Group C	1.1227	0.42874			1.1387	0.45336		
	Total	2.3074	0.96541			1.0760	0.44127		
Gingival Index	Group A	2.5293	0.24354	502.253	<0.001**	0.4257	0.12525	0.551	0.579
	Group B	2.5527	0.31437			0.3947	0.12235		
	Group C	0.3843	0.34599			0.3650	0.34609		
	Total	1.8221	1.06576			0.3951	0.22280		
Pocket probing depth	Group A	4.1113	0.39000	159.283	<0.001**	3.2377	0.25294	55.324	<0.001**
	Group B	3.8127	0.48002			3.1127	0.29867		
	Group C	2.4530	0.24280			2.3300	0.49042		
	Total	3.4590	0.81889			2.8934	0.53985		
Clinical attachment level	Group A	5.3107	0.50266	332.56	<0.001**	4.4363	0.42747	230.977	<0.001**
	Group B	5.2197	0.61782			4.3850	0.49957		
	Group C	2.4733	0.26326			2.4517	0.25768		
	Total	4.3346	1.40791			3.7577	1.01259		

Table 5: Comparison of blood sugar levels in terms of {Mean (SD)} among all the 3 groups at baseline and 3 months using ANOVA test.

Variables	Groups	Baseline				At 3 months			
		Mean	Std. Deviation	F value	P value	Mean	Std. Deviation	F value	P value
Fasting Blood sugar (mg/dl)	Group A	156.5833	35.60024	66.003	<0.001**	136.8243	41.31582	23.503	<0.001**
	Group B	102.5867	15.20873			102.9773	13.90636		
	Group C	94.8833	6.52523			93.9800	7.34375		
	Total	118.0178	35.55583			111.2606	31.31880		
Postpran-dial Blood sugar (mg/dl)	Group A	211.7723	55.82988	79.789	<0.001**	174.1467	56.46832	45.190	<0.001**
	Group B	115.6700	26.47394			109.0310	14.00917		
	Group C	103.4367	12.36799			100.7367	7.85509		
	Total	143.6263	60.55668			126.6381	47.85129		
HbA1c (%)	Group A	7.8900	1.64450	40.984	<0.001**	6.9000	1.15967	32.171	<0.001**
	Group B	5.7267	0.80126			5.6367	0.41893		
	Group C	5.6233	0.50082			5.5267	0.50646		
	Total	6.4133	1.50879			5.9878	1.00357		

Table 6: Comparison of micronutrient levels in terms of {Mean (SD)} among all the 3 groups at baseline and 3 months using ANOVA test.

Variables	Groups	Baseline				At 3 months			
		Mean	Std. Deviation	F value	P value	Mean	Std. Deviation	F value	P value
Zinc (µg/dL)	Group A	98.9257	41.59436	**4.223**	**0.018***	70.6553	45.45190	1.346	0.266
	Group B	88.4440	21.08982			71.9630	31.61214		
	Group C	72.8677	38.58686			72.7560	38.71645		
	Total	86.7458	36.19016			77.1248	39.15651		
Magnesi-um (mg/dL)	Group A	2.3670	0.55463	2.926	0.059	2.2503	0.49318	0.378	0.686
	Group B	2.4847	0.40769			2.2687	0.53715		
	Group C	2.2053	0.36211			2.3543	0.44878		
	Total	2.3523	0.45867			2.2911	0.49089		
Iron (mg/dL)	Group A	96.5340	37.13591	1.745	0.181	119.1793	46.64391	0.146	0.864
	Group B	100.5343	43.24751			125.6330	60.20252		
	Group C	118.5827	62.19238			118.2523	64.31809		
	Total	105.2170	49.11361			121.0216	56.99768		
Cop per (µg/ dL)	Group A	96.7737	17.32324	1.535	0.221	117.0250	29.30462	**6.045**	**0.003***
	Group B	88.0083	24.02115			98.8853	18.86305		
	Group C	96.3873	23.68865			97.0867	24.42084		
	Total	93.7231	22.02692			104.3323	25.92466		

Discussion

Periodontitis is a highly prevalent and complex disease, initiated by a dysbiotic plaque biofilm which progresses largely by an exaggerated host immune inflammatory response. The drivers of the latter include genetic make-up, lifestyle choices, environmental factors, and effects of medications, all of which may contribute as component causes to the development and progression of periodontitis.9 This local disruption of the homeostatic balance within the periodontal tissues may not be confined to the periodontium alone. Currently, research shows that the cells of the immune system can transmit inflammatory responses to other parts of the body when challenged by local periodontal stimuli.10 The possible mechanisms suggesting by which this can occur are by direct migration and colonization of the pathogenic microorganisms to distant organs, leading to an inflammatory reaction at sites distant from the point of infection leading to systemic inflammation as a result of metastatic periodontal inflammation.[10]

Factors contributing to this multifaceted local disease process in the oral cavity include a number of systemic diseases, especially diabetes that can exaggerate the host response to the local microbial factors, resulting in unusually destructive periodontal breakdown.11 Among all the systemic diseases studied, with association to

periodontitis, most evidence is available between Diabetes and periodontal diseases.

Studies on patients with diabetes and periodontal disease have revealed that both diseases have a synergistic effect when they coexist in an individual. However, the exact mechanisms by which hyperglycaemia can lead to increased periodontal tissue destruction is not yet fully understood. It is hypothesized that long-term hyperglycaemia, seen in diabetic patients, can lead to increased anaerobic infections in the periodontal tissues with a hyperactive immune response, which can cause chronic inflammation in various organ systems of the body including the periodontium.[12]

In this study, chronic periodontitis patients with type 2 Diabetes have been included as a separate group, to see the extent of the potentiated effect of the two diseases existing together on the micronutrient status. Patients taking oral hypoglycaemic drugs were included in this study because the mechanism of action of drug varies for oral hypoglycaemic drugs and insulin, which can influence the glycemic control.[13,14]

Nutrients play a regulatory role in preserving the health of the human body. Micronutrients play an essential role in coping with the oxidative stress and also for the adequate immune response. There is a continuous synergy between the nutrition and the integrity of the oral cavity in oral health and disease.15 When exposed to infections or inflammatory agents, the host responds not only by mounting appropriate specific and nonspecific immune responses but also by initiating a well characterized series of metabolic adjustments.[8]

In this study, micronutrients such as Zinc, Magnesium, Iron and Copper levels were analyzed because they play an essential role in various regeneration processes, combat the effects of oxidative stress and also maintain an adequate immune response. Deficiency of Zn, Mg,Fe and Cu increases the susceptibility to infection and disease, impairs the function of neutrophils and macrophages, reduces the antibody-mediated, cell-mediated, phagocytic and delayed type of hypersensitivity reactions with depletion of antioxidants.[16] Numerous studies have found alterations in the micronutrient status of diabetes and periodontitis.[7]

In this study to assess the micronutrient levels in Chronic Periodontitis patients, the serum of patients was chosen as compared to saliva or gingival crevicular fluid to better understand the hypothesis that chronic periodontal inflammation can be a contributing risk factor for systemic inflammation at distant sites.[16]

A period of 3 months was chosen for re-evaluation after non surgical periodontal therapy (Scaling and Root planing) for the assessment of clinical parameters and glycemic status and micronutrients levels. This is because, the appropriate time interval required for evaluating the effect of non-surgical periodontal therapy which aids in complete resolution of gingival inflammation and tissue repair is approximately 3 month.17 This resolution of the inflammation inturn improves the levels of micronutrient and glycemic status. Hence biochemical analysis was also done after a period of 3 months following Scaling and Root Planing.

In this Study, the following clinical parameters which were compared at baseline and after treatment showed: Mean scores of Plaque index of three groups i.e, A,B and C when compared at baseline and at 3 months, statistically significant difference was observed within all the groups and between the groups of A and B of after non-surgical periodontal therapy. However, No statistically significant difference was found in group C between baseline and follow-up period. Mean scores of Gingival index of three groups A, B and C showed highly statistically significant difference in between

baseline & follow-up period. When mean scores of three groups were compared at baseline, highly statistically significant difference was observed. However, At 3 months, no significant difference was seen between all the three groups. PPD and CAL mean scores of three groups i.e, A,B and C when compared at baseline and 3 months, highly and statistically significant difference was observed between all the groups and within the groups of A and B after non-surgical periodontal therapy. However, Group C showed no significant difference between baseline and follow-up period. With respect to Biochemical analysis,

In our study, the mean scores of FBS, PPBS and HbA1c levels was found to be highest at baseline and lowest at 3 months after non-surgical therapy for group A, B and C. No statistically significant difference found in mean scores of FBS, PPBS and HbA1c levels between baseline and follow-up period for group B and group C, However, group A patients showed statistically significant difference. When mean scores of FBS, PPBS and HbA1c levels of three groups i.e, A,B and C were compared at baseline and 3 months, highly statistically significant difference was observed within the groups after non-Surgical periodontal therapy.

The exact mechanism of association between periodontitis and HbA1c levels is still not clearly known. Non-enzymatic glycosylation of haemoglobin results from hyperglycaemia which occurs due to insulin resistance or low insulin levels causes inflammation and impede wound healing. The pro-inflammatory cytokines such as TNF-α, IL1-β, IL-6 and IF-γ are produced by inflamed periodontium. The insulin resistance caused by these pro-inflammatory cytokines interferes with glucose and lipid metabolism,18 resulting in decreased insulin production by causing apoptotic cell death of pancreatic β cells,[19] thereby leading to a vicious cycle. Nonsurgical, periodontal therapy decreases serum levels of these pro-inflammatory mediators,[19] thereby controls the inflammation and the systemic level of glucose profile and the non-enzymatic glycosylation of haemoglobin.

Zinc

In our study, mean scores of Zinc and Magnesium levels was found to be lowest at baseline and highest at 3 months after non-surgical therapy. Mean scores of Iron and Copper levels were found to be highest at baseline and lowest at 3 months after non-surgical therapy.

Zinc-In our study, mean scores of Zinc and Magnesium levels was found to be lowest at baseline and highest at 3 months after non-surgical therapy. Mean scores of Iron and Copper levels were found to be highest at baseline and lowest at 3 months after non-surgical therapy.

This was in accordance with study conducted by Sundaram et al.[16] which shows increase in the mean values of serum concentration of Zinc after 3 months of non-surgical therapy when compared with baseline.

In intergroup comparison, mean serum Zinc levels were seen to be highest in Group C, significantly decreased in Group B and least in Group A at baseline.

This was in accordance with studies conducted by Gulkazi et al.,20 Thomas B et al.,[21] Puspharani et al.[22] which showed that the mean values of Zinc were significantly reduced in diabetic participants as compared to nondiabetics.

When mean scores of serum Zinc level of three groups i.e, A, B and C were compared, no statistically significant difference was observed at baseline. However, at 3 months, statistically significant difference was observed between the groups after non-surgical periodontal therapy.

Zinc deficiency in gingiva causes an increase in the permeability of the gingival epithelium for bacteria and an inverse correlation between the Zinc levels and

alveolar bone causes reduced osteoblastic activity, decreased synthesis of collagen and subdued alkaline phosphatase activity.[23,24] Imbalance of Zinc in the serum can predispose an individual to the risk of developing chronic periodontitis.[25]Zn absorption is decrease in diabetic patients, causing intercellular depletion. Diabetes is associated with increased urinary excretion of Zn which leads to its lower level in serum. These decreased levels of Zn adversely affect the ability of the islet cell to produce and secrete insulin. Thus, it can be suggested that zinc deficiency can lead to suppressed immunity and increased oxidative stress. This poor regenerative capacity in an individual can be a contributing factor in the progression of diabetic complications.[26]

Magnesium-In our study, significant improvement in Magnesium levels was seen from baseline to 3 months after non-surgical therapy. However, No statistically significant difference was found in mean scores of serum

Magnesium

In our study, significant improvement in Magnesium levels was seen from baseline to 3 months after non-surgical therapy. However, No statistically significant difference was found in mean scores of serum Magnesium levels within all the groups and between baseline and follow-up period for group A,B and C.

In intergroup comparison, serum Magnesium levels were seen to be highest in Group C, lesser in Group B and least in Group A at baseline.

This was in accordance with studies conducted by Viktorínová A et al. ,[26] and Pushparani et al [27] in which the serum Magnesium level was found to be significantly decreased in type 2 Diabetes and Chronic Periodontitis patients when compared with healthy control group.

According to Walter et al., [28] Diabetic patients commonly have depletion of Mg, probably due to its urinary losses that accompany glycosuria, decreased intestinal absorption, and redistribution of Mg from the plasma into blood cells caused by insulin effect. It has been reported by Paolisso and Barbagallo,[29] that the less availability of intracellular Mg results in decreased tyrosine kinase activity, postreceptorial impairment in insulin action and worsening of insulin resistance in diabetic patients. Mg deficiency is also associated with low bone mass, manifested in the oral cavity as loss of alveolar crestal bone height and tooth loss, accompanied by the stimulation of pro-inflammatory cytokines.30

Iron-In our study, significant difference was found in mean scores of serum Iron level between baseline and follow-up period for group A and B. However, group C showed no significant difference between baseline and follow-up period.

In Intergroup comparison, at baseline, the mean serum Iron levels were found to be highest in group B, lesser in group A and least in group C. When mean scores of three groups i.e, A, B and C were compared, no statistically significant difference was observed at baseline and at 3 months between the groups after non-surgical periodontal therapy.

Fe plays an important role in oxidative burst, i.e. the release of reactive oxygen species (ROS) from macrophages and neutrophils. A shift in the levels may cause oxidative stress leading to periodontal destruction. This is mainly related to the conversion of hydrogen peroxide to ROS through Fenton reactions catalysed by free Fe. Subsequently, there is activation of matrix metalloproteinases (MMPs) which degrade the extracellular matrix components. This further activates the nuclear factor-kappa B (NF-κB) pathway stimulating the release of pro-inflammatory cytokines like IL-1β, IL-

6, IL-8 and TNF-α which destroy the periodontal tissues and alveolar bone.[31,32]The relationship between Fe and insulin is bidirectional as Fe influences insulin activity by getting involved in glucose uptake and consumption and insulin affects Fe uptake and storage by increasing the cell surface transferring receptors. 33 It is documented that Fe affects glucose metabolism and free iron concentrations in patients with Type 2 Diabetes could contribute to tissue damage that may potentially elevate the risk of Type 2 Diabetes.[34,35]

Copper

In our study, statistically significant difference was found in mean scores of serum Copper level between baseline and follow-up period for group A and B. However, in group C no significant difference.

This was in accordance to study conducted by Sundaram G et al.[16] who demonstrated the levels of Copper in diabetes and Non diabetes patients with Chronic Periodontitis were elevated at baseline and improved significantly 3 months following nonsurgical periodontal therapy, even in those participants with uncontrolled type 2 Diabetes.

In intergroup comparison, at baseline, mean serum Copper levels were seen to be highest in group A, lesser in group B and least in group C. When the mean scores were compared between the groups at baseline, statistically significant difference was observed. However, after 3 months of non-surgical periodontal therapy, no statistically significant difference was observed between the groups.

Serum Copper levels are seen to be elevated during inflammation. This is said to be due to an endogenous leukocyte mediator at the site of inflammation that acts as a feedback signal to mobilize Copper from the liver. Lysyl oxidase, a specific monoamine oxidase, is a Copper metalloenzyme which is involved in stabilizing collagen.[36,37] Elevated levels of Copper in the serum during chronic inflammation could be due to the accumulation of one or more of these enzymes in the serum.37 This mechanism explains the elevated serum Copper levels found in Chronic Periodontitis and type 2 Diabetes patients.

Thus, from the study conducted and the results obtained we can conclude that there is a change in the micronutrient levels of Zn, Mg, Fe and Cu following periodontal treatment in 3 groups. Nonsurgical periodontal treatment have improved the concentration of plasma micronutrients by reducing the levels of serum Iron and Copper levels and increased in serum Zinc and Magnesium levels thereby showing an improvement in periodontal parameters, as well as improvement in glycemic status such as Fasting Blood Sugar, Post Prandial Blood Sugar and Glycosylated Haemoglobin levels and has shown an improvement in overall health of the patients.

Limitations of the study

Some of the certain aspects which demand more detailed observations and elucidation of data and facts are

1. More longitudinal studies with a larger sample size and longer follow-up should be carried out to have a better understanding of the interrelationship between micronutrient imbalance and chronic disease processes like Periodontitis and Diabetes.
2. A comparative study where the micronutrient levels in serum, saliva, gingival crevicular fluid and gingival tissue samples can be compared, to better understand both the local and systemic nature of Chronic Periodontitis lesions and Diabetes.
3. There is variation in diet with different age group and religion.

4. Availability of cost-effective evaluation kits of micronutrient levels in regular monitoring of Diabetes and Chronic Periodontitis patient.

Conclusion

So within the limitations of the study, the results obtained in our study collectively supports and extend the views that the assessment of the micronutrients could serve as possible biomarkers for Chronic Periodontitis and Diabetes. Conversely, improvement in periodontal status by non-surgical therapy have improved the variation and concentration of plasma micronutrients thus maintaining the homeostasis of Zn, Mg, Fe and Cu levels and also improvement in the glycemic status, thereby preventing its various complications. Future researches are needed to focus on an evaluation of which nutrients may help to prevent the onset and the progression of Periodontal Disease and Diabetes.

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