Original Article

Indian Journal of Dental Education
Volume 13 Number 3, July–September 2020
DOI: https://dx.doi.org/10.21088/ijde.0974.6099.13320.2

Assessment of Homocysteine and Methylmalonic Acid in Saliva and its Correlation with Serum Vitamin B12 in Chronic Periodontitis Patients with or without Type 2 Diabetes

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How to cite this article:

Shruthy R, Ranganath Venugopal, Rachana C et. al., Assessment of Homocysteine and Methylmalonic Acid in Saliva and its Correlation with Serum Vitamin B12 in Chronic Periodontitis Patients with or without Type 2 Diabetes. J Dent Educ. 2020;13(3):107–115.

Abstract

Background: Chronic periodontitis and Diabetes are common chronic diseases in the world. The aim of this study was to investigate the role of two biomarkersnamely Homocysteine (Hcy) and Methylmalonic acid (MMA) in chronic periodontitis (CP) and type 2 diabetes mellitus (T2DM) patients and also to evaluate its correlation with serum vitamin B12 levels. *Methodology*: 85 adults were recruited and divided into five groups as follows: Group 1: 17 healthy individuals without CP and T2DM; Group 2: 17 subjects with CP and without T2DM; Group 3: 17 subjects with T2DM without CP; Group 4: 17 subjects with T2DM (controlled) and CP; Group 5: 17 subjects with T2DM (uncontrolled) and CP. Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD) and Clinical attachment level (CAL) were assessed. Fasting blood sugar (FBS) levels, HbA1c and Vitamin B12 levels were evaluated. Unstimulated salivary samples were collected for assessment of salivary Hcy and MMAusing ELISA. *Results*: The probing depth and clinical attachment level was found to be elevated in chronic periodontitis and chronic periodontitis with diabetes mellitus patients. The levels of Hcy were found to be elevated in group 5, followed by group 4, 3, 2 and 1. Elevated levels of MMA were seen in group 5 and 4equally. A statistically significant correlation between parameters such as PI,GI, PPD,CAL,FBS,HbA1c and salivary biomarkers were seen ($p < 0.05$). A negative correlation between Hcy, MMA and serum Vitamin B12 levels were recorded. *Conclusion*: Elevated levels of salivary Hcy and MMAseen in chronic periodontitis and diabetes mellitus patients, suggesting their interrelationship. The levels of homocysteine and methylmalonic acid are inversely correlated with serum vitamin B12 levels.

Keywords: Diabetes Mellitus; Chronic periodontitis; Biomarkers; Vitamin B12; Methylmalonic acid; Homocysteine.

Introduction

Periodontitis, a common inflammatory disease is initiated by bacterial infection and progresses due to deviant host response resulting in destruction of tooth supporting tissues. Chronic periodontitis hasan influence on systemic health procuring moderate association with several systemic diseases such as cardiovascular disease, diabetes and adverse pregnancy outcomes. Reactive oxygen species (ROS), the oxygen free radicals are overproduced by hyperactive neutrophils during periodontitis. Under physiological conditions, ROS are effectively neutralized by antioxidants, however during inflammation ROS production is markedly increased leading to tissue damage involving lipid peroxidation, DNA and protein damage. ROS also functions as signaling molecules or mediators of inflammation.[1]

Oral cavity serves as a continuous source of infectious agents and its condition often reflects the progressionof systemic pathologies. A change in paradigm has dismissed the concept of oral infections being localized to the oral cavity and a whole new notion of the status of oral cavity and its impact on systemic health and disease has emerged. Periodontal disease has already been reported as the sixth complication of diabetes and there are bounteous studies describing their bidirectional interrelationship.[2]

Diabetes mellitus represents a heterogenous group of metabolic disorders in which elevated blood glucose levels result in disturbance of carbohydrate, fat and protein metabolism. Chronic hyperglycaemia is attributed to complications seen in diabetes. Likewise, hyperglycemia also affects periodontal tissues by increasing the oxidative stress. An imbalance between ROS and antioxidants eventually lead to accumulation of advanced glycation end products (AGE's). Binding of AGE's to their receptors triggers intracellular events that boost the production of pro-inflammatorycytokines, chemokines and cell adhesion molecules.[3] Hence accumulation of AGEs as a result of chronic hyperglycaemia or diabetes coupled with presence of periodontitis may provide a possible explanation for the clinical outcomes observed in diabetic patients with periodontal disease.[2]

Vitamin B12 (cobalamin), an imperative water-soluble micronutrientacts as a coenzymefor cystolic methionine synthase (MS) and mitochondrial methylmalonyl CoA mutase (MCM). Therefore, deficiency of cobalamin (Cbl) inactivates MSand MCM leading to the accretion of homocysteine and MMA. Hence, Hcy and MMA are the favored serum biomarkers to determine B12 status.[4]

MMA in serum, plasma and urine is derived from the hydrolysis of D-methyl malonyl CoA, a metabolic intermediate in the conversion of propionic acid to succinic acid. The conversion to succinyl CoA is catalysed by the subsequent action of D,L methylmalonyl CoA racemase and L-methylmalonyl CoA mutase. The latter enzyme requires adenosylcobalamin as a cofactor. Hence, impaired cobalamin function leads to increased concentration of MMA, explaining its role being a sensitive and specific marker of cobalamin status.Homocysteine on the other hand is formed from methionine during S-adenosyl methionine dependent methylation reaction, which also requires cobalamin as cofactor during the process.[5]

There are numerous cross-sectional studies and case reports demonstrating that vitamin B12 deficiency is highly prevalent in adult diabetic patients, the most conspicuous risk factor being long-term and high dose metformin therapy. Metformin is one of the commonly used oral hypoglycaemic agents and it has for long beenshown to decrease vitamin B12 levels.[6]

Measurement of serum MMA or Hcy is a sensitive and specific approachfor screening type 2 diabetes mellitus patients with borderline serum vitamin B12 concentrations.[7]

The main objective of the present study was to find out the precise relationship between vitamin B12, Hcy and MMA in chronic periodontitis patients with and without type 2 diabetes. Saliva samples were chosen for the analysis of boththe biomarkers, as collection of salivary sample is non-invasive and does not cause patient discomfort.

Materials and Methods:

A total of 85 patients within the age range of 35-75 years were recruited for the study from the outpatient department of AECS Maaruti College of dental sciences and research centre, Bengaluru, India. Written consent was obtained from all the patients and the study was conducted in accordance with Helsinki declaration of 1975 as revised in 2013. The present study was reviewed and approved by the ethics and review committee of AECS Maaruti College of dental sciences, Bengaluru. Patients had to undergo a detailed questionnaire including age, gender, oral hygiene habits, diabetes history, smoking history, medications and family history. Comparison of biomarker levels of control andperiodontitis group with type 2 diabetes mellitus resulted into higher effectsize. Considering the effect size and power of the study, the total sample size obtained was 85. Therefore in this study, a sample of 17 patients per group was decided and accordingly the data generated.

Inclusion criteria were subjects with >20 teeth, attachment loss ≥5 mm at more than 30 % of sites and showing radiographic evidence of bone loss. Patients with type 2 diabetes mellitus (DM) were diagnosed based on the criteria of WHO.[8] Glycemic status of patients with type 2 DM was confirmed by their glycosylated Hb levels. Subjects were excluded if they suffered any other systemic diseases (except DM), if they were smokers, obese, pregnant or lactating, diagnosed with aggressive periodontitis and with history of antibiotics taken within 6 months. Patients were divided into 5 groups as follows:

Group I: 17 healthy individuals without chronic periodontitis and type 2 diabetes mellitus.

Group II: 17 subjects with chronic periodontitis and without type 2 diabetes mellitus.

Group III: 17 subjects with type 2 diabetes mellitus (controlled) without chronic periodontitis.

Group IV: 17 subjects with type 2 diabetes mellitus (controlled) and chronic periodontitis.

Group V: 17 subjects with type 2 diabetes mellitus (uncontrolled) and chronic periodontitis.

Periodontal parameters such as gingival index (GI), Plaque index (PI), Clinical attachment level (CAL), Probing pocket depth (PPD) were assessed. A single calibrated examiner completed a full mouth assessment of periodontal condition. Gingival status was assessed by Loe and Silness gingival index and plaque index by Silness and Loe.[9,10] PPD and CAL were measured on all six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) using a Williams periodontal probe. Systemic parameters such as BMI, FBS, total cholesterol, HDL (High density lipoprotein), LDL (Low density lipoprotein), TG (triglyceride) and Vitamin B12 were assessed. Unstimulated saliva samples were collected for the assessment of Hcy and MMA.

Laboratory analysis

4 ml of blood was collected from each patient by venipuncture using a 20 gauge needle from antecubital fossa for FBS, HbA1c, lipid profile and serum vitamin B12 analysis.

Unstimulated whole saliva was collected from patients before the oral examination. Sampling was performed in the morning around 10 AM to avoid a possible variation in peptide concentration due to circadian rhythm. Samples were collected in plastic disposable vials and were frozen immediately at -80^{o}C until analysis.Each saliva sample was assayed by commercially available ELISA kits (enzyme linked immunosorbent assay) to determine the level of MMA and Hcy, according to the manufacturer's recommendation. The Boster® Human Methylmalonic acid and Human Homocysteine ELISA kit was used.

Statistical analysis

The data was analyzed using statistical software (Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0 Released 2013).

Kruskal Wallis test followed by Mann whitney U test as post hoc analysis was used to compare the mean values and Spearmans correlation test was done to estimatethe relationship between different clinical, diagnostic, vitamin B12 and biomarkers levels between 05 study groups. Stepwise multiple linear regression analysis for hcy and MMA levels were done to measure the impact by diagnostic, vitamin B12 levels and periodontal parameters in different study groups. Level of significance was set at $p < 0.5$.

Results

Table 1 shows the mean values of different study parameters between five study groups done using Kruskal Wallis test. The findings imply that clinical parameters such as PI, GI were highest in group 4 followed by group 5. Serum vitamin B12 levels were highest in the control group and least in the diabetic groups (group 3, group 4 and group 5) suggesting that diabetes has an effect on the levels of serum Vitamin B12.

The mean and standard deviation scores of salivary homocysteine in group 1,group 2,group 3,group 4 and group 5 were 3.95 ± 1.82, 9.05 ± 1.76, 16.76 ± 4.29, 21.12 ± 3.66, 22.94 ± 5.95 respectively. The mean and standard deviation scores of salivary MMA in group 1, group 2, group 3, group 4 and group 5 were 0.02 ± 0.01, 0.04 ± 0.01, 0.06 ± 0.01, 0.08 ± 0.02, 0.08 ± 0.02 respectively. The concentrations of salivary Hcy and MMA were highest in group 5 and group 4 compared to control groups. This suggests that these biochemical parameters are increased in inflammatory conditions (graph 1 and 2). The highest values of serum vitamin B12 were seen in group 1 (healthy) and least in group 5 (graph 3).

Spearmans correlation between the biochemical, clinical and diagnostic parameters showed that with an increase in the periodontal parameters, the Hcy and MMA levels also increased suggesting a positive correlation of these biomarkers with chronic periodontitis (Table 2 and 3). It was also seen that as serum vitamin B12 levels increased, the biomarker levels decreased respectively inferring an inverse relationship. Multiple stepwise regression analysis was done with salivary homocysteine as the dependent variable and it was seen that the periodontal parameters, FBS and vitamin B12 influenced Hcy levels upto 86% and the difference was significant ($p < 0.001$).Regression analysis for the control groups had not shown any influence of the parameters to the biochemical parameters (hcy and MMA).

Table 1: comparison of mean values of different study parameters between the 05 study groups using Kruskal Wallis test

Parameters	Groups	N	Mean	SD	Min	Max	H	P-Value
BMI	Group 1	17	21.06	1.87	17.9	24.1	6.270	0.18
	Group 2	17	21.01	2.02	17.9	24.1		
	Group 3	17	22.13	2.13	19.1	25		
	Group 4	17	21.66	2.05	18.6	24.2		
	Group 5	17	22.35	2.10	18.6	25		
PI	Group 1	17	0.39	0.21	0.1	0.8	58.497	<0.001*
	Group 2	17	1.92	0.43	1.3	2.8		
	Group 3	17	0.73	0.56	0.2	2.2		
	Group 4	17	2.42	0.60	1.4	3		
	Group 5	17	2.40	0.60	1.4	3		
GI	Group 1	17	0.45	0.11	0.3	0.7	65.143	<0.001*
	Group 2	17	1.97	0.40	1.4	2.7		
	Group 3	17	0.94	0.58	0.3	2.7		
	Group 4	17	2.62	0.45	1.8	3.0		
	Group 5	17	2.62	0.47	1.8	3.0		
PPD	Group 1	17	1.46	0.23	1.0	1.8	61.420	<0.001*
	Group 2	17	5.36	0.85	3.6	6.1		
	Group 3	17	1.45	0.49	0.8	2.1		
	Group 4	17	5.86	1.14	4.1	7.3		
	Group 5	17	5.91	0.95	4.5	7.2		
CAL	Group 1	17	1.46	0.23	1.0	1.8	61.178	<0.001*
	Group 2	17	5.71	0.97	3.6	6.7		
	Group 3	17	1.45	0.49	0.8	2.1		
	Group 4	17	6.09	1.17	4.1	7.7		
	Group 5	17	6.23	0.95	4.8	7.6		
FBS	Group 1	17	87.29	6.36	76.0	98.0	70.055	<0.001*
	Group 2	17	86.24	8.54	72.0	100.0		
	Group 3	17	144.88	13.87	129.0	179.0		
	Group 4	17	150.12	20.98	128.0	195.0		
	Group 5	17	189.94	24.34	160.0	251.0		
HbA1c	Group 1	17	5.09	0.43	4.5	5.8	72.916	<0.001*
	Group 2	17	5.20	0.40	4.5	5.8		
	Group 3	17	6.71	0.41	6.1	7.3		
	Group 4	17	6.85	0.24	6.4	7.2		
	Group 5	17	10.04	1.03	8.2	12.0		
HDL	Group 1	17	41.77	3.15	38.0	48.0	30.802	<0.001*
	Group 2	17	53.47	11.64	36.0	78.0		
	Group 3	17	54.88	12.34	36.0	78.0		
	Group 4	17	59.06	5.09	48.0	68.0		
	Group 5	17	51.82	7.61	42.0	65.0		

Cont..../-

LDL	Group 1	17	93.47	5.11	89.0	110.0	2.963	0.56
	Group 2	17	93.47	5.11	89.0	110.0		
	Group 3	17	103.53	15.98	82.0	134.0		
	Group 4	17	96.59	10.01	88.0	120.0		
	Group 5	17	92.71	5.16	86.0	100.0		
TG	Group 1	17	136.41	40.84	54.0	200.0	15.340	0.004*
	Group 2	17	136.41	40.84	54.0	200.0		
	Group 3	17	103.00	49.24	45.0	198.0		
	Group 4	17	155.00	38.40	80.0	198.0		
	Group 5	17	107.11	42.72	54.0	195.0		
TC	Group 1	17	146.18	14.53	129.0	175.0	4.127	0.39
	Group 2	17	146.18	14.53	129.0	175.0		
	Group 3	17	138.82	23.82	104.0	178.0		
	Group 4	17	138.29	9.73	124.0	156.0		
	Group 5	17	138.35	11.86	110.0	160.0		
S. Vit B12	Group 1	17	664.00	87.84	544	824	71.352	<0.001*
	Group 2	17	480.65	86.13	332	639		
	Group 3	17	210.82	43.80	156	333		
	Group 4	17	168.77	15.44	140	190		
	Group 5	17	150.00	25.09	120	200		
SHC	Group 1	17	3.95	1.82	1.2	8.1	67.351	<0.001*
	Group 2	17	9.05	1.76	5.8	12.3		
	Group 3	17	16.76	4.29	8.9	24		
	Group 4	17	21.12	3.66	16.1	32.1		
	Group 5	17	22.94	5.95	15.8	34.6		
SMA	Group 1	17	0.02	0.01	0.01	0.04	69.091	<0.001*
	Group 2	17	0.04	0.01	0.03	0.05		
	Group 3	17	0.06	0.01	0.04	0.08		
	Group 4	17	0.08	0.02	0.05	0.13		
	Group 5	17	0.08	0.02	0.06	0.12		

* - Statistically Significant; SD – Standard deviation; N – Group sample size; GI – Gingival Index; PI – Plaque Index; PPD – Probing Pocket Depth; CAL- Clinical attachment loss; HbA1c – glycated haemoglobin; FBS – Fasting Blood Sugar; LDL –low density lipoprotein, HDL- high density lipoprotein, TC- total cholesterol, TG- triglycerides, S.Vit B12- serum vitamin B12, SHC- salivary homocysteine, SMA- salivary methylmalonic acid

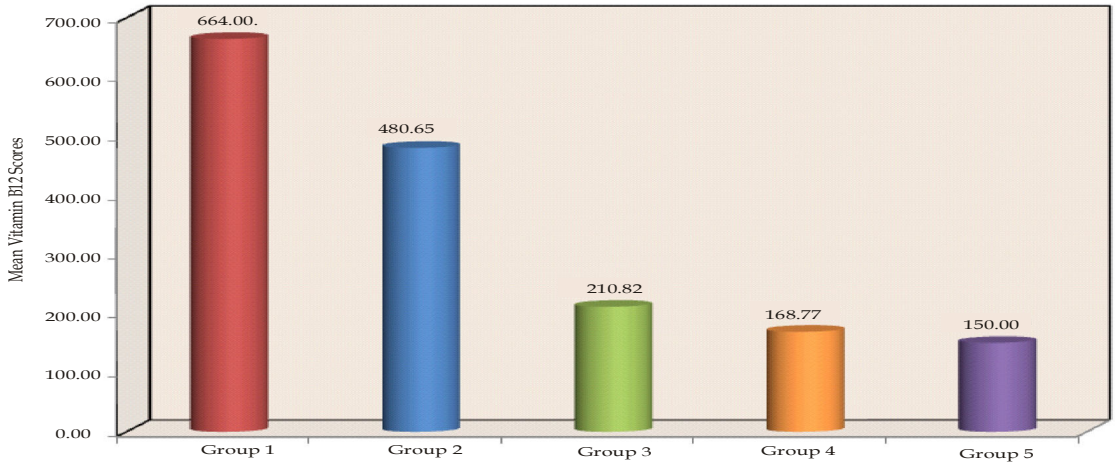

Fig. 1: Comparison of Mean Vitamin B12 Levels among 05 study groups. Serum vitamin B12 levels highest in group 1 (healthy) and least in group 5 (control).

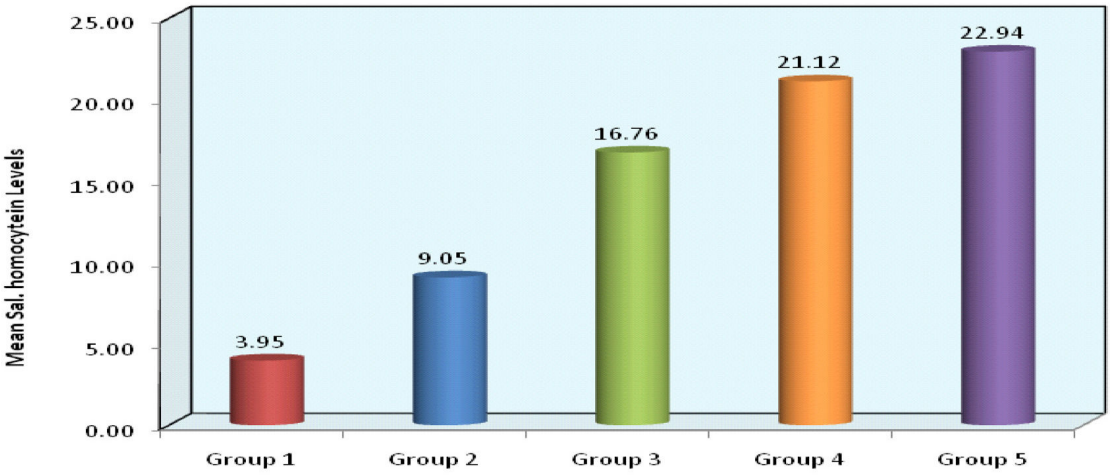

Fig. 2: Comparison of Mean Salivary Homocystein scores among 05 study groups. Increased levels of Salivary homocysteine seen in uncontrolled diabetes patients with chronic periodontitis.

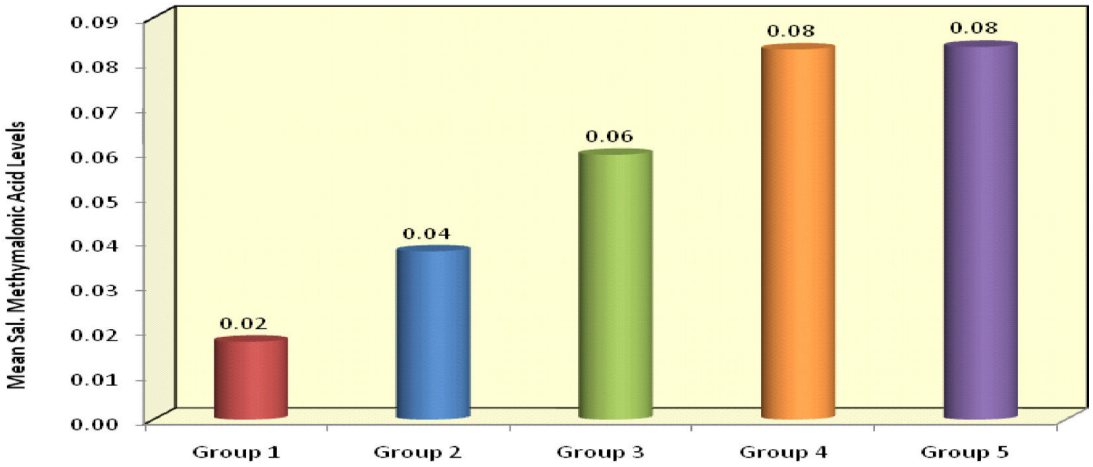

Fig. 3 Comparison of Mean Salivary Methymalonic Acid levels among 05 study groups shows that in chronic periodontitis patients with controlled and uncontrolled diabetes (group 4 and group 5), the mean salivary methylmalonic acid levels were increased followed by group3, group 2 and group1.

Table 2: Spearman's correlation between Salivary Homocysteine levels, diagnostic and clinical parameters in Overall Samples and in each study group.

Group	Values	Age	BMI	PI	GI	PPD	CAL	FBS	HbA1c	HDL	LDL	TG	TC	Vit B12
Overall	rho	0.12	0.19	0.63	0.52	0.63	0.60	0.48	0.44	0.56	-0.06	0.10	0.07	-0.71
	P-Value	0.29	0.08	<0.001*	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.62	0.36	0.58	<0.001*
Group 1	rho	-0.35	0.25	-0.08	-0.50	-0.18	-0.18	-0.31	-0.35	0.28	0.31	0.32	0.18	-0.32
	P-Value	0.17	0.33	0.75	0.04*	0.49	0.49	0.23	0.17	0.27	0.23	0.21	0.48	0.21
Group 2	rho	-0.39	0.10	0.59	0.51	0.42	0.40	0.52	0.12	0.59	0.29	0.59	0.18	-0.67
	P-Value	0.02	0.71	0.007*	0.005*	0.003*	0.008*	0.01*	0.66	0.001*	0.25	0.01*	0.48	0.002*
Group 3	rho	0.05	-0.09	-0.23	-0.11	0.11	0.11	-0.30	-0.22	-0.09	0.48	0.01	0.60	-0.43
	P-Value	0.85	0.73	0.37	0.68	0.69	0.69	0.24	0.41	0.74	0.06	0.97	0.01*	0.001*
Group 4	rho	-0.29	0.27	0.51	0.57	0.64	0.48	0.52	0.53	0.51	0.30	0.47	0.23	-0.50
	P-Value	0.26	0.30	0.003*	0.008*	<0.001*	0.005*	0.03*	0.03*	0.04*	0.25	0.01*	0.38	0.04*
Group 5	rho	0.10	-0.24	0.54	0.55	0.68	0.63	0.54	0.52	0.55	0.45	0.49	0.33	-0.50
	P-Value	0.99	0.36	0.003*	0.006*	<0.001*	0.001*	0.01*	0.01*	0.01*	0.06	0.02*	0.19	0.04*

The correlation coefficients are denoted by 'rho'

Minus sign denotes negative correlation

Table 3 Spearman's correlation between Salivary Methymalonic Acid levels, diagnostic and clinical parameters in Overall Samples and in each study group

Group	Values	Age	BMI	PI	GI	PPD	CAL	FBS	HbA1c	HDL	LDL	TG	TC	Vit B12
Overall	rho	0.07	0.22	0.70	0.78	0.65	0.63	0.45	0.47	0.56	-0.10	0.12	0.15	-0.60
	P-Value	0.50	0.04*	<0.001*	<0.001*	<0.001*	<0.001*	0.003*	0.003*	0.004*	0.39	0.30	0.09	0.007*
Group 1	rho	-0.19	-0.03	-0.01	-0.41	0.12	0.12	-0.32	-0.34	0.49	0.13	0.025	0.427	-0.106
	P-Value	0.459	0.92	0.99	0.10	0.64	0.64	0.22	0.18	0.04*	0.62	0.925	0.087	0.686
Group 2	rho	-0.36	0.30	0.55	0.57	0.41	0.31	0.53	0.39	0.47	0.41	0.55	0.19	-0.61
	P-Value	0.16	0.24	0.02	0.02	0.03*	0.04*	0.03*	0.97	0.001*	0.10	0.02*	0.47	0.009*
Group 3	rho	-0.40	-0.22	0.30	0.38	0.05	0.07	-0.41	-0.20	-0.156	-0.011	0.286	0.06	0.07
	P-Value	0.11	0.40	0.24	0.13	1.00	1.00	0.11	0.45	0.549	0.967	0.266	0.818	0.79
Group 4	rho	-0.36	0.22	0.63	0.55	0.69	0.56	0.54	0.55	0.53	-0.32	0.47	0.26	-0.55
	P-Value	0.16	0.40	0.007*	0.02*	0.002*	0.02*	0.03*	0.02*	0.03*	0.21	0.001*	0.32	0.02*
Group 5	rho	0.01	-0.18	0.69	0.61	0.60	0.48	0.58	0.47	0.49	-0.41	0.54	0.241	-0.54
	P-Value	0.97	0.48	<0.001*	<0.001*	0.001*	0.003*	0.002*	0.006*	0.004*	0.106	0.001*	0.352	0.03*

The correlation coefficients are denoted by 'rho'
Minus sign denotes negative correlation.

Discussion

In our study which included 85 subjects the objective was to find the association between MMA, Hcy, Vitamin B12, type 2 DM and chronic periodontitis. To the best of our knowledge this study is a first of its kind.

The interrelationship between periodontitis and type 2 DM has been extensively studied in the past and hence the finding of our study was no different. The periodontal parameters were elevated in poorly controlled diabetic group when compared to the controlled diabetic group. Recent study by Teew et. al. [11]on the influence of glycemic control on subgingival pathogens depicted similar results. As a result of chronic hyperglycemic state, accumulation of AGEs coupled with presence of infection provides a viable explanation for the clinical outcomes observed in diabetic patients with periodontal disease.

In our study, Hcy was detected in all the saliva samples, contradictory to another study by Dillon et. al.[12] including 69 subjects,where Hcy was detected only in two saliva samples. On the other hand, MMA was also detected in all the saliva samples collected.

The results of our study showed a statistically significant negative correlation of salivary Hcy and MMA with serum vitamin B12 levels. There are many other studies demonstrating an inverse relationship between these biomarkers and serum vitamin B12.[13-16] Conversion of Hcy to Methionineby methionine synthase (MS) depends on the availability of serum vitamin B12 and folate and hence deficiency of these leads to accumulation of Hcy in serum. Vitamin B12 deficiency also leads to inactivation of AdoCbl (adenosylcobalamin) dependent MCM (methylmalonyl CoA mutase) leading to a buildup of its substrate methylmalonyl CoA which enters circulation as free MMA. Hence, MMA and Hcy are considered as specific and sensitive markers of vitamin B12 deficiency.[4]

A positive correlation of salivary Hcy with periodontal parameters in the proposed study suggested its role in chronic periodontitis which was in accordance to several other studies.[17-19] reporting elevated plama Hcy levels in patients with chronic periodontitis. The levels of salivary Hcy was also seen elevated in diabetic groups compared to healthy group in our study which was in accordance to the results of another study by Ramachandra et. al.,[20] in which elevated Hcy levels were reported in patients with type 2DM. One of the possible mechanisms behind elevated Hcy levels and chronic periodontitis may be the acute phase reactants in the systemic circulation followed by IL-6 release from the inflamed periodontal pockets.[18] Other one being the activation of TH1 response by IL-6 leading to release of IF-γ which in turn stimulates ROS production. Overproduction of ROS as a consequence of chronic immune activation makes Vitamin B12 (oxidative sensitive molecule) a target for ROS, thereby explaining the elevated Hcy levels.

In our study salivary MMA levels showed a positive correlation with periodontal parameters. There are no other studies to our knowledge detecting MMA in saliva as ours except for a study by Bassim[21] wherein salivary methylmalonate levels were assessed in patients with methylmalonic acidemia.

Serum Vitamin B12 levels were decreased in the chronic periodontitis group and the diabetic groups compared to control group in the present study. Reportedly there are two case reports.[22,23] depicting patients with severe vitamin B12deficiency had generalized alveolar bone loss suggesting the role of Vitamin B12 and periodontitis. The exact mechanism explaining this is still unclear. Thorough medical history examination of each patient in the study revealed that more than half of the diabetic population was on metformin therapy. Our findings were inconsistent when compared to a study by Shtaynberg et. al.,[24] latter reporting Vitamin B12 deficiency was no greater in patients with diabetes on metformin therapy. Whereas our findings were consistent with the results of Roy R P et. al.[25] and Damiao C P et. al.[26] The possible mechanism underlying this association include: alterations in small bowel motility stimulating bacterial overgrowth and consequential vitamin B12 deficiency, alterations in IF levels, inhibiting calcium dependent absorption of the vitamin B12-IF complex. Metformin is believed to givea positive charge to the surface of the ileal cell surface receptor which acts to displace divalent cations such as calcium. Limitations of this study are that information regarding dosage of metformin was not determined, effect of periodontal therapy with the levels of biomarkers and severity of the disease not being assessed, smaller sample size and variation in age.

Therefore, from our study it is now evident that the levels of these biomarkers increase during inflammatory conditions. There are several studies reporting that elevated Hcy level is a risk for cardiovascular diseases.[27-29] Early interventions to reduce the levels of Hcy could be beneficial in the future.

Conclusion

In conclusion two novel biomarkers (MMA and Hcy) were detectable in all the salivary samples. This study showed an upregulation of MMA and Hcy in the saliva of subjects with chronic periodontitis with/without well controlled type 2diabetes and poorly controlled type 2 diabetes mellitus when compared to healthy subjects.Decreased levels of vitamin B12 in patients with chronic periodontitis suggest an underlying link between them. Hence, the novel association between Vitamin B12 deficiency, the biochemical parameters, type 2 diabetes and chronic periodontitis could provide a possible link amongst them.

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