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CORRELATION AMONG CYSTATIN C, OSTEOCALCIN, AND FIBRONECTIN SERUM AND SALIVARY LEVELS IN SUBJECTS WITH DIABETES AND\OR PERIODONTITIS COMPARED TO CONTROLS.

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ABSTRACT

Background: Aim: To determine the correlation among salivary and serum levels of cystatin C, osteocalcin, and fibronectin in subjects with diabetes and periodontitis alone or in combination compared with control. **Study Design:** Prospective case control study. **Materials and Methods:** A total of 226 subjects recruited from Endocrinology Unit, Samara General Hospital during the period from 1st October 2014 to end of October 2015. Their age ranged from 32 to 75 years. The study population depending on their clinical and laboratory findings divided in to non- diabetic without periodontitis as control, diabetic, periodontitis without diabetes, and diabetic with periodontitis groups. Blood and salivary samples collected from subjects and serum isolated. Salivary and serum samples tested for cystatin C, osteocalcin and fibronectin using enzyme linked immunosorbant assay. **Results:** There was a significant correlation between serum and salivary cystatin, osteocalcin and fibronectin in control group, periodontitis group, diabetic group and periodontitis subjects with diabetes. In addition, in diabetic group, there was a significant correlation between serum osteocalcin and serum cystatin; serum osteocalcin and serum fibronectin; salivary osteocalcin and salivary fibronectin. While in those with diabetes and periodontitis, there was a significant correlation between serum osteocalcin and salivary cystatin; salivary osteocalcin and serum cystatin; salivary osteocalcin and salivary fibronectin. **Conclusion:** Patterns of significant correlation are the same in control and periodontitis groups, while it is different in

diabetic group and Periodontitis with diabetes. Presence of periodontitis with diabetes alter the trend of association.

KEYWORDS: Diabetes, periodontitis, cystatin, osteocalcin, fibronectin, saliva, serum.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease with abnormal inflammatory and immunologic responses. Previous studies suggest an association between diabetes and periodontal disease with a bidirectional effects.[1] Both conditions are characterized with presence of many changes in oxidants\ antioxidants, lipid profile, cytokines, and in inflammatory responses.[2-5] Diabetes and periodontitis are polygenic disease with dysfunction of immune regulation.[6] The presence of one condition may potentiate others and well control of each of them will improve and control the other.[7] Thus this study was conducted to illustrate the correlation between serum and salivary markers in diabetes and periodontitis alone or in combination and compared to matched control.

MATERIALS AND METHODS

Study design: Prospective case control study.

Study population

A total of 226 subjects recruited from Endocrinology Unit, Samara General Hospital during the period from 1st October 2014 to end of October 2015. Their age ranged from 32 to 75 years. The study population depending on their clinical and laboratory findings divided in to the following groups:

Group I: (50) subjects who were non-diabetic without periodontal disease (apparently healthy) as a control group.

Group II: (56) subjects who were non-diabetic with periodontal disease.

Group III: (120) diabetic subject who were subdivided into:

- Diabetics with periodontitis : (60) subjects.
- Diabetics without periodontitis :(60) subjects.

The study was approved by the Ethical Committee of Tikrit University College of Medicine and informed consent taken from each participant before their inclusion in the study.

Sample collection

Blood and saliva samples were collected from subjects attended to Endocrine Unit in Samarra General Hospital after an overnight fasting in plain tube in the absence of any anticoagulants for blood samples, and serum had been harvested by allowing the sample to clot within 30 minutes then centrifugation for 10 minutes at 500 rpm, the sera supernatant of serum and saliva were divided into aliquots and stored at -20 until assayed.

Exclusions criteria

Patients with acute or chronic illnesses apart from diabetes mellitus has been excluded from the study.

Methods

Fasting blood sugar was determined using enzymatic method kits purchased from Biolabo, France. While salivary and serum cystatin C, osteocalcin, and fibronectin were determined using enzyme linked immunosorbent assay kits [Elabscience, China].

STATISTICAL ANALYSIS

Data were translated into codes using a specially designed coding sheet, and then converted to computerized database. An expert statistical advice was sought and statistical analyses were done using SPSS (Statistical Package for Social Science) version 22. Frequency distributions for variables were done first and plotted on histograms which fail to show distribution of normality and confirmed by using the chi square test. As no assumption about the normality of distribution of study variables was made, nonparametric methods were used to assess the statistical significant of associations. The statistical significant of difference in means of a quantitative continuous variable between two groups was assessed by Mann-Whitney test, while between more than two groups Kruskall-Wallis test was used. P value less than 0.05 level of significant was considered significant.

RESULTS

Correlation within Control group.

Serum cystatin, osteocalcin and fibronectin were significantly positively correlated with salivary cystatin, osteocalcin and fibronectin respectively., Table -1 and figures .1 and 2.

Table -1: Correlation between Serum Cystatin, Osteocalcin and Fibronectin with Salivary Cystatin, Osteocalcin and Fibronectin within the Control group.

Variable		Serum Cystatin	Salivary Cystatin	Serum Osteocalcin	Salivary Osteocalcin	Serum Fibronectin	Salivary Fibronectin
Serum Cystatin	**r- value**	1	**.654****	.269	-.250	-.110	.121
	P-value		**.000**	.452	.486	.763	.740
Salivary Cystatin	**r- value**	**.654****	1	-.609	.454	.395	.411
	P-value	**.000**		.062	.187	.258	.238
Serum Osteocalcin	**r- value**	.269	-.609	1	**.857****	-.270	-.319
	P-value	.452	.062		**.002**	.451	.369
Salivary Osteocalcin	**r- value**	-.250	.454	**.857****	1	.243	-.081
	P-value	.486	.187	**.002**		.499	.825
Serum Fibronectin	**r- value**	-.110	.395	-.270	.243	1	**.551***
	P-value	.763	.258	.451	.499		**.05**
Salivary Fibronectin	**r- value**	.121	.411	-.319	-.081	**.551***	1
	P-value	.740	.238	.369	.825	**.05**	
*. Correlation is significant at the 0.05 level (2-tailed).							

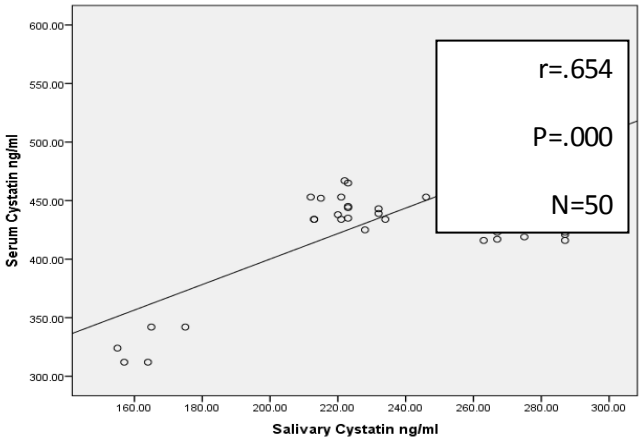

Figure. 1: Correlation between Serum Cystatin and Salivary Cystatin within Control group.

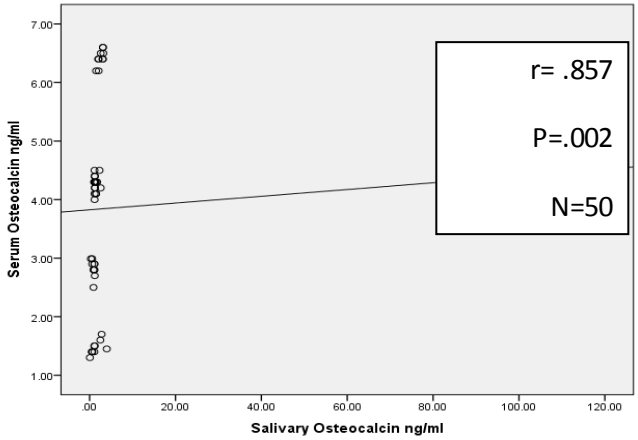

Figure. 2: Correlation between Serum Osteocalcin and Salivary Osteocalcin within Control group.

Correlation within Periodontitis group.

There was a significant correlation among serum and salivary cystatin, osteocalcin and fibronection in periodontitis group, Table -2 and figures .3, 4 and 5.

Table -2: Correlation between Serum Cystatin, Osteocalcin and Fibronectin with Salivary Cystatin, Osteocalcin and Fibronectin within the periodontitis group.

Variable		Serum Cystatin	Salivary Cystatin	Serum Osteocalcin	Salivary Osteocalcin	Serum Fibronectin	Salivary Fibronectin
Serum Cystatin	**r- value**	1	**.586***	-.255	-.108	.479	.001
	P-value		**.028**	.378	.713	.083	.998
Salivary Cystatin	**r- value**	**.586***	1	-.017	-.181	-.485	.099
	P-value	**.028**		.953	.537	.079	.736
Serum Osteocalcin	**r- value**	-.255	-.017	1	**.842****	-.102	-.088
	P-value	.378	.953		**.000**	.729	.766
Salivary Osteocalcin	**r- value**	-.108	-.181	**.842****	1	.068	.003
	P-value	.713	.537	**.000**		.816	.991
Serum Fibronectin	**r- value**	.479	-.485	-.102	.068	1	**.717****
	P-value	.083	.079	.729	.816		**.004**
Salivary Fibronectin	**r- value**	.001	.099	-.088	.003	**.717****	1
	P-value	.998	.736	.766	.991	**.004**	

**. Correlation is significant at the 0.01 level (2-tailed).

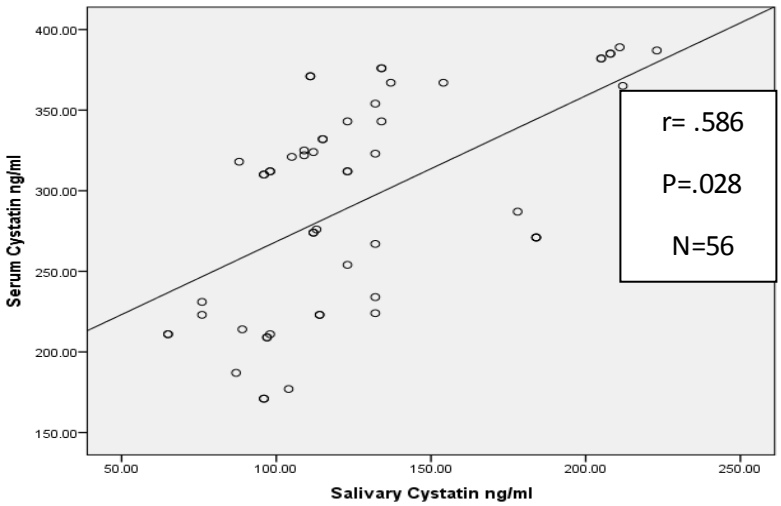

Figure. 3: Correlation between Serum Cystatin and Salivary Cystatin within Periodontitis group.

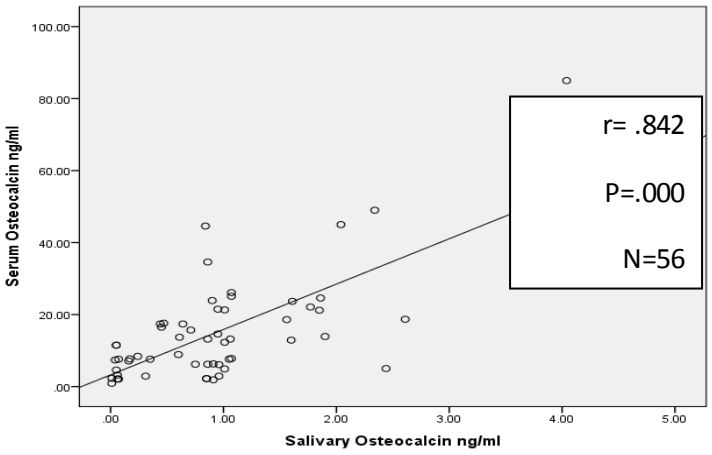

Figure. 4: Correlation between Serum Osteocalcin and Salivary Osteocalcin within Periodontitis group.

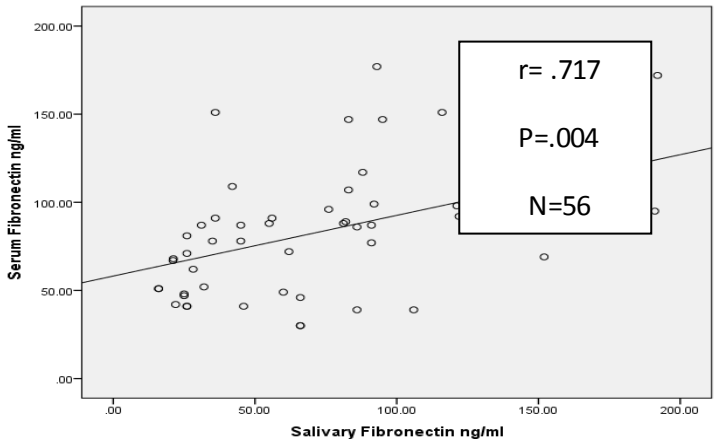

Figure. 5: Correlation between Serum Fibronectin and Salivary Fibronectin within Periodontitis group.

Correlation within Diabetic group.

Serum cystatin, osteocalcin and fibronectin significantly correlated with salivary cystatin, osteocalcin and fibronectin respectively, within the diabetic group, Table -3 and figures 6,7 and 8.

Table -3: Correlation between Serum Cystatin, Osteocalcin and Fibronectin with Salivary Cystatin, Osteocalcin and Fibronectin within the Diabetic group.

Variable		Serum Cystatin	Salivary Cystatin	Serum Osteocalcin	Salivary Osteocalcin	Serum Fibronectin	Salivary Fibronectin
Serum Cystatin	**r-value**	1	**.652****	**.408***	-.017	.040	.147
	P-value		**.000**	**.025**	.931	.835	.438
Salivary	**r-value**	**.652****	1	.318	.200	-.198	-.027

Cystatin	**P-value**	**.000**		.087	.288	.294	.889
Serum	**r-value**	**.408***	.318	1	**.372***	**.497****	.353
Osteocalcin	**P-value**	**.025**	.087		**.043**	**.005**	.056
Salivary	**r-value**	-.017	.200	**.372***	1	**.377***	**.441***
Osteocalcin	**P-value**	.931	.288	**.043**		**.040**	**.015**
Serum	**r-value**	.040	-.198	**.497****	**.377***	1	**.828****
Fibronectin	**P-value**	.835	.294	**.005**	**.040**		**.000**
Salivary	**r-value**	.147	-.027	.353	**.441***	**.828****	1
Fibronectin	**P-value**	.438	.889	.056	**.015**	**.000**	
**. Correlation is significant at the 0.01 level (2-tailed).							
*. Correlation is significant at the 0.05 level (2-tailed).							

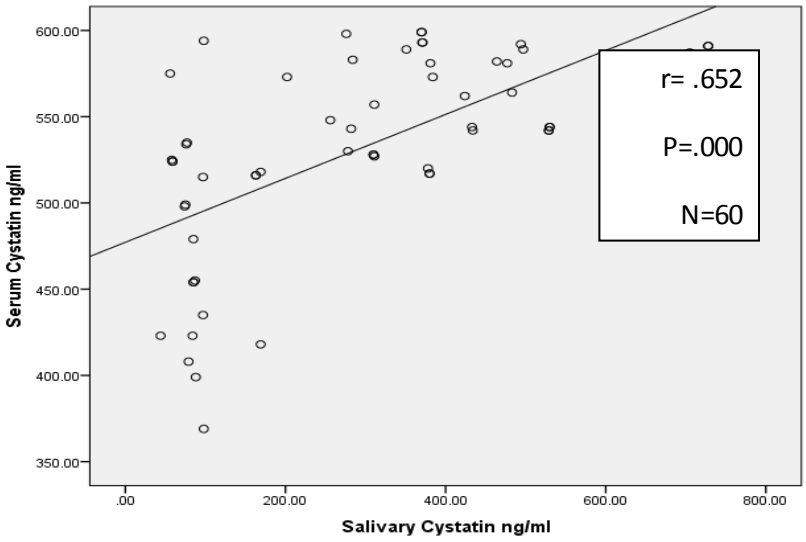

Figure. 6: Correlation between Serum Cystatin and Salivary Cystatin within Diabetic group.

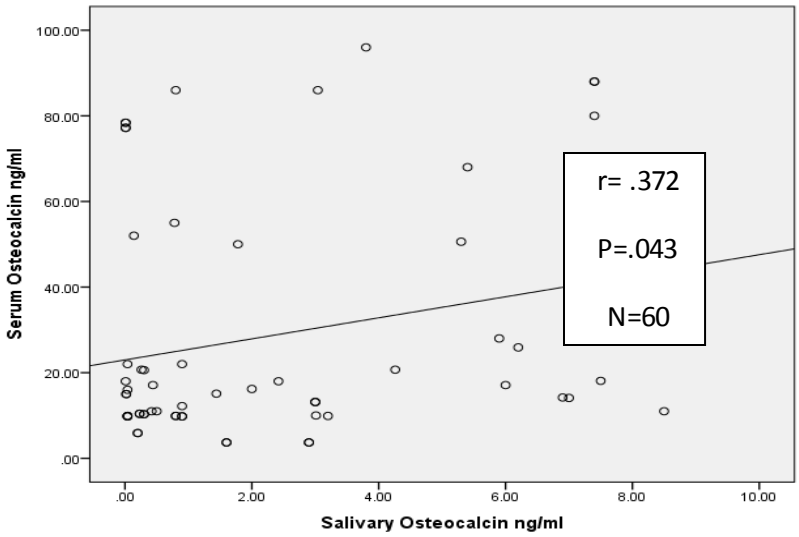

Figure. 7: Correlation between Serum Osteocalcin and Salivary Osteocalcin within Diabetic group.

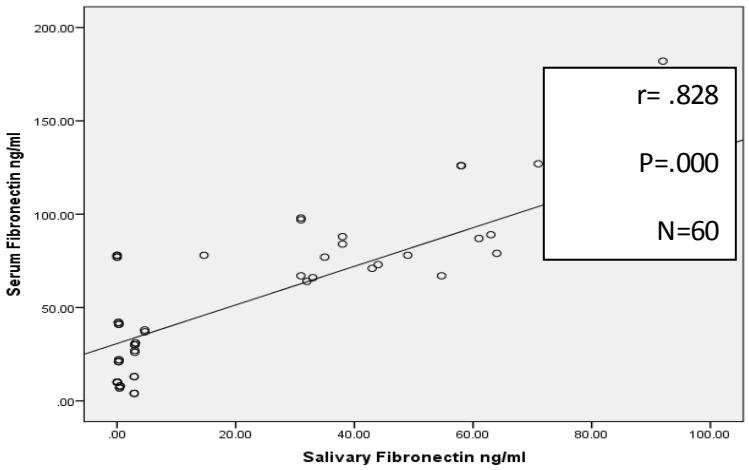

Figure. 8: Correlation between Serum Fibronectin and Salivary Fibronectin within Diabetic group.

Correlation within Diabetic with periodontitis group.

Serum Cystatin, Osteocalcin and Fibronectin significantly correlated with Salivary Cystatin, Osteocalcin and Fibronectin respectively within the diabetic with periodontitis group, Table -4 and figures. 9, 10 and 11.

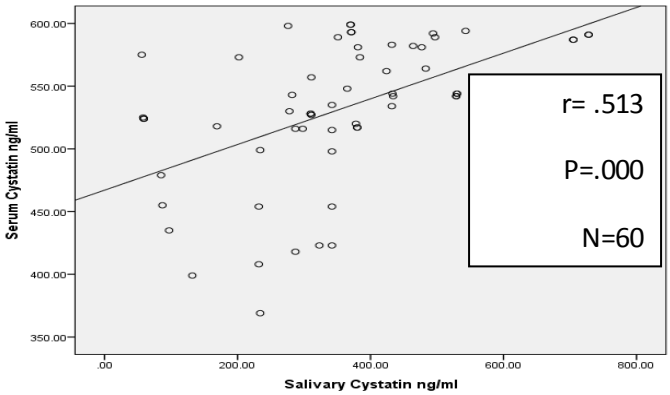

Figure. 9: Correlation between Serum Cystatin and Salivary Cystatin within Diabetic with Periodontitis group.

Table -4: Correlation between Serum Cystatin, Osteocalcin and Fibronectin with Salivary Cystatin, Osteocalcin and Fibronectin within the Diabetic with periodontitis group.

Variable		Serum Cystatin	Salivary Cystatin	Serum Osteocalcin	Salivary Osteocalcin	Serum Fibronectin	Salivary Fibronectin
Serum Cystatin	**r- value**	1	**.513***	.126	**-.431***	.246	.171
	P-value		**.000**	.508	**.017**	.190	.367
Salivary	**r- value**	**.513***	1	**-.453***	.160	-.087	-.091

Cystatin	**P-value**	**.000**		**.012**	.398	.647	.633
Serum	**r- value**	.126	**-.453***	1	**.365**	.175	.006
Osteocalcin	**P-value**	.508	**.012**		**.004**	.355	.977
Salivary	**r- value**	**-.431***	.160	**.365**	1	.265	**.391***
Osteocalcin	**P-value**	**.017**	.398	**.004**		.157	**.033**
Serum	**r- value**	.246	-.087	.175	.265	1	**.917****
Fibronectin	**P-value**	.190	.647	.355	.157		**.000**
Salivary	**r- value**	.171	-.091	.006	**.391***	**.917****	1
Fibronectin	**P-value**	.367	.633	.977	**.033**	**.000**	
*. Correlation is significant at the 0.05 level (2-tailed).							
**. Correlation is significant at the 0.01 level (2-tailed).							

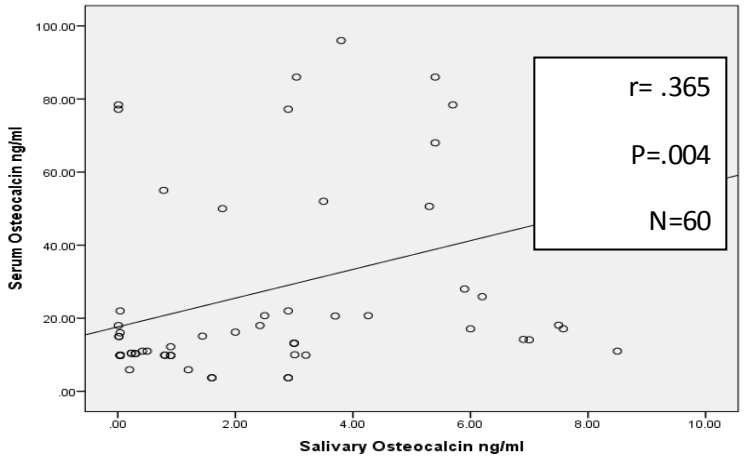

Figure. 10: Correlation between Serum Osteocalcin and Salivary Osteocalcin within Diabetic with Periodontitis group.

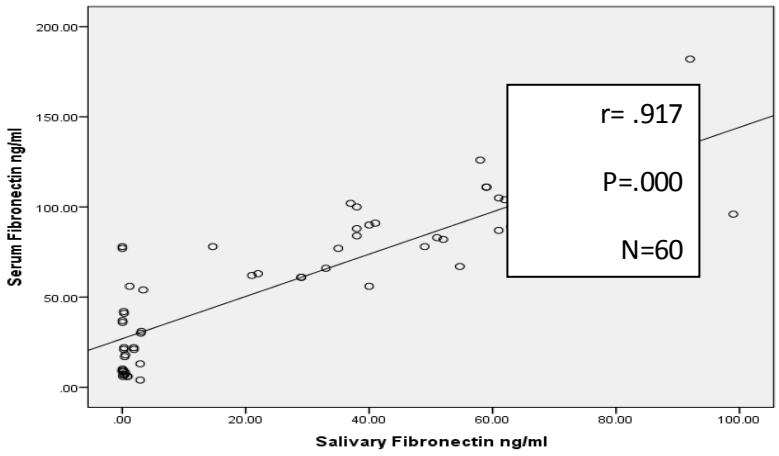

Figure. 11: Correlation between Serum Fibronectin and Salivary Fibronectin within Diabetic with Periodontitis group.

In control group, FBS was significantly correlated with serum osteocalcin [r=0.779, P=0.008] and inversely significantly correlated with salivary osteocalcin [r=-0.685, P=0.029], Tables

17 an and inversely significantly correlated with salivary osteocalcin [r=-0.685, P=0.029], Tables 5 and 6.

In periodontitis group, FBS not show a significant correlation with serum cystatin, osteocalcin and fibronectin, however, there was a significant correlation with salivary cystatin [r=0.59, P=0.026] and salivary osteocalcin [r=0.559, P=0.038], Tables 5 and 6.

In diabetic group, FBS demonstrated significant correlation with serum osteocalcin [r=0.599, P=0.000], and serum fibronectin [r=0.386, P=0.036]. In addition, FBS show a significant correlation with salivary osteocalcin [r=0.577, P=0.001] and fibronectin [r=0.599, P=0.000], Tables 17 and 18. However, in diabetic and periodontitis group, FBS was with a significant association serum [r=0.420, P=0.021] and salivary fibronectin [r=0.446, P=0.009], Tables 5 and 6.

Table. 5. Correlation between fasting blood sugar and serum cystatin ,osteocalcin, and fibronectin.

Variable		Control	Periodontitis	Diabetic	Diabetic with periodontitis
Serum Cystatin	r- value	.391	-.372	.197	.068
	P- value	.264	.190	.296	.722
Serum Osteocalcin	r- value	**.779****	-.453	**.599**	.002
	P- value	**.008**	.104	**.000**	.990
Serum Fibronectin	r- value	-.075	.107	**.386**	**.420**
	P- value	.837	.715	**.036**	**.021**
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

Table .6. Correlation between fasting blood sugar and salivary cystatin ,osteocalcin, and fibronectin.

Variable		Control	Periodontitis	Diabetic	Diabetic with periodontitis
Salivary Cystatin	r- value	-0.427	**0.590**	0.199	0.148
	P- value	0.218	**0.026**	0.292	0.437
Salivary Osteocalcin	r- value	**-0.685**	**-0.559**	**0.577**	0.102
	P- value	**0.029**	**0.038**	**0.001**	0.592
Salivary Fibronectin	r- value	-0.206	0.455	**0.599**	**0.466**
	P- value	0.568	0.102	**0.000**	**0.009**
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

DISCUSSION

Serum and salivary Cystatin, osteocalcin and fibronectin were significantly correlated in patients with periodontal disease. This correlation indicated that determination of such

biomarkers in saliva was with predictive value in diagnosis of periodontitis and co-effective, and non-invasive procedures. This finding was in line of using saliva as sample for determination of large number of disease biomarkers.[8-13]

Salivary and serum osteocalcin, cystatin and fibronectin were significantly positively correlated in control group, periodontitis group, diabetes group and those with diabetes with periodontitis. These finding in subject with diabetes indicated that saliva sample was practical and suitable as alternative for blood sampling to determine the above mentioned biomarkers.
FBS shows a significant correlation with serum and salivary osteocalcin in control group; salivary cystatin and salivary osteocalcin in periodontitis group; serum and salivary osteocalcin and serum and salivary fibronectin in diabetic group; serum and salivary fibronectin in diabetic with periodontitis group. However, FBS was with marginal significant correlation with serum and salivary cystatin in control group and salivary fibronectin in periodontitis group. Serum cystatin significantly correlated with serum fibronectin in periodontitis group; and with serum osteocalcin in diabetic group. In addition, salivary osteocalcin was significantly correlated with salivary fibronectin in diabetes with periodontitis group; and diabetic group. The above data of correlation were varied among the studied group, which suggest that, although both periodontitis and diabetes use bidirectional effect on each other, still there was a difference in the magnitude of changes in biomarkers for both conditions. A large scale study to clarify the interaction between the two conditions is warranted.

In diabetic patients, serum osteocalcin (total and undercarboxylated) was with negative correlation to HbA1c[14]. In addition, Zhou *et al* [15] reported a significant association between osteocalcin and triglycerides and diabetes. Furthermore, Kantas *et al*[16] reported a significant association between fibronectin and diabetes. Sarkar and Choudhury[17] found that serum osteocalcin levels were significantly negatively associated with FBS in diabetic patients. In addition Kanazawa et al [18], found a negative significant correlation between osteocalcin and HbA1c in diabetic individuals.

Salivary urea and glucose were two biomarkers that show significant predictivity in diagnosis of metabolic abnormality and correlated to each other.[19]

The significant positive correlation of serum osteocalcin and FBS in periodontitis, diabetes and diabetic with periodontitis subjects indicate that these entities must be taken in consideration since they are more reliable to develop cardiovascular disease.[20]

Kanazawa *et al.*[18] reported a significant negative correlation between serum levels of osteocalcin and % of fat in men with diabetes. This finding may suggest that osteocalcin was associated with lipid metabolism in diabetic subjects. In addition, the association between osteocalcin and development of cardiovascular diseases was supported by a previous reports in patients with osteoporosis.[21-25] and other studies.[26-29].

In conclusion, the patterns of significant correlation are the same in control and periodontitis groups, while it is different in diabetic group and Periodontitis with diabetes. Presence of periodontitis with diabetes alter the trend of association.

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