

Assessment of Salivary Lactoferrin and pH Levels and Their Correlation with Gingivitis and Severity of Chronic Periodontitis (Part: 2)

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ABSTRACT

Background: Periodontal diseases are bacterial infections of the gingiva, bone and attachment fibers that support the teeth and hold them in the jaw. Lactoferrin is a multifunctional glycoprotein and it is the main component of neutrophil polymorphonuclear leukocytes that activated during inflammatory processes such as Periodontal diseases

Aims of the study: Determine the salivary levels of Lactoferrin and pH and their correlations with clinical periodontal parameters (Plaque Index, Gingival Index, Bleeding on Probing, Probing Pocket Depth, and Clinical Attachment Level) and the correlation between Lactoferrin with potential of hydrogen ion (PH), flow rate and α -amylase of study groups that consisted of patients had gingivitis and patients had chronic periodontitis with different severities (mild, moderate, severe) and control group.

Materials and Methods: Salivary Lactoferrin and pH levels were measured from 75 males, age ranged (30-45) years old, that divided into study groups (group of 45 chronic periodontitis patients with different severities which sub-grouped into (Mild=15, Moderate=15 and Severe=15), group of 15 patients with gingivitis) and control group comprised 15 subjects had clinically healthy periodontium.

Results: The levels of salivary Lactoferrin in patients had severe chronic periodontitis were the highest followed by moderate chronic periodontitis then the mild chronic periodontitis then patients had gingivitis. Highly significant differences were demonstrated among the control, gingivitis and chronic periodontitis subgroups and between each pairs of chronic periodontitis subgroups. pH increased in gingivitis group and decreased in chronic periodontitis group with its different severities. Highly significant strong positive correlations between Lactoferrin with clinical periodontal parameters at all groups and subgroups.

Conclusions: The findings of the present study suggest that salivary Lactoferrin can help to monitor the progression of the periodontal disease.

Keywords: gingivitis, chronic periodontitis, Lactoferrin, saliva, pH. (J Bagh Coll Dentistry 2018; 30(1): 46-52)

INTRODUCTION

Periodontal diseases (PD) are bacterial infections of the gingiva, bone and attachment fibers that support the teeth and hold them in the jaw ⁽¹⁾. The two common forms of periodontal diseases are gingivitis and periodontitis.

Gingivitis is an inflammatory response of the gingiva to plaque bacteria, it represents the reversible part of periodontal disease ⁽²⁾, whereas periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament (PDL) and alveolar bone with pocket formation, recession, or both ⁽³⁾.

Chronic periodontitis (CP) has been defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss ⁽³⁾.

Saliva is a unique complex and important body fluid that contains number of systems which serves a wide spectrum of physiological needs Which protect the oral mucosa and the whole body from infection ⁽⁴⁻⁶⁾.

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Due to the non-invasive and simple nature of salivary sample collection, saliva is also considered to be useful for the screening tests for

PD, and a mean of monitoring the response to treatment ⁽⁷⁾.

Salivary potential hydrogen ion (pH), is the measure of the acidity or alkalinity of saliva. The normal salivary pH is from 6 to 7, increasing with increasing alkalinity and decreasing with increasing acidity ^(8,9).

The Lactoferrin (LF) is a multifunctional glycoprotein originally isolated in 1939 from bovine milk. In 1960, it was isolated from human milk. Furthermore, LF is the main component of neutrophil polymorphonuclear leukocytes that, when activated during inflammatory processes, such as PD, they experience degranulation of granules containing proteases, carbohydrates, and antimicrobial substances like lactoferrin, lysozyme and myeloperoxidase as well as other mediators that release directly into the plasma ^(10,11).

The LF is produced by various mammal species, and fulfills diverse functions like antimicrobial, anti-inflammatory, immuno-modulating, anti-oxidant and anti-tumor activities. For all these reasons, this protein is considered an important component for the innate defense response ⁽¹²⁾. Hence, such salivary marker LF and pH can help to enhance oral defense mechanism.

Based on these detectable changes, this study was conducted to find the correlation between salivary levels of LF and pH with severity of PD.

MATERIALS AND METHODS

The human sample included 75 males age range from 30-45 years old. Subjects recruited for this

study were from the Department of Periodontics at the Teaching Hospital of College of Dentistry, University of Baghdad as well as from blood bank in Baghdad. From each subject, unstimulated whole saliva sample was harvested. The salivary pH was measured by using the (DF universal test paper) by immersing the strip into the saliva for about 2 seconds, then waited for color changes for 15 seconds and compared to the color chart present on the plastic case of the product, then each sample cold centrifuged at 1000 rpm for 20 minutes in the Teaching Laboratories of Baghdad Medical City, then the clear supernatant saliva was collected by micropipette into eppendorf tubes and store at -20°C until biochemical analysis of LF.

Full examination of clinical periodontal parameters including plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). Salivary levels of α -amylase and flow rate (FR) were previously assessed in part 1 study⁽¹³⁾. According to clinical periodontal examination, the subjects generally were divided into three main groups:

- 1. (CP) Group:** Consisted of 45 males had chronic periodontitis. This was defined by the presence of at least four sites with PPD $\geq 4\text{mm}$ and clinical attachment loss of 1-2 mm or more⁽¹⁴⁾. Patients in this group subdivided into three subgroups according to the severity of clinical attachment loss⁽¹⁵⁾ into:
 - Mild CP: Consisted of 15 males with clinical attachment loss of 1-2mm.
 - Moderate CP: Consisted of 15 males with clinical attachment loss of 3-4mm.
 - Severe CP: Consisted of 15 males with clinical attachment loss of ($\geq 5\text{mm}$).
- 2. Gingivitis Group:** Consisted of 15 males with gingivitis, this was defined by the presence of signs and symptoms of gingival inflammation and without periodontal pocket or clinical attachment loss.
- 3. Control Group:** Consisted of 15 males with clinically healthy periodontium, this was defined by the absence of any signs and symptoms of gingival inflammation and without periodontal pocket or clinical attachment loss. This group presents a baseline data for the levels of salivary LF.

The inclusion criteria were apparently systemically healthy subjects or patients and at least 20 teeth present.

The exclusion criteria were females, smokers, alcohol drinkers, patients undergone periodontal treatment and /or used a course of anti-inflammatory, antimicrobial or other medications

in the last 3 months before the study and presence of systemic disease, e.g. Diabetes mellitus, cardiovascular disease, rheumatoid arthritis ..etc. For LF enzyme analysis used kit manufactured by MYBioSource which utilizes Enzyme Linked Immuno-Sorbent Assay (ELISA) technique. The procedure was made in the Teaching Laboratories of Baghdad Medical City.

Statistical Analysis:

Descriptive statistics in the form of means, standard deviation (S.D.) and inferential statistics in the form of one-way ANOVA test, LSD test and Pearson's correlation coefficient test (r) were used in this study.

In the statistical evaluation, the following levels of significance (Sig.) were used:

Non-significant	NS	$P > 0.05$
Significant	S	$0.05 \geq P > 0.01$
Highly significant	HS	$P \leq 0.01$

We certify that this study involving human subjects is in accordance with the Helsinki declaration of 1975 as revised in 2000 and that it has been approved by the relevant Institutional Ethical Committee.

RESULTS

In table 1, the biochemical analysis of salivary LF level revealed that severe CP subgroup presented the highest mean value (18.55) followed by moderate CP subgroup (16.31) then the mild CP subgroup (12.49) then gingivitis group (7.79) and lastly the control group showed the minimum mean value (3.90). A highly significant statistical difference was observed among control, gingivitis and CP subgroups with $p \leq 0.000$. On the other hand, physical parameter analysis showed increase in mean values of pH in gingivitis group and decreased in CP subgroups as compared to control group. In addition to that the mild CP subgroup presented the highest mean value (5.53) among the CP subgroups followed by moderate CP subgroup (4.47) and the severe CP subgroup showed the minimum mean value (2.47). Again a highly significant statistical difference was observed among the control, gingivitis and CP subgroups ($p \leq 0.001$).

Regarding salivary LF and pH, highly significant differences were revealed between all pairs of CP subgroups and between each one of CP subgroups with gingivitis and control groups as well as both groups with each other (Table 2). The results of correlations (Table 3) between mean values of PLI and GI for control, gingivitis groups and CP subgroups with the LF levels were highly significant strong positive correlations. The correlations between mean values of BOP

score 1, PPD, CAL of CP subgroups with the LF were highly significant strong positive, the same result was revealed between mean values of BOP score 1 of gingivitis group with the LF. The correlations between mean values of salivary pH and FR for control, gingivitis groups and CP subgroups with the LF were highly significant strong negative, except the highly significant strong positive correlation of salivary pH at gingivitis group.

From table 4, the correlations between pH with clinical periodontal parameters were almost

highly significant strong negative at CP subgroups, while they were highly significant strong positive correlations between pH with PLI, GI and BOP score 1 at gingivitis group. The correlations between FR with pH were highly significant strong which were positive at CP subgroups while it was negative at gingivitis group.

From table 5 the correlations between LF and α -amylase at control, gingivitis and CP subgroups were highly significant strong positive

Table 1: Descriptive statistics of salivary LF concentrations (ng/mL) and pH for groups and subgroups with difference among Control, Gingivitis and CP subgroups

Groups and Subgroups	Lactoferrin				pH			
	Mean	±S.D.	F-test	p-value Sig.	Mean	±S.D.	F-test	p-value Sig.
Control	3.90	0.64	828.662	0.000 HS	7	0	405.807	0.000 HS
Gingivitis	7.79	0.85			8.67	0.49		
Mild	12.49	1.14			5.53	0.52		
Moderate	16.31	0.47			4.47	0.52		
Severe	18.55	0.79			2.47	0.52		

Table 2: Mean differences of salivary LF and pH between all pairs of groups and subgroups.

Groups and Subgroups		Lactoferrin		pH	
		Mean Difference	p-value Sig.	Mean Difference	p-value Sig.
Mild	Moderate	-3.82	0.000 (HS)	1.06	0.000 (HS)
	Severe	-6.06	0.000 (HS)	3.06	0.000 (HS)
Moderate	Severe	-2.24	0.000 (HS)	2.00	0.000 (HS)
Control	Gingivitis	-3.89	0.000 (HS)	-1.67	0.000 (HS)
	Mild	-8.59	0.000 (HS)	1.47	0.000 (HS)
	Moderate	-12.41	0.000 (HS)	2.53	0.000 (HS)
	Severe	-14.65	0.000 (HS)	4.53	0.000 (HS)
Gingivitis	Mild	-4.70	0.000 (HS)	3.14	0.000 (HS)
	Moderate	-8.52	0.000 (HS)	4.20	0.000 (HS)
	Severe	-10.76	0.000 (HS)	6.20	0.000 (HS)

Table 3: Correlations between the levels of salivary LF with the clinical parameters.

Parameters	Statistical Analysis	Control	Gingivitis	Mild	Moderate	Severe
PLI	r	0.927	0.964	0.861	0.991	0.838
	P	0.000	0.000	0.000	0.000	0.000
GI	r	0.892	0.961	0.898	0.894	0.955
	P	0.000	0.000	0.000	0.000	0.000
BOP Score 1	r	-	0.940	0.871	0.947	0.891
	P	-	0.000	0.000	0.000	0.000
PPD	r	-	-	0.876	0.903	0.930
	P	-	-	0.000	0.000	0.000
CAL	r	-	-	0.796	0.990	0.915
	P	-	-	0.000	0.000	0.000
pH	r	-	0.867	-0.788	-0.865	-0.902
	P	-	0.000	0.000	0.000	0.000
FR	r	-0.954	-0.902	-0.886	-0.952	-0.964
	P	0.000	0.000	0.000	0.000	0.000

Table 4: Correlations between the levels of salivary pH with the clinical parameters.

Parameters	Statistical Analysis	Control	Gingivitis	Mild	Moderate	Severe
PLI	r	-	0.808	-0.785	-0.836	-0.727
	P-value	-	0.000	0.001	0.000	0.002
GI	r	-	0.851	-0.831	-0.688	-0.818

	P	-	0.000	0.000	0.005	0.000
BOP Score 1	r	-	0.835	-0.635	-0.840	-0.816
	P	-	0.000	0.011	0.000	0.000
PPD	r	-	-	-0.778	-0.771	-0.799
	P	-	-	0.001	0.001	0.000
CAL	r	-	-	-0.638	-0.872	-0.777
	P	-	-	0.010	0.000	0.001
FR	r	-	-0.866	0.818	0.818	0.828
	P	-	0.000	0.000	0.000	0.000

Table 5: Correlations between salivary levels of α-Amylase with LF at groups and subgroups

Parameters	Statistical Analysis	Control	Gingivitis	Mild	Moderate	Severe
Lactoferrin with α-Amylase	r	0.898	0.968	0.890	0.951	0.909
	P	0.000	0.000	0.000	0.000	0.000

DISCUSSION

Concerning the LF levels, highly significant difference showed among the CP subgroups and in inter- subgroups comparisons. On the other hand, comparisons revealed highly significant differences between the control with gingivitis groups, also each of them with each one of CP subgroups. So increased level of LF with increased severity of PDs.

These results in accordance with other studies which indicate that significantly higher levels of LF in Gingival Crevicular Fluid (GCF) have been found at sites with gingivitis and CP compared to healthy sites (16-18).

Also in agreement with study that was conducted by Friedman et al. (19), who evaluated the levels of LF in GCF at localized aggressive periodontitis, CP and gingivitis patients, the three PD groups had significantly higher levels of LF as compared with the control group.

In addition, others found the concentration of LF in GCF of sites with CP significantly higher than the healthy sites and its quantification detects the degree of periodontal tissue inflammation. Sites with PD had been shown to have larger volumes of crevicular fluid compared with healthy sites (20) Also, the results of this study were consistent with previous study that conducted by Glimvall et al.,2012 (21) who detected elevated levels of salivary LF, which is a marker for increase in severity of inflammation in CP patients as compared to subjects with clinically healthy periodontium.

The increased levels of LF can explained by increased secretion from the salivary glands and/or increased leakage from the GCF (20).

Where as in PD, an increase in the number of neutrophils in the gingival crevice or pocket and adjacent connective tissue had been detected, once these neutrophils are activated they experience degranulation of granules containing

proteases, carbohydrases, and antimicrobial substances like LF, lysozyme, myeloperoxidase as well as other mediators. Thus, increasing inflammatory mediators which consequently will define the destination of the response towards resolution or tissue damage hence LF is the main component of neutrophil granules, and is present in saliva and GCF and interacts with pathogenic periodontal pathogenes, also LF can be an important element for host defense against PD(22). The results showed that pH level increased in gingivitis group and decreased in CP subgroups with highly significant differences among the CP subgroups and at inter subgroups comparisons.

At the same time, highly significant differences between the control with gingivitis groups, also between the control and gingivitis groups with each one of CP subgroups were demonstrated.

These results were in agreement with the previous study (23), which evaluated salivary pH as a diagnostic biomarker and observed a correlation between pH of saliva and presence of PD when compared with healthy group. Salivary pH in patients with chronic generalized gingivitis was more alkaline than that in patients with clinically healthy periodontium. While in patients with chronic generalized periodontitis, the salivary pH was more acidic than the control group.

Salivary pH above 7.0 usually indicates alkalinity. Excessive alkalinity can bring about the same anaerobic conditions as acidemia, but it is much rare condition hence, plaque bacteria take calcium compounds in the environment and use the minerals to protect them from the high pH. The two key factors for plaque formation are, first; there must be oral bacteria to attack food particles and elevate the pH. Second, the pH must elevate above 7.6 to form dental plaque crystals that cause PD. Thus, alkaline pH is essential for plaque growth that explain the mildly alkaline pH of the saliva obtained from the subjects with generalized chronic gingivitis (23).

On the other hand, in patients with chronic generalized periodontitis, there was increase acidity (pH below 7) with the increasing severity of periodontal tissue inflammatory condition⁽²³⁾.

Some studies^(24,25) detected that in CP patients, there was significant increase in the pH while other⁽²⁶⁾, revealed non-significant increase in the pH as compared to control group.

Other study⁽²⁷⁾, demonstrated that in CP patients, there was non-significant decrease in the pH while, it was significant decrease in the pH according to previous study⁽²⁸⁾ as compared to control group.

Kudva et al.⁽⁹⁾, revealed that the higher the concentration of hydrogen ions, the lower the pH, and vice versa. There are various sources of hydrogen ions in oral fluids: secretion by the salivary glands in the form of organic and inorganic acids, production by the oral microbiota or acquisition through foods. These ions influence the equilibrium of calcium phosphates in the enamel. Where during CP there is increase in salivary gland secretion and increase in oral bacteria, thus causing increase in the concentration of hydrogen ions, with the lower the pH.

The pH level negatively correlated with proportion of periodontal pathogens, that grow in mildly acidic pH, either utilize or create products that are mild to moderately acidic in nature⁽²⁹⁾.

The correlations between mean values of PLI and GI for control, gingivitis and CP groups and CP subgroups with the LF levels were highly significant strong positive correlations.

Ozdemir et al.⁽¹⁸⁾ showed that PLI and GI scores increased during the experimental gingivitis period of 14 days and positively correlated with LF level.

Significantly higher LF levels in GCF were found in CP sites with higher PLI and GI values⁽²⁰⁾ which indicated the strong correlations of the clinical periodontal parameters with the levels of LF (ng/site) and suggests that increasing severity of periodontal inflammation was accompanied by an increase of LF in GCF. Thus, an increasing in plaque accumulation leads to more severe gingival inflammation with higher level of LF.

The correlations between mean values of BOP score 1, PPD, CAL of CP group and CP subgroups with the LF were highly significant strong positive, the same result was revealed between mean values of BOP score 1 of gingivitis group with the LF.

These could be due to increase in the bacterial invasion and the amount of plaque that caused destruction of periodontal ligament fibers and surrounding alveolar bone with apical migration

of junctional epithelium⁽³⁾. Thus, increasing of mean values of BOP, PPD and CAL with the increasing intensity of inflammation and numbers of neutrophils as a response to inflammatory process and the degranulation of neutrophils cause increase in the level of LF⁽²⁰⁾.

Glimvall et al.⁽²¹⁾ found that in CP patients, the salivary concentrations of LF were positively correlated with percentage of BOP sites and the number of sites with PPD \geq 6 mm. Hence, the BOP showed increase levels during gingivitis and positively correlated with LF level⁽¹⁸⁾.

In addition, the correlation between mean values of salivary pH for gingivitis group with the LF was highly significant strong positive, hence, in gingivitis more alkaline pH (above 7)⁽²³⁾ and increasing in LF level occur⁽¹⁸⁾ as a result of inflammatory process.

While the correlation between mean values of salivary pH for CP group and CP subgroups with the LF were highly significant strong negative thus, increasing the severity of inflammation result in more acidic pH⁽²³⁾ with higher LF level^(20,21).

The correlations between mean values of salivary FR for control, gingivitis and CP groups and CP subgroups with the LF were highly significant strong negative.

During the inflammation increase the LF level through the increase number of neutrophils were demonstrated^(18,20,21) with decline in salivary FR^(30,31).

The correlations between pH and all clinical periodontal parameters at CP group and subgroups were almost highly significant strong negative, while they were highly significant strong positive correlations with PLI, GI and BOP Score 1 at gingivitis group.

Although, other study⁽²⁷⁾, detected that the correlations between pH with CAL was non-significant weak positive but, non-significant weak negative correlations with PLI, GI, BOP Score 1 and PPD in patients with CP.

The increased intensity of inflammation during PD which manifested by increased mean values of clinical periodontal parameters resulting in pH more acidic at CP group and more alkaline at gingivitis group⁽²³⁾.

In addition, the correlations between FR with pH were highly significant strong which were positive that means both physical markers decreased at CP group and subgroups while it was negative at gingivitis group which means that pH increased and FR decreased at this group.

Again, previous study⁽²⁷⁾, revealed that the correlation between FR with pH was non-significant weak positive in patients with CP.

The correlation between LF and α -Amylase at control, gingivitis and CP groups and CP subgroups were highly significant strong positive. There were no other studies that correlate α -Amylase with LF to compare the results of this study with them.

Hence, with PD progression and increased periodontal tissue destruction resulting in the increased release of α -Amylase⁽³³⁾ accompanied by higher LF level^(18,20,21).

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الخلاصة:

الخلفية: أمراض اللثة هي التهابات البكتيرية في اللثة والعظام والألياف الرابطة التي تدعم الأسنان وتثبيتهم في الفك، اللاكتوفيرين هو بروتين سكري متعدد الوظائف وهذا هو العنصر الرئيسي في الكريات البيض العذلة النوى التي تتفاعل أثناء عمليات التهابات مثل أمراض اللثة.

أهداف الدراسة: تحديد المستويات الاعيانية من اللاكتوفيرين وعلاقته مع مؤشرات ما حول الأسنان السريرية (مؤشر الصفيحة الجرثومية، مؤشر التهاب اللثة، مؤشر النزف عند التسبير، عمق جيوب اللثة ومستوى الانسجة الرابطة سريريا)، تقدير مستوى درجة الحموضة وعلاقته مع مؤشرات ما حول الأسنان السريرية واللاكتوفيرين والفلا-أميليز ومعدل التدفق في مجموعات الدراسة التي تتكون من (المرضى لديهم التهاب اللثة و التهاب اللثة المزمن مع الشدة المختلفة (طفيفة، معتدلة، شديدة)) والمجموعة الضابطة (دواعم صحية سريريا).

المواد وطرق: مستويات درجة الحموضة و اللاكتوفيرين تم قياسها من ٧٥ من الذكور, تتراوح أعمارهم (٣٠-٤٥) سنة, التي قسمت إلى مجموعتين الدراسة (المجموعة الاولى يتألف من ٤٥ مريض لديهم التهاب اللثة المزمن مع الشدة المختلفة التي قسمت إلى فروع (طفيف = ١٥، معتدل = ١٥ وشديد = ١٥)، وتألفت المجموعة الثانية من ١٥ مريضا مع التهاب اللثة) و تتألف المجموعة الضابطة الثالثة من ١٥ شخصا لديهم دواعم صحية سريريا.

النتائج: كانت المستويات اللعابية للاكتوفيرين في المرضى الذين يعانون من التهاب اللثة المزمن الشديد أعلى تليها التهاب اللثة المزمن المعتدل ثم التهاب اللثة المزمن الطفيف ثم المرضى الذين يعانون من التهاب اللثة وأظهرت اختلافات كبيرة جدا بين كل زوج من المجموعات الفرعية من التهاب اللثة المزمن و أعلى مستوى له عند مرضى التهاب اللثة المزمن الشديد . ارتفاع مستوى درجة الحموضة في مجموعة التهاب اللثة و انخفاضها في مجموعة التهاب اللثة المزمن مع الشدة المختلفة. الارتباطات معنوية عالية إيجابية قوية للغاية بين اللاكتوفيرين مع مؤشرات ما حول الاسنان السريرية في جميع المجموعات والمجموعات الفرعية.

الاستنتاجات: نتائج الدراسة تشير إلى أن اللاكتوفيرين اللعابي يمكن أن يساعد على مراقبة تطور امراض اللثة

