



Estimation of the concentration of some biochemical parameters and bacterial profile in patients with periodontitis and its relationship with cardiovascular disease

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ABSTRACT

Background: Concerning the widespread occurrence of hyperlipidemia, a significant risk factor for cardiovascular disease, and unclear findings on the connection between periodontal disease and the levels of lipids in the blood. **Aim of the study:** This study aimed to isolation different bacterial type from periodontitis patients and evaluated level of lipid profile in the same patients **Material and methods:** The study included a collection age group of 90 samples swaps (patient and control), 60 samples from patients with periodontitis attending outpatient clinics and Balad General Hospitalthe sample were collected for different ages between 15-55 years old and both sexes, from july 2023to December 2024. The sample was taken using a sterile cotton swab (transport cotton swab). The blood samples were taking from their arms from (cubital vien), 5cc of venous blood taken from patients & control healthy individual, Samples were cultured directly using the plotting method on culture media suitable for bacterial growth, which included blood agar, MacConkey agar, mannitol salt agar, MSB agar, and chromo agar. The plates were incubated upside down at 37°C for 18–24 days. The bacterial isolates were identified based on physiological tests and biochemical tests.

Result: The present study's result showed About (60%) showed positive bacterial growth which were *Prophyromonas gingivalis* (30%), *Staph. aureus* (16.7%), *Strep. pneumonia* (11.7%), *Staph. epidermidis* (10%), *Strep. pyogenes*, *Strep. Viridans*, *Strep. mutans* (8.3% %) for them, and (6.7%) for *Enterococcus fecalis* that was diagnosed based on biochemical tests, and the diagnosis of some bacterial species was confirmed with the VITEK device., whereas (40%) showed no growth, And there were a high significant differences between the periodontitis patient compared with control group in mean level of cholesterol, HDL and LDL which scored (151.1±42.7, 139.9±41.4, 184.9±25.4) respectively in patient compared with controls (80.0±16.8, 46.2±14.6, 99.1±27.1). While Triglyceride and VLDL showed no differences between studied groups. **Conclusion:** This study concluded increase cholesterol and low-density lipoprotein in periodontitis patient, While no differences in Triglyceride and VLDL level

تقدير تركيز بعض المعايير الكيموحيوية والخصائص البكتيرية لدى مرضى التهاب اللثة وعلاقتها بأمراض القلب والأوعية الدموية

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الملخص

الخلفية: فيما يتعلق بانتشار مرض ارتفاع الدهون في الدم على نطاق واسع، وهو عامل خطر كبير لأمراض القلب والأوعية الدموية، ونتائج غير واضحة حول العلاقة بين أمراض اللثة ومستويات الدهون في الدم. هدف الدراسة: هدفت هذه الدراسة إلى عزل أنواع بكتيرية مختلفة من مرضى التهاب اللثة وتقييم مستوى الدهون في نفس المرضى.

المواد والطرق: شملت الدراسة مجموعة من 90 عينة (المرضى والأصحاء)، 60 عينة من مرضى المصابين بالتهاب اللثة تم تشخيصهم في العيادات الخارجية ومستشفى البلد العام تم جمع العينة لمختلف الأعمار ولكلا الجنسين للفترة من يوليو 2023 إلى ديسمبر 2024. وتم أخذ العينة باستخدام قطعة قطن معقمة (مسحة قطنية للنقل). تم أخذ عينات من منطقة الذراع سعة 5 سم من الدم الوريدي المأخوذ من المرضى والأفراد الأصحاء، وتمت زراعة العينات مباشرة باستخدام طريقة التخطيط على أوساط الزراعة المناسبة للنمو البكتيري، والتي شملت أجار الدم، أجار ماکونكي وملح المانيتول. أجار، أجار MSB، وأجار كرومو. تم تحضير الأطباق رأسًا على عقب عند درجة حرارة 37 درجة مئوية لمدة 18-24 يومًا. تم تشخيص العزلات البكتيرية بناء على الاختبارات الفسيولوجية والكيميائية الحيوية

النتائج: أظهرت النتائج الحالية ان اعلى نسبة سجلت كانت لبكتريا Porphyromonas gingival بنسبة 30% تليها بكتريا Staph. aureus بنسبة 16.7% و Strep. pneumonia بنسبة 11.7% و Strep. pyogenes، Staph. epidermidis (10%)، Staph. Viridans، Strep. mutans (8.3%) و Enterococcus fecalis بنسبة 6.7% وتم تأكيد تشخيص بعض الأنواع البكتيرية بجهاز VITEK، في حين لم تظهر (40%) أي نمو. أظهرت نتائج الدراسة الحالية وجود فروق ذات دلالة إحصائية عالية بين مريض التهاب اللثة ودواعم الأسنان مقارنة مع مجموعة الأصحاء في المستوى المتوسط من الكوليسترول، HDL و LDL الذي سجل (42.7±151.1، 41.4±139.9، 25.4±184.9) على التوالي في المريض مقارنة مع مجموعة الأصحاء. الضوابط (80.0 ± 16.8، 14.6 ± 46.2، 27.1 ± 99.1). بينما لم تظهر الدهون الثلاثية والبروتين الدهني منخفض الكثافة (VLDL) أي فروق بين المجموعات المدروسة. الاستنتاج: لخصت هذه الدراسة إلى زيادة الكوليسترول والبروتين الدهني منخفض الكثافة لدى مرضى التهاب اللثة، في حين لا توجد فروق في مستوى الدهون الثلاثية والبروتين الدهني منخفض الكثافة (VLDL).

1. Introduction

Periodontal disease is a prevalent disorder that primarily targets the oral cavity in humans [1]. Periodontal problems are prevalent in both developed and developing nations, affecting around 20-50% of the worldwide population. It is prevalent in youth, adults, and elderly individuals, making it a subject of public health significance. Periodontal problems are linked to multiple risk factors, such as smoking, insufficient dental hygiene, diabetes, medication usage, advanced age, hereditary susceptibility, and stress [2]. Hyperlipidaemia is a condition that leads to the development of ischemic heart disease, atherosclerosis, and stroke. Additionally, elevated levels of triglycerides in the blood can result in pancreatitis [3]. The primary focus in hyperlipidemia is determining the underlying cause of this disorder. The most commonly acknowledged causes include genetic disorders, a diet high in lipids and hydrogenated fats, insufficient intake of antioxidant agents, and a lack of adequate physical exercise due to an unhealthy lifestyle [3]. A recent study has shown a causal link between elevated levels of lipids in the blood and the development of periodontal disease. Recent studies demonstrate a correlation between periodontal diseases and hyperlipidemia, suggesting that periodontal disease may be an underlying factor for hyperlipidemia. The theory is outlined in the study conducted by Losche et al, which showed a significant increase in Total serum Cholesterol and LDL levels among patients with periodontitis compared to the control group [4]. This finding has been supported by several other

studies [5-8]. However, there were also some studies that did not find a significant relationship [9-11]. This study was done to investigate the relationship between chronic periodontitis and serum lipid levels, due to the contentious evidence and increasing trend of serum lipid levels, which may lead to unknown complications.

2. Materials and methods

Collection of Samples

The study included a collection of 90 samples (patient and control), 60 samples from patients with **periodontitis** attending outpatient clinics and Balad General Hospital. The samples were collected for different ages and both sexes, from July 2023 to December 2024. The sample was taken using a sterile cotton swab (transport cotton swab). The questionnaire information included: Chronic diseases of the patient such as diabetes and heart disease, Patient name, age, gender, etc

Collection of Blood Sample and Plasma Preparation

The blood samples were taken from their arms from (cubital vein), 5cc of venous blood taken from patients & control healthy individual and put it in gel tubes. Blood samples preserved in a cool box containing ice packs until transferring the samples to the laboratory. Plasma separated from blood samples using centrifuge at speed 4000rpm for (10min) then plasma samples preserved immediately into other plain tubes and stored in freeze at (- 20° C) until they were assayed. The measurement of lipid profile was done by spectrophotometer.

Specimens Culturing

The diagnosis of bacteria based on Sequential steps, initiated with culture on blood agar, MacConkey agar, mannitol salt agar, MSB agar, and chromo agar, microscopic examination, biochemical analysis, finally diagnosed with VITEK device, according to[12].

Statistical methods

Data analysis was conducted using version 19 of the Statistical Package for Social Sciences (SPSS). The examination of the findings utilised a t-test sample. The data was displayed in a suitable format. P-values below 0.05 were considered to be statistically significant.

3. Result

Isolation & identification of bacteria from periodontitis patients

A total of 60 clinical samples were obtained from patient's Periodontitis. After collecting the

samples all samples were grown on blood agar and MacConkey agar, mannitol salt agar and Chromogenic Medium and other simple media plates and incubated aerobically at 37 °C for 24 hours.

About (60%) showed positive bacterial growth which were *Prophyromonas gingivalis* (30%), *Staph. aureus* (16.7%), *Strep. pneumonia* (11.7%), *Staph. epidermidis* (10%), *Strep. pyogenes*, *Strep. Viridans*, *Strep. mutans* (8.3%) for them, and (6.7%) for *Enterococcus fecalis* that was diagnosed based on biochemical tests, and the diagnosis of some bacterial species was confirmed with the VITEK device., whereas (40%) showed no growth, which might be attributed to antibiotic treatment or the presence of other types of causative agents which may need specialized diagnostic tests

Table 1: Bacterial percentage in periodontitis

Bacteria	No. of isolate	Percentage
<i>Strep. pyogenes</i>	5	8.3%
<i>Strep. Viridans</i>	5	8.3%
<i>Strep. pneumonia</i>	7	11.7%
<i>Strep. mutans</i>	5	8.3%
<i>Staph. aureus</i>	10	16.7%
<i>Staph. epidermidis</i>	6	10%
<i>Prophyromonas gingivalis</i>	18	30%
<i>Enterococcus fecalis</i>	4	6.7%
Total	60	100%

Study means of Lipid profile in Periodontitis patients compared with control group

The present study's result showed there were a high significant difference between the periodontitis patient compared with control group in mean level of cholesterol, HDL and LDL

which scored (151.1±42.7, 139.9±41.4, 184.9±25.4) respectively in patient compared with controls (80.0±16.8,46.2±14.6,99.1±27.1). While Triglyceride and VLDL showed no differences between studied groups, table (2).

Table 2: Mean of Lipid profile in Periodontitis patients compared with control group

Transactions	Triglyceride mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Patients	114.5±22.5	151.1±42.7	139.9±41.4	184.9±25.4	23.22±9.03
Control	107.1±24.9	80.0±16.8	46.2±14.6	99.1±27.1	20.25±7.51
T-test	1.93	11.27	15.69	14.44	1.65

Study the relation between the means of lipid profile according to Age groups.

The study showed comparison between three age group, G1(20-40 years), G2(41-60 years), G3(61—80 years) with the mean level of lipid profile. There was a high significant difference (P<0.05) in G1, G2, G3 of patients, cholesterol (151.03±21.00, 152.30±24.47, 147.70±22.50) compared with control groups (79.55±11.06, 88.17±7.310, 70.00±12.80), in HDL

(140.63±26.17, 140.04±18.15, 136.40±24.90) compared with control (42.15±8.370, 53.67±9.180, 40.00±7.590), in LDL (184.70±28.24, 183.39±21.50, 190.90±27.60) compared with controls (99.79±13.16, 90.00±18.70, 99.40±20.20) respectively. While showed no significant differences (p>0.05) between triglyceride and VLDL patients and control according to the ages groups, table (3).

Table 3: Relationship between lipid profile and age groups in periodontitis patients compared with control.

Age group		Cholesterol	Triglyceride	HDL	LDL	VLDL
G1	P	151.03±21.00 a	116.70±25.39a	140.63±26.17a	184.70± 28.24a	23.34±4.33a
	C	79.55±11.06 b	103.15±18.97a	42.15±8.370b	99.79±13.16b	20.83±2.48 a
G2	P	152.30±24.47 a	114.65±21.21a	140.04±18.15a	183.39±21.50a	23.89±3.76 a
	C	88.17±7.310b	102.68±20.08a	53.67±9.180b	90.00±18.70b	20.67±4.01a
G3	P	147.70±22.50a	104.90±17.8a	136.40±24.90a	190.90±27.60a	20.51±3.45a
	C	70.00±12.80b	106.00±24.10a	40.00±7.590b	99.40±20.20b	21.20±3.81a
P-value		0.0006	0.5	0.0008	0.0009	0.5

Study relation between lipid profiles in periodontitis and control groups according to Genders

The study showed comparison between Gender groups with the mean level of lipid profiles. There was a high significant difference (P<0.05) in male and female groups of patients cholesterol (154.06±24.18, 147.79±21.55) compared with control groups (78.86±17.89, 81.00±16.27), in HDL (138.69±28.45a, 141.32±25.26) compared

with control (43.86±7.830,48.19±8.420), in LDL (183.91±26.86, 186.07±24.13) compared with controls (96.00±18.93,101.82±19.01), VLDL showed difference in female patients (25.68±8.62) compared with control (20.06±7.88) respectively. While showed no significant differences (p>0.05) between triglyceride patients and control according to the genders, table (4).

Table 4: Relationship between lipid profile and gender in periodontitis patients compared with control.

Gender		Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Male	P	154.06±24.18 a	116.13±24.20a	138.69±28.45a	183.91±26.86 a	21.07±8.96 ^b
	C	78.86±17.89 b	106.07±20.34a	43.86±7.830b	96.00±18.93b	20.46±7.36 ^b
Female	P	147.79±21.55a	112.71±21.16a	141.32±25.26a	186.07±24.13a	25.68±8.62a
	C	81.00±16.27b	108.01±19.51a	48.19±8.420b	101.82±19.01b	20.06±7.88b
P-value		0.0008	0.3	0.00001	0.00004	0.05

Study means of lipid profile according to bacterial causes

The study showed comparison between bacterial causes with the mean level of lipid profiles. There was a high significant difference (P<0.05) in patients' cholesterol, HDL and LDL that

infected with aerobic and anaerobic bacteria compared with control groups. While showed no significant differences (p>0.05) between triglyceride and VLDL patients and control according to the bacterial causes, table (5).

Table 5: Relationship between lipid profile and bacteria in periodontitis patients compared with control.

Transactions	Triglyceride mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Aerobic bacteria	115.21±25.23 ^a	152.43±20.28 ^a	134.95±29.62 ^a	183.33±27.23 ^a	23.24±4.61 ^a
Anaerobic bacteria	112.94±26.41 ^a	148.10±29.10 ^a	151.50±24.30 ^a	188.61±20.88 ^a	23.17±5.22 ^a
Control	106.10±24.95 ^a	80.00 ±16.78 ^b	46.17 ± 8.620 ^b	99.11±17.13 ^b	20.25±3.51 ^a
P-value	0.23	0.0008	0.00004	0.0007	0.308

4. Discussion

Periodontal disease is an inflammatory condition that happens when the biofilm of bacteria in the mouth interacts with the immune-inflammatory response of the body, leading to changes in the balance of bone and connective tissues [13].

The results of the current study showed here were a high significant difference between the periodontitis patient compared with control group in mean level of cholesterol, HDL and LDL. While Triglyceride and VLDL showed no differences between studied groups. These study agreements with the research conducted by Zhang *et al.*, [14] demonstrated that patients with chronic periodontitis exhibited a notable modification in their lipid profile when

compared to healthy individuals. Patients with chronic periodontitis had a significant increase in the levels of TC, VLDL cholesterol, and TGL. The study compared the mean level of lipid profiles with bacterial causes. When comparing the cholesterol, HDL, and LDL of patients infected with aerobic and anaerobic bacteria to the control groups, there were significant changes (P<0.05). However, according to the bacterial causes, there were no significant differences (p>0.05) between the control group and the triglyceride and VLDL patient, according to the study Chen *et al.*, and Mirzaei *et al.*, [15,16], there is evidence to show that periodontitis is linked to a higher likelihood of developing dyslipidemia. Thus, addressing

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periodontitis could potentially enhance dyslipidemia, namely by improving HDL and triglyceride levels. Mikam *et al.*, [17] demonstrated that periodontitis patients exhibited significantly elevated levels of mean serum LDL cholesterol (126.62) and total cholesterol (173.32) compared to the control group. Anomalous blood levels of LDL and HDL were identified in various age cohorts. The study JIA *et al.*, [18] found that individuals with periodontitis who were 40 years and older had greater levels of blood triglycerides (TG) compared to the control group.

Gram-negative bacteria like *Aggregatibacter Actinomycetemcomitans* and *Porphyromonas gingivalis* (*P. gingivalis*). These bacteria, along with less harmful organisms, form highly organised complex communities called biofilms. The results of the current study showed that the

highest percentage of bacterial isolates was *Porphyromonas gingivalis*, at 30%, followed by *Staph. aureus*, which amounted to 16.7% compared to other isolates causing periodontitis, and this study was consistent with the researchers' study Pirih *et al.*, [19]. A notable correlation between being female and PISA values in relation to high-density lipoprotein cholesterol level was observed in cases of *Porphyromonas gingivalis* infection. There was a negative correlation between periodontal inflammation and greater levels of high-density lipoprotein cholesterol, particularly in females [20].

Conclusion

This study concluded increase cholesterol and low density lipoprotein in periodontitis patient, While no differences in Triglyceride and VLDL level

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