The influence and assessment of E-cadherin sera levels with periodontitis and gingivitis

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Abstract

Periodontal disease occurs as a result of microorganisms and inflammation derived from the host, which is considered the main reason to teeth loss worldwide. E-cadherin is a protein present in the tissue of the gingiva that acts as a barrier against bacteria, so it is necessary to find it in the normal range, and their deficiency facilitates the entry of bacteria and other organisms penetrability to the tissue. The present study aims to investigate the possibility of using E-cadherin (E-cad) protein as a new marker for the diagnosis and progression of periodontal disease. This study was conducted at the University of Baghdad, College of Dentistry, Department of Periodontics, in the Periodontics clinic received 80 new patients 40 gingivitis cases, and 40 periodontitis. Periodontal parameters were measured for every patient. In order to assess the level of E-cadherin & vitamin C, and Ca2+.The result showed that significantly increased levels of E-cad in patients with Periodontal disease & vitamin C levels were significantly decreased in periodontitis & gingivitis patients compared with a control group. In conclusion, high levels of E-cadherin found in periodontal disease patients may be assessed and used as a marker for diagnosis and progression of the disease, vitamin C & Ca2+ deficiency is considered a risk factor for periodontal disease.

Keyword: *E-cadherin, Vitamin C, Ca2+, periodontal disease, periodontitis, gingivitis.*

INTRODUCTION

Periodontal disease affects a large number of people, as periodontal inflammation leads to the destruction of the alveolar bone as well as the hard tissues, and leads to tooth loss, where it is considered a risk factor for other diseases [1]. periodontitis destruction in soft and hard tissue [1], while gingivitis destruction occurs only in soft tissue [2], periodontitis is one of the leading causes of tooth loss, which can affect the quality of life, mastication, and esthetics also it has long-term effects on public health [3].

The gingival epithelium serves as a physical barrier to separate the biofilm from the gingival tissue [4], acting as the initial line of defense against bacterial invasion periodontal disease [4], tissue damage mediated by host cells may be caused by cytokines and bacterial agents [5], building and regulating cell adhesion is important for the maintenance and development of tissue organization in living organisms [6]. All epithelial cells express the E-cad member [6], and expression of E-cadherin (encoded by Cdh1) [7] cadherins consist of transmembrane cell-surface glycoproteins that play a role in Ca2+-dependent cell-cell adhesion [8], they are essentially polypeptides that undergo alterations to become proteins [9], E-cadherin, which is in the middle between type IV collagen that found in gingiva and epithelial cells [10].

E-cadherin in the epithelial-mesenchymal transition (EMT) spectrum, loss of E-cadherin expression is typically regarded as a diagnostic for a mesenchymal phenotype and indicates a reasonably favorable prognosis for cancer patients [6], in which cancer cells alter in shape and extracellular matrix, which results in loss of expression of adhesion molecules like E-cadherin and epithelial-mesenchymal transition.[11], in diverse malignancies, loss of E-cadherin expression or function leads to diminished cell-cell interaction, enhanced invasion, and finally metastasis [12]. cell-cell connections are broken down as a result of the production of Soluble E-Cadherin (sE-cad) by different proteases. consequently, cleaved E-cadherin indicates a major proteolytic event activity, as well as adherens junction disruption [6], traditional mammalian cadherins comprise five extracellular domains numbered from EC1 to EC5, each of which contains calcium-binding sites, these locations each include negatively charged motifs that can bind three Ca2+ ions, increasing the connections between the extracellular domains [15]

In the absence of Ca2+, cadherins are easily destroyed by proteolysis due to their high Ca2+ sensitivity [16], E-cad membrane proteins that are dependent on calcium [16], which the maintenance of periodontal health when enough calcium [15], as well as it necessary to build bone and muscles [16]. Vitamin C commonly known as ascorbic acid [17], the presence of vitamin C is necessary to reduce reactive oxygen species (ROS) [18], as well as suppress cellular senescence, and promotes the self-renewal and maturation of periodontal stem/progenitor cells [19], it is one of the key elements in the production of collagen, which makes up a large portion of the connective tissue in alveolar bone, cement, gingiva, and periodontal ligaments. When inflammation happens, the tissue's antioxidant levels fall quickly, and the formation of free radicals rises near the inflammatory site in particular inflammatory conditions like periodontal diseases [20].

The hypothesized of this study is that an increased level of serum E-cad with periodontal disease may be used as a potential marker for diagnosis and progression of that disease and to find a relationship between vitamin C & Ca in the periodontal disease, so it is an important existence of adequate levels vitamin C because it has a role in wound healing and to ensures that there is no tissue loss.

Materials and Methods

Study subjects

The case-control study consists of 120 patients divided into three groups, group I with 40 patients 20 males & 20 females in the gingivitis group with a mean age 31.8±7.014 years and a range of 20-45years. group II with 40 patients 20 males & 20 females in the periodontitis group with a mean age 35.8±9.065 and a range of 20-50 years. group III with 40 healthy periodontium (control group) 20 males &20 females to serve as the

control group with a mean age 32.13±7.219 and a range of 21-48 years.

This study was approved gave from the Ethics Committee in the Applied Science Department / University of Technology and Ministry of Health, Baghdad, Iraq (Ref. No. AS 2132 -3-11- 2021) in accordance with the Helsinki Declaration of 1975 revised in 2000, Additionally all participants verbal and written consent was obtained from all participants in this research.

Inclusion Criteria

The cases that have been taken are gingivitis (moderate &severe), generalized periodontitis, and healthy periodontium.

Exclusion Criteria

The cases that were excluded are a history of any systemic disease, previously having smoked either used alcohol, pregnant or lactating women, menopausal women, any taking systemic antibiotic or anti-inflammatory therapy within the last three months, persons with any periodontal treatment containing deep scaling and root planning or local antimicrobial treatment within the last three months, stable periodontitis, patients wearing orthodontic appliances, intake of supplements, and mild gingivitis.

Collection and storage of blood samples

Blood is taken from the periodontal disease patients and the healthy control group, and the samples were centrifuged to separate them. Serum samples were kept until analysis at -40° C.

Sandwich ELISA (Enzyme-Linked Immunosorbent Assay) kits were used to assess the serum concentrations of E-cadherin, and vitamin C (MyBioSource-U.S.A), the technique is based on the idea that an antigen (such as proteins or hormones) binds to a particular antibody, enabling the detection of tiny small levels of the antigen in a fluid sample [21], Ca2+ (Roche- Germany).

Statistical Analyses

Applying biochemical data analysis SPSS (statistical package for social sciences) software, version (26). One-Way ANOVA was used in order to assess the results of this study, after normally distributed results were as mean ±SD.The receiving operating characteristic (ROC) curve was used to find cutoff values and the area under the curve (AUC). Data were considered significant at $P < 0.05$.

Results

The result of this study includes there was a highly significant elevation ($p < 0.001$) in the serum levels of E-cad in the periodontitis group (2.0641±0.2895 ng/mL) as compared with the healthy control group (1.1733 ±0.2930 ng/mL), which also shows highly significant elevation ($p < 0.001$) in the serum levels of E-cad in the gingivitis group (1.5402±0.1868 ng/mL) compared with a healthy control group (1.1733 ±0.2930 ng/mL), as well as show highly significant elevation ($p < 0.001$) when compared periodontitis group (2.0641±0.2895 ng/mL) with gingivitis group (1.5402 ±0.1868 ng/mL) as shown in table (1).

Table 1: Serum levels E-cad, Vitamin C, and Ca2+ measured in patients and controls.

Parameter	Periodontitis group Mean ± SD N=40	Gingivitis group Mean ± SD N=40	healthy control group Mean ± SD N=40	Periodontitis & healthy control (LSD)	gingivitis& healthy control (LSD)	Periodontitis & gingivitis (LSD)
E-cadherin (ng/mL)	2.0641±0.2895	1.5402±0.1868	1.1733 ±0.2930	0.001**	0.001**	0.001**

Vitamin C (ug/mL)	**3.7686±0.5968**	**3.9734±0.6575**	**4.8320 ±1.0166**	**0.001****	**0.001****	**NS**
Ca^{2+} (mg/dL)	**8.9333±1.2628**	**9.4190 ±0.9971**	**10.0367±1.0531**	**0.001****	**NS**	**NS**

SD: standard deviation, N: number of subjects, ** High significance at P⩽ 0.001 level, * Significant at ⩽0.05, NS: Non-significant.

Fig. 1: Serum level of E-cadherin (ng/mL) in Periodontal disease patients and control group.

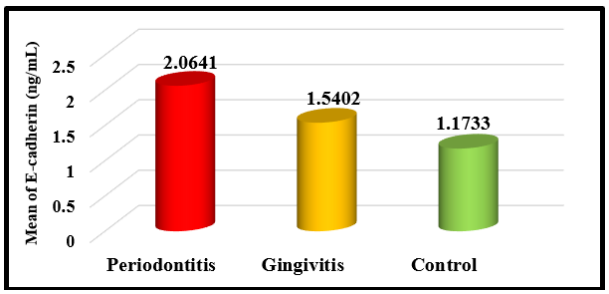
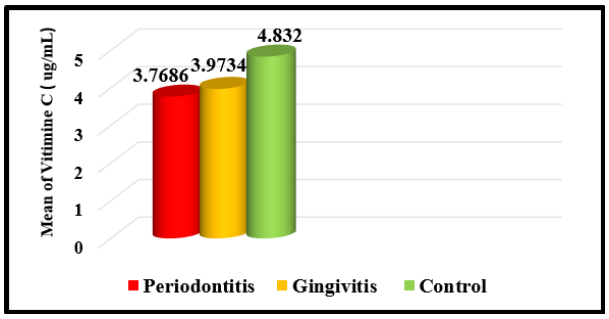
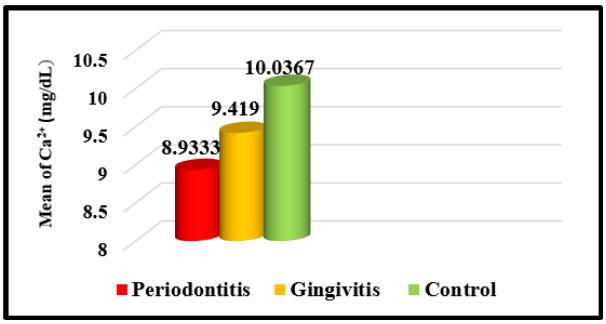


Fig. 2: Serum level of Vitamin C (ug/mL) in Periodontal disease patients and control group.



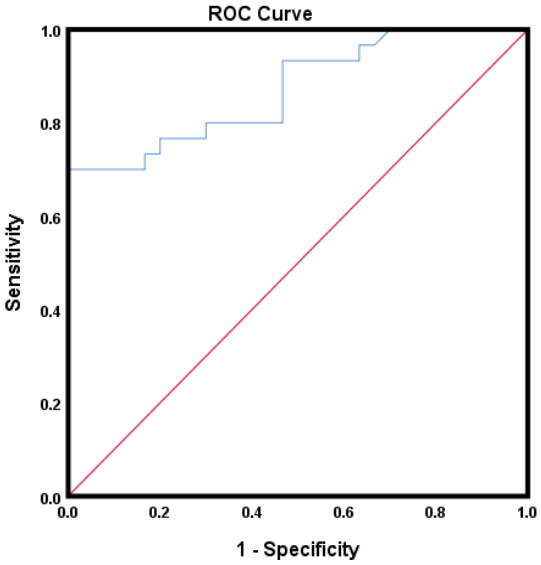
Levels of vitamin C also show a highly significant decrease ($p < 0.001$) between periodontitis groups (3.7686±0.5968 ng/mL) with the healthy control group (4.8320 ±1.0166 ng/mL), and between the gingivitis group (3.9734±0.6575 ng/mL) with the healthy control group (4.8320 ±1.0166 ng/mL), but they found was no significant difference ($p > 0.05$) between periodontitis group (3.7686±0.5968 ng/mL) with the gingivitis group (3.9734±0.6575 ng/mL) as shown in table (1).

Fig. 3: Serum level of Ca2+ (mg/dL) in Periodontal disease patients and control group.

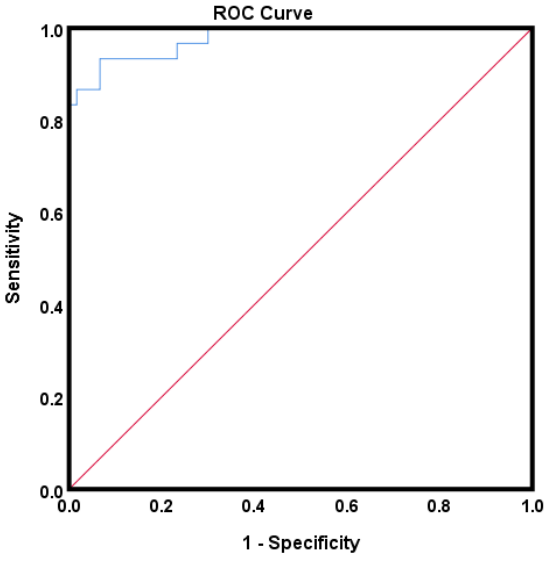


The levels of Ca2+ showed a highly significant decrease ($p < 0.001$) between the periodontitis group (8.9333±1.2628 mg/dL) with the healthy control group (10.0367±1.0531 mg/dL), whereas there was show was no significant difference ($p > 0.05$) between the gingivitis group (9.4190 ±0.9971 mg/dL) with the healthy control group (10.0367±1.0531 mg/dL), as well as between the periodontitis group (8.9333±1.2628 mg/dL) and the gingivitis group (9.4190 ±0.9971 mg/dL) as shown in table (1).

Fig 4: ROC curve for E-cad high levels of E-cad, can give an indication for the presence of Periodontal disease, A E-cad level in the gingivitis group, B E-cad level in the periodontitis group.



A



B

Analysis of the receiving operating characteristic (ROC) curve was used for investigating the potential of using E-cadherin levels in the serum to diagnose Periodontitis. The results appear that, the area under the curve (AUC) value was 0.977 the 95% confidence interval (CI) was from 0.950 to 1.000 at P<0.001, and Cut off value was 1.7360 ng/mL calculated at maximum sensitivity and specificity (93.3).

Analysis of the receiving operating characteristic ROC curve was used for investigating the potential of using E-cadherin levels in the serum to diagnose gingivitis. The results appear that, the area under the curve (AUC) value was 0.872 the 95%-confidence interval (CI) was from 0.782 to 0.961 at P<0.001, and Cut off value was1.456 ng/mL calculated at maximum sensitivity and specificity (70% and 100% respectively).

Discussion

The current study showed that the assessment of the E-cad level was significantly elevated in patients with periodontitis compared to patients with gingivitis and Healthy (control) group, however, vitamin C and Ca2+ show lower levels in periodontal disease compared to healthy subjects. There was no correlation between E-cad, vitamin C, and Ca2+ also between E-cad, vitamin C, and Ca with demographic parameters, or between periodontal parameters, whereas we found a positive correlation between Ca2+ with pocket depth.

A large number of the world's population90% suffers from the periodontal disease [22]. The oral

epithelium protects the oral cavity from microbial threats through physical, chemical, and immunological barriers [23], and also from bacterial invasion into the underlying tissues to the oral cavity which then transforms into the blood [24]. E-cad is present in all epithelial cells and has a major function in intercellular adhesion, cellular polarity maintenance, and tissue architecture [8], in addition to the defense function in epithelial cells against pathogens [25].

According to studies, pathogenic oral bacteria have additional unusual ways to break down cell adhesion and enhance penetration through the mouth mucosa.[5], so the lower in E-cadherin expression shows that the protective barrier has been destroyed [26], sE-Cad is generated by various proteases consequently disrupting cell-cell communication [6], they found pathogenic bacteria have many techniques to circumvent the natural defenses of the host, including using the transcellular pathway, acting on cell-to-cell connections, and exploiting damaged tissues [27], in this search study few Pg gingipains are released by strains to cause the breakdown of (E-cad), which facilitates the entrance of paracellular bacteria across the epithelial cell junction complexes. the gingipains role in changing the mucosal barrier by the usage of a mutant Pg strain that lacks to express gingipain [25], where there is little Ecad expression in gingival keratinocytes of periodontitis patients, which leads to a disruption that functions as a barrier between the oral microbiome and the periodontium, where extracellular cleavage of E-cad causes a disruption in the integrity of the oral mucosal epithelial layer [28], the report of this study was the expression of E-cadherin was highest in the control gingiva, while it was lowest in the mild, moderate, and severe periodontitis subgroups [29], Our results agree with this study that found a decrease in the expression of E-cad in inflammatory gingival tissue, as well as bacteria and their products contributing to the loss of the epithelial barrier function in human gingival epithelial cells, also they found by scavenging ROS antioxidants especially ascorbic acid that will suppress the reduction in E-cad expression. [30]. This study found when primary epithelial cell cultures were infected with periodontal pathogens, a significant decrease (3-fold) in E-cad expression was found [31], that distractive tissue will be released E-cad from the tissue into the blood as (sE-cad), so that increase in serum of periodontal disease. To our knowledge there are no published studies that assess levels of E-cad in sera of the periodontal disease this warrants further studies to assess (sE-cad) in sera of patients with gingivitis and periodontitis.

Collagen is the most abundant extracellular matrix component in the periodontal ligament (PDL), periodontal ligament cells (PDLCs) produce extracellular matrix components like collagen, which help to form PDL collagen fibers and play an important role in PDL collagen remodeling [32], by increasing the host's matrix metalloproteinases, gingipains can promote collagen lysis [5], additionally, periodontitis is linked with oxidative stress, and antioxidants such as ascorbic acid do scavenging of ROS that may be important for the prevention of periodontal disease [33], and advanced periodontal disease is connected with low vitamin C levels [34], hence periodontitis act as serves as a reduction of vitamin C levels, that consider a risk factor for cardiovascular disease [35], where that recent research suggests that lower serum vitamin C levels may be caused by NO signaling inactivation [35], this study consists of groups are 36 patients with periodontitis, 35 patients with ischemic heart disease (CAD), 36 patients with periodontitis plus CAD, and 36 healthy controls, they found that patients with periodontitis and CAD had lower serum and salivary vitamin C levels than patients with periodontitis and healthy control [35], Vitamin C is essential for the prevention and treatment of many diseases [36], in practically all cross-sectional investigations vitamin C intake and blood levels were found to be negatively associated to periodontal disease, where Patients with decreased dietary consumption or blood levels of vitamin C had a faster progression of periodontal disease than healthy control group [36]. Our results agree with this study they found low vitamin C levels were associated with severe periodontal disease, whereas vitamin deficiency was

associated with more severe periodontal disease and increased systemic inflammation [37], this review investigated vitamin C levels in plasma with periodontitis, whereas periodontitis patients showed significantly reduced plasma levels when compared to healthy control, and consuming grapefruit improves sulcus bleeding scores and raises plasma vitamin C levels [38], in two of the case-control trials patients with periodontitis had greater blood vitamin C levels than a healthy control group, these data support the hypothesis that vitamin C from the diet is delivered to periodontal tissue via blood circulation, lowering the incidence of periodontal disease. However, the cross-sectional studies' relationships suggested a non-linear- relationship. Thus, the relationships could affect the impact of periodontal disease on vitamin C intake due to decreased mastication rather than the effect of vitamin C intake on periodontitis [36], this study found in lower calcium levels increase the cause of developing periodontitis due to increased inflammation and bone loss, while adequate calcium intake is associated with a lower rate of dental plaque [39], our findings support this finding, as there was no difference between the healthy control and gingivitis group and show differences between periodontitis group and healthy control group, but this lower within the normal range of calcium level in the blood [40], because the presence of calcium within the normal level in the blood will maintain and strengthen the work and presence E-cad in the tissues and it deficiency will weaken E-cad, so the results obtained in this study the low E-cad and destroyed in tissue absolutely by bacteria and their products, not by lower levels of Ca2+, also E-cad link with the development of periodontal disease, therefore, its levels in the blood will rise indicating its loss in the tissues, and deficiency of vitamin C causes weak tissue because it is among the components of the gingiva, also has a role in collagen production and exposes tissue to injury and bleeding besides difficulty in healing.

Conclusion

In this study, the periodontitis group had higher levels of E-cad in sera comparison with the gingivitis group and healthy control group, this result can use as a diagnostic biomarker for Periodontal disease, also the study's cutoff values can be applied to estimate the health case or periodontal disease patient. Lower levels of vitamin C& Ca2+ have a risk factor for to increase and development of periodontal disease It is necessary for it to be within the normal level to protect from damage and heal the tissue. There were no highly decreased levels of Ca in all groups, so there was no effect to reduce the E-cad level in tissue because of the concentration of Ca2+.

Limitations of the study

The limitations of this study are the small sample size because the disease is complex and different according to different sampling methodologies, disease diagnosis and measurement methods, and periodontal disease criteria, with the exclusions in this study, it has become difficult to find suitable samples for the study and a longer time is needed to finish the study.

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Conflicts of interest

None declared.

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