Assessment of Adenosine deaminase specific activity in serum and saliva of patients with chronic gingivitis

تحديد الفعالية النوعية لانظيم الادينوسين دي امينيز في مصل ولعاب المرضى المصابين بالتهاب اللثة المزمن

Hazema Mossa Khalil **Zahraa Hussein M. Kadri** **Hadeel Khlil Rahman**
College of Education Ibn Al-Haitham/ University of Baghdad.
حازمة موسى خليل **زهراء حسين محمد قدري** **هديل خليل رحمن**
كلية التربية-ابن الهيثم/ جامعة بغداد

Abstract

Adenosine deaminase (ADA) is an enzyme that catalyzes the deamination of adenosine to inosine. The enzyme is widely distributed in human tissues and work as a marker of cellular immunity, and its activity is found to be elevated in those diseases in which there is a cell-mediated immune response. The aim of this study was to explore the usefulness of ADA specific activity in serum and saliva as a biomarker of chronic gingivitis (CG). Thirty CG patients and 15 CG-free controls were enrolled in the study, and they were attendant of the Dental Clinic at the College of Dentistry Medicine (University of Baghdad) during the period January-March 2013. The results demonstrated that the ADA mean specific activity was significantly ($P \leq 0.001$) increased in serum (17.58 ± 0.81 *vs*. 0.75 ± 0.03 U/mg protein) and saliva (85.43 ± 2.43 *vs*. 0.11 ± 0.03 U/mg protein) of CG patients as compared with controls. Accordingly, it is possible to conclude that ADA specific activity might be a good biomarker for CG, especially in saliva, and can reflect inflammatory and destruction processes in the periodontal tissue.

Keywords: **Chronic gingivitis, Adenosine deaminase enzyme**

المستخلص

يحفز انظيم ادينوسين دي امينيز على إزالة مجموعة الأمين من الادينوسين ويحولها الى اينوسين. ينتشر الانظيم بشكل واسع في أنسجة الإنسان ويعد كأحد عوامل المناعة الخلوية. وقد وجد بأن فعالية هذا الانظيم تزداد في الأمراض التي تتوسطها الاستجابة المناعية الخلوية. هدفت هذه الدراسة إلى تسليط الضوء على الأهمية النوعية لانظيم الادينوسين دي امينيز في المصل واللعاب واعتباره احد العوامل الحياتية لتقييم التهاب اللثة المزمن. تم دراسة 30 شخص مصاب بالتهاب اللثة المزمن و 15 شخص سليم (سيطرة) وقد أخذت عينات المرضى من كلية طب الأسنان/جامعة بغداد خلال المدة من كانون الثاني إلى آذار من عام 2013. أظهرت النتائج إرتفاع معدل الفعالية النوعية لانظيم الادينوسين دي امينيز معنويا (احتمالية ≥ 0.001) في مصل (17.58 ± 0.81 مقابل 0.75 ± 0.03 وحدة/ملغم بروتين) ولعاب (85.43 ± 2.43 مقابل 0.11 ± 0.03 وحدة/ملغم بروتين) المرضى المصابين بالتهاب اللثة المزمن مقارنة بالسيطرة. بموجب هذه النتائج فأنه من الممكن أن تعد الفعالية النوعية لانظيم ادينوسين دي امينيز كعامل حياتي جيد للكشف عن التهاب اللثة المزمن خاصة في اللعاب والذي عن طريقه ممكن معرفة درجة الالتهاب والتحطيم الحاصل لأنسجة الأسنان.

الكلمات المفتاحية: التهاب اللثة المزمن، ادينوسين دي امينيز

Introduction

Periodontal disease is an inflammatory process that affects the protective and supportive tissues around the tooth, and bacterial plaque accumulation on the tooth surface leads to a marginal tissue inflammation, known as gingivitis [1]. On an initial bacterial infection, the disease progresses as a loss of collagen fibers and attachment to the cemented surface, followed by apical migration of the junctional epithelium, formation of deepened periodontal pockets, and resorption of the alveolar bone. The disease then continues with progressive bone destruction and, if left untreated, can lead to tooth loss [2]. In addition, the initiation and progression of periodontal disease were reported to be the result of complicated interactions between specific subgingival bacteria and host immuno-inflammatory response [3,4]. The immuno-inflammatory response of the host against periodonto-pathogens consists of immune cells and their products (especially cytokines), and their complex interactions have led several studies to focus on the components of the host immune response [5], and the resolution of inflammation depends on the balance between pro- and anti-inflammatory cytokines [6,7]. Furthermore, a number of studies have proposed an association between periodontal diseases and endothelial dysfunction, atherosclerosis, coronary artery disease, and stroke [8,9]. These have been attributed to the inflammatory nature of periodontitis and systemic dissemination of locally produced

inflammatory mediators such as interleukin IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) [10,11,12].

As gingivitis is a disease of mouth, studies have been elaborated to assess the usefulness of saliva as a source of biomarkers, especially enzymes, for chronic gingivitis CG; for instance, lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), creatine kinase (CK), alkaline phosphatase (ALP), and acid phosphatase (ACP) [13,14]. A further enzyme, which has not been investigated, is adenosine deaminase (ADA). It is an enzyme that catalyzes the deamination of adenosine to inosine . The enzyme is widely distributed in human tissues and work as a marker of cellular immunity, and its activity is found to be elevated in those diseases in which there is a cell-mediated immune response [15]. Accordingly, the present study was designed to assess the specific activity of ADA in serum and saliva of chronic gingivitis of Iraqi patients, and to determine its usefulness as a diagnostic biomarker.

Materials and Methods

Patients and Controls: A total of forty five adult individuals of both gender (age range: 25 -50 years) attending the Dental Clinic at the College of Dentistry Medicine (University of Baghdad) during the period January-March 2013, were enrolled in the study. They were clinically examined by the dental medical staff, and based on their evaluation; the subjects were distributed into two groups; CG (30 patients) and CG-free controls (15 individuals). From each participant, 2 ml of venous blood and saliva were collected. The blood was left for 15 minutes to clot at room temperature, and then it was centrifuged (2000 rpm for 15 minutes) and the separated serum was frozen at -20°C until assessment. The saliva was also centrifuged, and supernatant.

Adenosine deaminase specific activity in serum and saliva: The total activity of ADA in serum or saliva was first determined, and after protein estimation [16], the specific activity of ADA was obtained and expressed as Unit/mg protein [17]. The method is based on the enzymatic deamination of adenosine to inosine, which is converted to hypoxanthine by purine nucleoside phosphorylase. Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase. Hydrogen peroxide is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline and 4-aminoantipyrine in the presence of peroxidase to generate quinone dye which is monitored in a kinetic manner.

Statistical analysis: Data are presented as mean ± standard error (S.E.), and differences between means were assessed by the Student t test. The analyses were carried out using the statistical package SPSS version 13.

Results and Discussion

The mean ADA specific activity was significantly ($P \leq 0.001$) increased in serum (17.58 ± 0.81 *vs.* 0.75 ± 0.03 U/mg protein) and saliva (85.43 ± 2.43 *vs.* 0.11 ± 0.03 U/mg protein) of CG patients as compared with controls (Tables 1 and 2). However, such activity was 23.4 times of the serum control value, but it was much higher in the saliva (776.63 times).

Table(1): Specific activity of adenosine deaminase in serum of chronic gingivitis patients.

Groups	No.	Specific Activity of ADA (Unit/mg protein)		
		Mean ± S.E.	Minimum	Maximum
Patients	30	17.58 ± 0.81	12.14	25.10
Controls	15	0.75 ± 0.03	0.60	0.90

t test = 14.61; D.F. = 43; P ≤ 0.001 (95% C.I: 14.506-19.154)

Table (2): Specific activity of adenosine deaminase in saliva of chronic gingivitis patients.

Groups	No.	Specific Activity of ADA (Unit/mg protein)		
		Mean ± S.E.	Minimum	Maximum
Patients	30	85.43 ± 2.43	66.43	100.30
Controls	15	0.11 ± 0.03	0.02	0.32

t test = 24.68; D.F. = 43; P ≤ 0.001 (95% C.I: 78.349-92.291)

These results clearly suggest the diagnostic potential of ADA, especially in term of a saliva biomarker for CG; therefore, the identification of biomarkers of specific pathologic processes is very useful for the early detection of the disease. Saliva is a complex body fluid composed by a mixture of secretions of multiple salivary glands and by numerous minor glands in the lip, cheek, tongue and palate, containing also serum products, nasal and bronchial secretions, epithelial cells and microbes or their products [18]. Saliva is nowadays becoming more and more interesting as a clinical tool because of its potential to reflect both oral and systemic health conditions [19]. In human medicine a wide number of studies have sought to develop a complete catalogue of proteins in saliva that are relevant not only for basic research but also for its use as a diagnostic tool [20]. Moreover, several disease-specific biomarkers have been reported in human saliva proteomic analysis; for instance for breast cancer [21], head and neck squamous cell carcinoma [22], Sjogren's syndrome [23], and type 2 diabetes mellitus [24] diagnosis. Accordingly, and based on the current study results, ADA can serve as a saliva biomarker to identify the pathological status of CG.

Adenosine deaminase is a cytoplasmic enzyme involved in purine metabolism that catalyzes the hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine. The purine salvage pathway is primarily responsible for the intracellular disposition of transported adenosine [15], and although it is found in most tissues, ADA activity is greatest in the lymphoid tissues, and also it has been found to be localized in other parts of human body; for instance, pancreatic acinar cells in animal and human studies. Furthermore, human ADA also appears on the cell surface as an ecto-enzyme, anchored to CD26, which is a marker for T and B lymphocytes, as well as, macophages [25]. Adenosine is an immunomodulator with anti-inflammatory properties, such as the promotion of endothelial barrier function and the regulation of cytokine production by macrophages, superoxide production by neutrophils, and mediator release by mast cells [26]. Accordingly, CG might have induced a strong inflammatory response in patients that resulted in local activation of the oral immune system, and inflammatory mediators (i.e. cytokines) appear to play a critical role in the pathogenesis of CG. In this regard, ADA gene expression has been demonstrated to be enhanced in inflamed human gingival tissues, compared with control healthy gingival tissues, and consequently, it has been suggested that the enzymatic activity of ADA is increased in periodontitis lesions to enhance the biological effects of adenosine at inflamed sites [27]. However, further studies will certainly be required to address the molecular mechanisms by which adenosine regulates inflammatory reactions in periodontitis, especially if the findings are interpreted in ground of cytokine profile in the saliva.

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