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The Influence of Hydroxychloroquine on Adenosine Deaminase Activity in Systemic Lupus Erythematosus: A Potential Marker for Disease Management

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Abstract

Adenosine deaminase (ADA) is essential for immune function, facilitating the conversion of adenosine to inosine. Elevated ADA activity has been observed in several autoimmune disorders, including systemic lupus erythematosus (SLE), which is characterized by the production of autoantibodies and dysregulation of T and B lymphocytes. This study involved thirty-five females diagnosed with SLE disease classified according to the American College of Rheumatology (ACR) criteria and 70 age-matched healthy controls. Blood and saliva samples were collected from all participants both before and after 4 weeks of hydroxychloroquine (HCQ) treatment. Results demonstrated that serum and saliva levels of TADA, ADA1, and ADA2 activities were noticeably higher than those of healthy controls ($p < 0.001$). During HCQ therapy, the activities of the above enzymes showed a decrease in SLE patients. Although the presence of this decreased, the activities remained high as compared to controls. After treatment, serum and saliva levels of ADA2 activity, which is associated with monocytes and macrophages, were increased significantly compared to controls. In addition, ADA1 activity, which is linked to lymphocyte function, was increased significantly in serum, while in saliva it did not significantly alter as compared to controls. The study highlights the potential of ADA, particularly ADA2, as a biomarker for monitoring disease activity and therapeutic response in SLE. Although HCQ lowers inflammation, ADA levels do not return to baseline, suggesting persistent chronic immunological dysregulation. Salivary ADA as a non-invasive marker for SLE management needs more investigation.

Keywords: Adenosine, Adenosine deaminase (ADA), Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), Hydroxychloroquine (HCQ), Systemic lupus erythematosus (SLE).

تأثير هيدروكسي كلوروكين على نشاط أدينوسين دي أمينيز في مرض الذئبة الحمامية الجهازية: مؤشر محتمل لإدارة المرض.

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الخلاصة

أدينوسين دي أمينيز (ADA) ضروري لوظيفة المناعة، حيث يُسهّل تحويل الأدينوسين إلى إينوسين. وقد لوحظ ارتفاع نشاط ADA في العديد من اضطرابات المناعة الذاتية، بما في ذلك الذئبة الحمامية الجهازية (SLE)، والتي تتميز بإنتاج الأجسام المضادة الذاتية واختلال تنظيم الخلايا الليمفاوية التائية والبائية. شملت هذه الدراسة خمسًا وثلاثين أنثى تم تشخيصهن بمرض الذئبة الحمامية الجهازية (SLE) والمصنف وفقًا لمعايير الكلية الأمريكية لأمراض الروماتيزم (ACR) وسبعين من الضوابط الصحية المتطابقة في العمر. تم جمع عينات الدم واللعاب من جميع المشاركين قبل وبعد 4 أسابيع من علاج هيدروكسي كلوروكين (HCQ). أظهرت النتائج أن مستويات أنشطة TADA و ADA1 و ADA2 في المصل واللعاب كانت أعلى بشكل ملحوظ من تلك الموجودة لدى الضوابط الصحية ($p < 0.001$). خلال العلاج بـ HCQ، أظهرت أنشطة الإنزيمات المذكورة أعلاه انخفاضًا لدى مرضى الذئبة الحمامية الجهازية. وعلى الرغم من وجود هذا الانخفاض، إلا أن الأنشطة ظلت مرتفعة مقارنةً بالضوابط. بعد العلاج، ارتفعت مستويات نشاط ADA2 في المصل واللعاب، المرتبط بالخلايا الوحيدة والبلعميات، بشكل ملحوظ مقارنةً بمجموعة الضبط. كما ارتفع نشاط ADA1، المرتبط بوظيفة الخلايا الليمفاوية، بشكل ملحوظ في المصل، بينما لم يتغير بشكل ملحوظ في اللعاب مقارنةً بمجموعة الضبط. وتُبرز الدراسة إمكانات ADA، وخاصةً ADA2، كمؤشر حيوي لرصد نشاط المرض والاستجابة العلاجية في مرض الذئبة الحمامية الجهازية. على الرغم من أن هيدروكسي كلوروكين يُخفِّف الالتهاب، إلا أن مستويات ADA لا تعود إلى مستوياتها الأساسية، مما يُشير إلى استمرار اختلال التنظيم المناعي المزمن. إن استخدام ADA في اللعاب كعلامة غير جراحية لإدارة مرض الذئبة الحمامية الجهازية يحتاج إلى مزيد من التحقيق.

1. Introduction

Adenosine deaminase (ADA) is a hydrolytic enzyme found in various tissues that catalyses the conversion of 2'-deoxyadenosine into 2'-deoxyadenosine or adenosine into inosine[1]. ADA plays a crucial role in the development, function, and regulation of immune responses, making its measurement a valuable tool for generating informative data in diseases characterized by immune system alterations [2]. The relationship between several immunodeficiency illnesses and serum ADA activity has received a lot of research [2-4]. The extensive distribution of ADA in the majority of human tissues may suggest that it is involved in a variety of illnesses and conditions [5, 6].

Numerous investigations have demonstrated elevated ADA activity in diseases such rheumatoid arthritis (RA) and systemic lupus erythematosus SLE [7-10]. Autoimmune disorders, which can be either organ-specific or non-organ-specific, cause the body to harm its tissues [11]. The SLE, a connective tissue disease, with a wide variety of clinical and laboratory signs and an idiopathic etiology [12]. Although the etiology of SLE is thought to be complex, autoantibodies are created in response to the disease [13]. SLE, a well-known example of a non-organ-specific autoimmune illness, produces a large number of autoantibodies, especially against nuclear DNA. Moreover, T-cell/B-cell dysregulation is one of the primary features of SLE [14-16]. Hydroxychloroquine (HCQ) is frequently prescribed to SLE patients because of its superior efficacy and tolerability [17]. Further research in the 1990s aimed to determine HCQ's effects on glucocorticoid (GC) dosages, disease activity, and lupus flares [18]. Alarcon *et al.*'s 2007 study [19] demonstrated that HCQ use enhanced survival in SLE patients and was linked to a long-term protective impact against end-organ damage. Subsequent cohort studies have confirmed these findings. Additionally, it has been discovered that HCQ has pleiotropic beneficial effects on the metabolic profile and endothelial dysfunction, which lowers the risk of cardiovascular disease

and thrombosis [20]. Moreover, HCQ has been shown to improve pregnancy outcomes (including reducing congenital heart block), osteoporosis rates and infection, and may ultimately aid in the prevention of neoplasia [21].

Because SLE affects multiple organs, no single diagnostic test currently exists. Therefore, identifying a test with greater specificity and sensitivity for diagnosing and monitoring disease activity is crucial. While increased ADA activity has been documented in autoimmune illnesses, the specific alterations in ADA1 and ADA2 activities in SLE patients, especially in response to HCQ medication, have not been completely explored. Most studies use blood samples, but there is little research on using saliva as a non invasive indicator for SLE therapy. While HCQ is known to lower inflammation in SLE, it is unknown if it entirely restores ADA activities or if residual immunological dysregulation remains. This study aims to compare the activities of ADA and its isoenzymes (ADA1 and ADA2) in the serum and saliva of patients with SLE before and after receiving HCQ treatment. The study looked at changes in ADA activity to see if it might be used as an indicator for SLE disease activity and how well a therapy is working.

2. Materials and Methods

Materials (Patients and samples)

The study was carried out between December 2023 to May 2024, involving 35 female patients with SLE attending the Rheumatology Clinic at Baghdad Teaching Hospital, affiliated with the Ministry of Health. For comparison, 70 age-matched healthy controls were also included. Individuals exhibiting the clinical symptoms were diagnosed by a panel of rheumatology specialists. Every patient met the eleven standards set forth by the American College of Rheumatology (ACR) [22].

Samples were obtained from these newly diagnosed patients in two phases: before treatment initiation and about four weeks after treatment. Serum and saliva supernatant were obtained by centrifuging blood and saliva samples at 3,000 rpm for 10 minutes without the use of any anticoagulants. Before analysis, all samples were kept at -20°C.

Methods (ADA activity detection and protein assay)

Total ADA (TADA) in serum and saliva was measured using a kinetic method, which involves the formation of colored indophenol complexes from ammonia released from adenosine, followed by spectrophotometric quantification at 550 nm. Sigma-Aldrich (St. Louis, MO, USA) provided the 0.1 mmol/l erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), which was used to assess the ADA2 activity. By deducting the ADA2 activity from the TADA activity, the ADA1 activity was determined.

Protein concentration in serum was determined according to the Biuret method, and in saliva was determined according to the Hartree method. The standard curve of bovine serum albumin (BSA) was used at different concentrations to find the concentration of the unknown protein [23].

Statistical analysis

All results were expressed as mean ± standard deviation (SD). Differences in mean values were analyzed for statistical significance using a one-way ANOVA test. All statistical analyses were performed using the Statistical Package for the Social Sciences (Prism, version 8.4.3).

3. Results and Discussion

The study included 103 participants in total: 70 healthy controls and patients diagnosed with SLE, all of whom met the American College Rheumatology (ACR) criteria (at least 4 out of 11). The patient group was categorized into two groups based on treatment status at the time of sampling: newly diagnosed patients and the same patients after undergoing HCQ therapy. Figures 1 and 2 show the outcomes of the ADA activity and specific activity, including the TADA and its isoenzymes in serum and saliva. Figure 1 shows the values, activities and specific activities of TADA, ADA1, and ADA2 in the serum before and after therapy for all SLE patients as well as healthy controls. The overall mean of serum TADA in SLE patients was considerably greater ($p< 0.001$) than in healthy controls (both before and after medication). When comparing the mean ADA1 and ADA2 activities of patients with SLE before and after therapy to those of healthy controls, there was a substantial increase in all three groups (before, after therapy, and control) ($p< 0.001$).

ADA enzyme potential as a measure of disease activity is highlighted by elevated ADA activity in SLE patients, both before and after medication. By changing adenosine into inosine, ADA controls the immunological response and is essential for the growth and maturation of T cells. Because adenosine has an anti-inflammatory function, an increase in ADA activity can exacerbate inflammation by depleting adenosine stores and speeding up its breakdown [24]. There is evidence from various research studies that autoimmune disorders are associated with higher levels of ADA. Similar to the current findings, an investigation on SLE patients found that their ADA activity was much higher than that of healthy controls [8]. The SLE overactive immune system is thought to be the cause of the elevated ADA activity in the condition. In chronic inflammatory diseases like SLE, there is a clear dysregulation of both humoral and cellular immunity [25]. Both isoenzymes are implicated in the pathogenesis of SLE, as evidenced by the increased ADA1 and ADA2 activity seen in SLE patients, even after taking HCQ treatment. ADA1 is primarily associated with lymphocyte function, whereas ADA2 is linked to monocytes and macrophages, which are cells involved in the inflammatory response [26]. These isoenzymes continue to increase after taking HCQ medication, which is suggestive of the chronic inflammatory nature of SLE as well as the treatment's partial immune system control. have suggested that ADA2 in particular could be a sign of inflammation.

The HCQ well-known anti-inflammatory properties and immune suppressive aid in the treatment of SLE. By cytokine production and altering toll-like receptor signaling, HCQ can inhibit the immunological response, claims [27]. The significant reduction in ADA activities that occurred after therapy in this trial demonstrated the efficacy of HCQ therapy in reducing inflammation. The prolonged elevation of baseline ADA activities in untreated patients

indicates that HCQ does not completely restore baseline ADA activities, even while it reduces immunological hyperactivity.

Due to the chronic nature of SLE, long-term medication is frequently necessary to manage disease activity. This partial reduction in ADA activity is consistent with this. According to similar results, HCQ decreased inflammatory markers in other studies, although it did not fully bring them back to normal levels [28].

The elevated ADA activity in SLE patients before therapy and its reduction after HCQ treatment highlights ADA as a potential biomarker for monitoring disease activity and treatment efficacy. The ADA could serve as a complementary marker alongside traditional measures, such as complement levels and anti-dsDNA antibodies, to assess disease flare ups or remission in SLE [5]. Furthermore, the specific elevation of ADA2 suggests a potential role for this isoenzyme in monitoring vascular complications in SLE, such as vasculitis, which is a common manifestation of the disease [29].

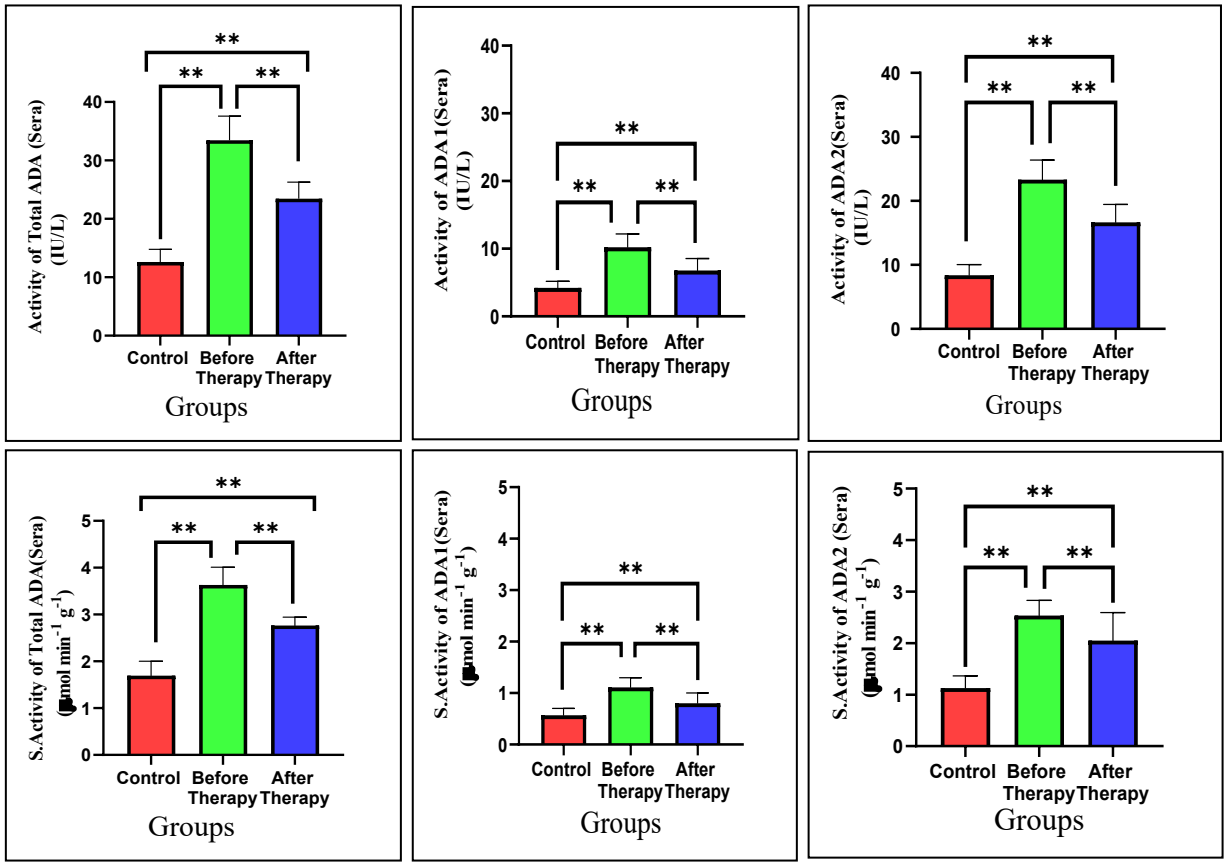

Figure 1: Serum TADA, ADA1, and ADA2 activities and specific activities in patients with SLE (before and after therapy) and healthy controls

Figure 2 shows the values of TADA, ADA1, and ADA2 activities in the saliva before and after therapy for all SLE patients as well as healthy controls. Overall mean saliva TADA and ADA2 in SLE patients was considerably greater ($p< 0.001$) than in healthy controls. Moreover, the decrease in saliva ADA1 activity in patients with SLE was not statistically significant. While specific activity TADA and ADA1 in healthy controls was considerably greater ($p<0.001$) than in SLE patients, and between patients before and after therapy was not

statistically significant. Moreover, the decrease in saliva ADA2 specific activity in patients with SLE and controls was not statistically significant.

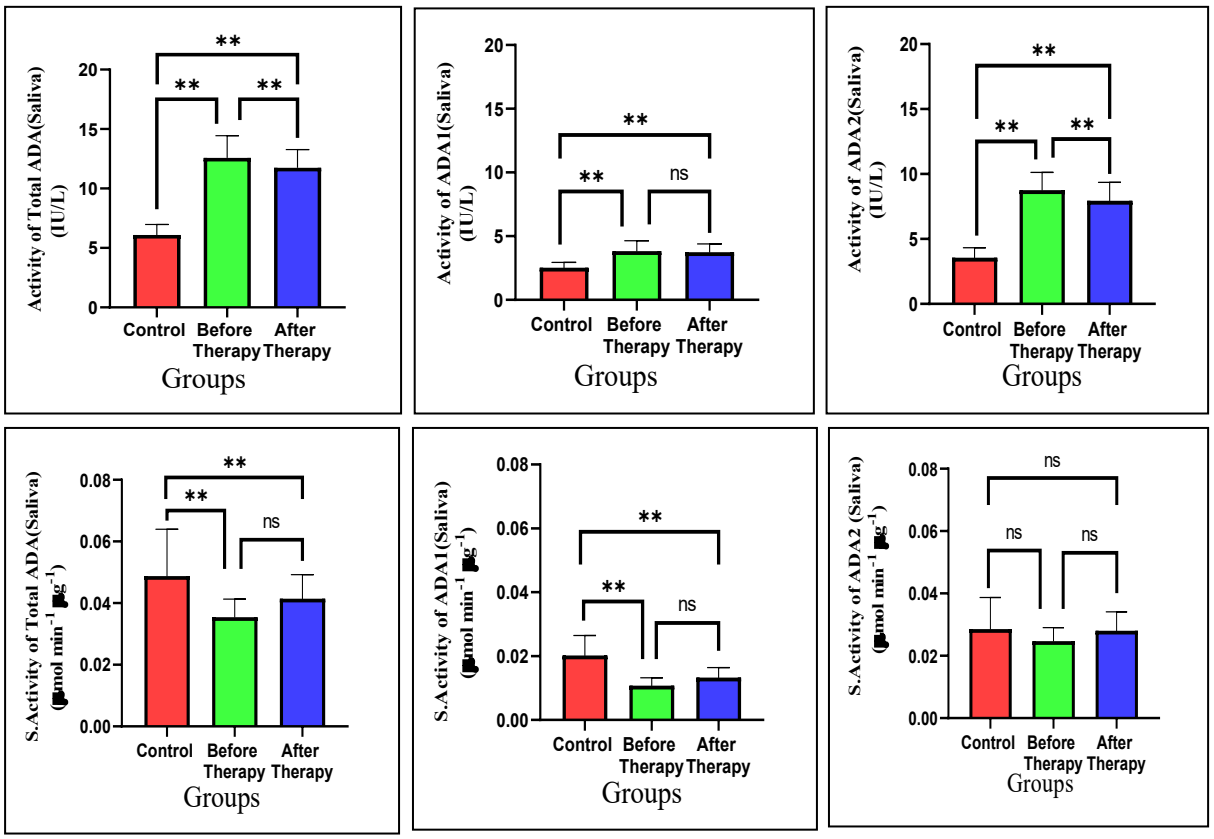

Figure 2: Saliva TADA, ADA1, and ADA2 activities and specific activities in patients with SLE (before and after therapy) and healthy controls (**$p < 0.001$) (ns $p > 0.05$)

Increased TADA and ADA2 in SLE Patients' Saliva. When comparing SLE patients to healthy controls, the overall mean values of TADA and ADA2 in saliva were considerably greater ($p< 0.001$). This increase is likely linked to SLE's increased inflammatory and immunological response, which is a frequent characteristic of autoimmune diseases. Notably, monocytes and macrophage cells that are essential to inflammation secrete ADA2 more frequently than other secretions. In autoimmune illnesses, elevated ADA2 levels have been previously noted, and its activity is correlated with both immunological activation and the severity of the disease [26].

Interestingly, there was no statistically significant decrease in salivary ADA1 activity in SLE patients (both before and after therapy). The ADA1, which is associated more closely with lymphocytes, may not reflect acute inflammatory changes as sensitively as ADA2. This lack of significant change suggests that ADA1 might be a less reliable indicator for monitoring therapeutic efficacy in SLE, as observed in a previous study that questioned its responsiveness to anti-inflammatory therapies [30].

Specific activities of TADA and ADA1 were significantly higher in healthy controls than in SLE patients (both before and after therapy), suggesting that SLE patients had decreased enzyme efficiency or activity concerning protein content. This decreased enzymatic

efficiency in SLE may be caused by chronic inflammation and immunological dysregulation. To support the hypothesis that these markers might not be sensitive enough to identify therapy-induced alterations, the study also demonstrated that the differences in specific activities for both TADA and ADA1 between SLE patients before and after therapy were not statistically significant.

Despite having greater absolute ADA2 activities than healthy controls, SLE patients may not be significantly affected by the disease's effects on the enzyme's activity or functionality with its concentration. This is implied by the fact that neither group's ADA2-specific activity changed significantly. These results indicate that while a drug's therapeutic efficiency may not be directly related to its modulation of this enzyme activity, higher ADA2 activities most likely reflect immune system activation. Research has shown that immune system dysfunction can result in elevated ADA2 activities in ADA2-specific diseases, including ADA2 insufficiency [31].

Conclusion

The results of this study are in line with earlier studies that demonstrated that higher inflammation and immunological activity in SLE lead to increased ADA activity. Because SLE is a chronic inflammatory disease, HCQ therapy reduces ADA activity but does not fully return it to normal levels. It may be possible to monitor disease activity and the efficacy of treatment interventions by using ADA and its isoenzymes, especially ADA2. While TADA and ADA2 are significantly elevated in the saliva of SLE patients, ADA1 does not show significant variation after therapy. Moreover, the specific activity of these enzymes does not reflect significant therapeutic changes, highlighting the potential limitations of ADA1 and specific activity as biomarkers in SLE. ADA2, however, remains a promising marker, particularly as it aligns with inflammatory processes in SLE. Further research is needed to confirm the utility of salivary ADA as a non-invasive tool for monitoring disease progression and treatment efficacy in SLE.

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Ethics Statements

The Research Ethics Committee for Scientific Research has been approved by the Iraqi ministries of scientific research, higher education, health, and the environment.

Disclosure and conflict of interest

There is no conflict of interest.

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