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Adenosine Deaminase and Guanine Deaminase: The Potential Role in Diabetic Foot Ulcers

Ali W. Al-Ani
Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq

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Abstract

The present study explored the possible role of adenosine deaminase and guanine deaminase in diabetic foot ulcers which is considered one of the main chronic complications of diabetes mellitus. Serum adenosine deaminase, guanine deaminase, and some biochemical parameters were investigated in 54 patients with diabetic foot ulcers and 40 healthy individuals. According to our findings, adenosine deaminase and guanine deaminase activities are much higher in diabetic foot ulcer patients than in healthy individuals. A strong positive correlation was found between adenosine deaminase and guanine deaminase activities. Adenosine deaminase and guanine deaminase had 100% sensitivity and specificity for diagnosing diabetic foot ulcers, and their area under the receiver operating characteristic curve was 1.0. Kinetic study displayed significant changes in Vmax and Km between patients and healthy individuals for both adenosine deaminase and guanine deaminase. Based on results, it can be concluded that the increase of adenosine deaminase and guanine deaminase activities may negatively contribute in diabetic foot ulcers progression.

Keywords: Adenosine, adenosine deaminase, diabetic foot ulcers, guanosine, guanine deaminase.

أدينوسين دي أمينيز وجوانين دي أمينيز: الدور المحتمل في تقرحات القدم السكرية

علي وليد العاني
قسم الكيمياء, كلية العلوم, جامعة بغداد, بغداد, العراق

الخلاصة

استكشفت الدراسة الحالية الدور المحتمل للأدينوسين دي أمينيز والجوانين دي أمينيز في قرح القدم السكرية التي تعتبر واحدة من المضاعفات المزمنة الرئيسية لمرض السكري. تمت دراسة الأدينوسين دي أمينيز، الجوانين دي أمينيز، وبعض المتغيرات البيوكيميائية في مصل 54 مريضاً يعانون من قرح القدم السكرية و 40 شخصاً أصحاء. وفقًا للنتائج التي توصلنا إليها ، فإن أنشطة الأدينوسين دي أمينيز والجوانين دي أمينيز أعلى بكثير في مرضى قرحة القدم السكرية مقارنة بالأفراد الأصحاء. تم العثور على علاقة إيجابية قوية بين أنشطة الأدينوسين دي أمينيز والجوانين دي أمينيز. الأدينوسين دي أمينيز والجوانين دي أمينيز لهما حساسية وخصوصية بنسبة 100٪ لتشخيص قرح القدم السكرية، وكانت المنطقة الواقعة تحت منحنى الخاصية العملياتية

*Email: ali.w@sc.uobaghdad.edu.iq

للمستقبل 1.0. أظهرت الدراسة الحركية تغيرات كبيرة في Vmax و Km بين المرضى والأفراد الأصحاء لكل من الأدينوسين دي أمينيز والجوانين دي أمينيز. بناءً على النتائج، يمكن أن نستنتج أن زيادة أنشطة الأدينوسين دي أمينيز والجوانين دي أمينيز قد تساهم سلبًا في تطور قرح القدم السكرية.

Introduction

Adenosine deaminase (ADA) and guanine deaminase (GDA) are essential enzymes for regulation the catabolic pathway of purines and are stringently controlled the pool of nucleobase derivatives. These enzymes are involved in the modulation of purinergic responses for several physiological disorders or even can considered as markers for some diseases [1].

Adenosine aminohydrolase (E.C.3.5.4.4), or ADA, is ubiquitously expressed in tissues and body fluids which irreversibly catalyzes the deamination of adenosine/deoxyadenosine to form inosine/deoxy inosine, [2]. The specific role of ADA is in lymphoid tissues, including lymphocytic proliferation and differentiation, and maturation and maintenance of immunological responses [3]. To date, several studies reported that the increase in ADA level is strongly related to many inflammatory and autoimmune diseases such as systemic lupus erythematosus [4], rheumatoid arthritis [5], Behcet's disease, celiac disease, Graves' disease, tuberculosis, ulcerative colitis [6], lung diseases [6], cancer diseases [7], type 2 diabetes mellitus [8], and autoimmune hepatitis [9] that means the presence of an association between ADA and cell mediated immunity.

Similarly, GDA is classified as deaminase enzyme that catalyzes the irreversible conversion of guanine to xanthine [10]. Guanine aminohydrolase (E.C.3.5.4.3) or GDA, also known as cypin, is expressed essentially in human cells of liver, kidney, brain, small intestine,and central nervous system. It is mainly contributed in the neuronal morphology development by promotion the branching of dendrites. Previous studies claimed that GDA activity is specifically increased in liver diseases, as well as indicated that there is a relationship betweenGDA activity and skin diseases [1, 11, 12].

Diabetic foot ulcers (DFU) are characterized by ulcers and/or deep tissue injury as a result of nerve disorders and vascular lesions in the distal lower limbs of patients [13]. It has been usually diagnosed as vascular complications of diabetes mellitus which is accompanied by a high ratio of morbidity and mortality [14]. The World Health Organization defines DFU as an "ulceration of the foot (distally from the ankle and including the ankle) associated with neuropathy and different grades of ischemia and infection" [15]. Pathogenic processes which enable to induce DFU are multifactorial. It is scientifically considered that abnormal foot pressures, abnormal joint mobility, foot deformity, peripheral neuropathy, peripheral artery disease and trauma are the possible commonest reasons of DFU [16]. Among these, the peripheral neuropathy is the most important causes that induce of DFU pathogenesis. Peripheral neuropathy drastically suppresses the nerve activity and can threat autonomic, motor, and sensory roles through the body [17, 18]. Another main cause involves in development of DFU is an immune-inflammatory system. According to previous studies, an immune activation plays a vital role in development several stages of chronic wounds. Diabetic foot ulcers incidence may precede by the upregulation of immune-inflammatory in similar way when it precedes some major cardiovascular diabetic disorders such as coronary artery disease [19, 20].

The studying of relationship between ADA and GDA with DFU may therefore help to understand the role of these enzymes in the development of DFU. Where the activities of both enzymes have been characterized to be increased in inflammatory diseases as a marker of T-cell activation and proliferation [19]. To the best of our knowledge, there is no data regarding to the role of ADA and GDA activities in the pathogenesis event of DFU. Thus, this study aimed to investigate the relationship of ADA and GDA activities with DFU, and the possible role of these enzymes in the DFU deterioration.

1. Patients and Methods

1.1. Patients

This study was carried out between February 2022 and May 2022 and involved total 94 individuals in order to estimate the relationship between ADA, GDA, and DFU progression. Out of these 54 (20 male and 34 female) newly diagnosed DFU patients who were admitted to Abu Ghraib Hospital, Baghdad, Iraq and 40 (24 male and 16 female) age- and sex-matched healthy controls. The study was conducted after obtaining ethical approval from the ethical committee at scientific research by ethical approval of environmental, health, higher education, and scientific research ministries in Iraq and informed consent was taken from the participants. The criteria excluded in this study were alcohol and smoking consumption, heredity diseases, taking medications and liver disorders.

1.2. Assessment of Serum ADA Activity

The activity of ADA was determined according to Giusti and Galanti method [21]. The principle of this method was based on Berthelot reaction. This reaction based on the detection of ammonia formed by ADA action on adenosine. Ammonia is quantified by a reaction with phenol, which generates an indophenol derivative with an intense blue color. The blue indophenol complex was quantified using spectrophotometer (UV-1800 UV-Vis spectrophotometer, Shimadzu,Japan) at 628nm. ADA activity was expressed as IU/L serum, where one unit of enzyme activity was defined as the amount of ADA required to generate 1µmol of ammonia/minutes under the assay conditions.

1.3. Assessment of Serum GDA Activity

GDA activity was estimated as described by Jones *et al.* [22]. Determination of GDA activity was carried out using guanosine in phosphate buffer. Ammonia released during 30 minutes of incubation at 37^{o}C was colorimetrically determined using a modified phenol- alkaline hypochlorite method[23].

1.4. Assessment of Some Biochemical Parameters

Analysis of some biochemical parameters, included glycated hemoglobin A1c (HbA1c), iron level, total protein concentration (Tp), and uric acid were determined using commercially available kits by Linear Chemicals S.L. company (Barcelona, Spain).

1.5. Enzyme Kinetic Analysis

Pooled serum was prepared by combining 100 µL of serum from individuals in each studied groups. Pooled sera for DFU patients and healthy controls were incubated with various concentration of adenosine (0, 0.5, 1, 5, 10, 21, 42mM) and guanosine (0, 0.25, 0.5, 1,7, 14, 28mM) separately. Then the activities of ADA and GDA were determined as previously mentioned. The substrate concentration (Km) at the half of Vmax was calculated from the equation of Michaelis-Menten curve.

1.6. Statistical Analyses

Statistical analyses were performed using GrphPad prism software version 6. All variables were expressed as Mean±standard deviation (SD). One-way analysis of variance (ANOVA) and T-test analyses were used for study of differences among the groups. The $P \leq 0.05$ was used as significant.

2. Results

Characteristics of healthy and patients with some biochemical parameters are listed in Table-1.

Table 1: Characteristics and some biochemical parameters of DFU patients and control

Parameters	Male		Female		*P*-value
	Controls	DFU Patients	Controls	DFU Patients	
Age(year)	42±2.9 43 (38-45)	46.8±5.1 49 (37-50)	48±2.45 48.5 (44-51)	55.29±12.04 53 (32-65)	M (0.093) F (0.107)
Gender; n(%)	24(60)	20(37)	16(40)	34(63)	---
HbA1c %,(mmol/mol)	5.4±0.2,(36) 5.4,(36) (5.2-5.9), (33-41)	9.0±2.8,(75) 8.5,(69) (5.9-13.4), (41-123)	5.6±0.1,(38) 5.6,(38) (5.5-5.8), (37-40)	8.9±2.1,(74) 8.2,(66) (5.5-13,8), (37-127)	M (0.014) F (<0.001)
Iron (µmol/L)	18.98±7.8 17.82 (15.04-25.79)	3.58±1.46 3.8 (1.7-5)	18.85±2.63 20.15 (14.86-21.31)	3.15±1.03 2.3 (1.2-3.9)	M (<0.001) F (<0.001)
Tp (g/dL)	8.3±1.58 8.33 (5-10.42)	11.81±1.46 11.25 (10.83-14.58)	8.85±0.62 8.75 (8.33-9.58)	9.97±2.43 10.42 (7.08-14.17)	M (<0.001) F (0.2652)
Uric acid (mg/dL)	4.83±0.32 4.78 (4.63-5.21)	4.14±0.9 4.39 (3.16-5.06)	3.98±0.76 3.8 (3.2-4.8)	4±2.07 2.92 (2.05-7.48)	M (0.479) F (0.810)

Data are presented as mean±SD, median (min–max), M: male, F: female, *P-value ≤0.05 is considered to be statistically significant.

The HbA1c level of male and female was significantly increased in DFU patients compared to control group. Iron level was dramatically decreased in both male and female who were suffering from DFU compared to healthy individuals. From the results, the Tp concentration increased in patients with DFU compared to the control group, and this increasewas most noticeable in male patients. There was no significant alteration observed in uric acidlevel between patients and control group. Table 2 shows that both male and female groups displayed highly significant increase in the activity and specific activity of ADA in patients with DFU compared to control group. This was also observed for GDA activity and specific activity.

Table 2: Activities and specific activities of ADA and GDA in DFU patients and control group

Parameters	Male		Female		*P*-value
	Control	DFU patients	Control	DFU patients	
ADA activity (IU/L)	12.11±3.98 11.33 (5.4-17.27)	33.69±5.84 33.46 (24.28-41.01)	14.3±4.66 12.95 (8.64-19.97)	40.93±13.91 35.89 (28.06-55.58)	M (<0.001) F (<0.001)
ADA specific activity (IU/g)	0.145±0.032 0.138 (0.108-0.201)	0.272±0.043 0.309 (0.224-0.324)	0.149±0.054 0.136 (0.104-0.208)	0.394±0.117 0.375 (0.241-0.586)	M (<0.001) F (0.0128)
GDA activity (IU/L)	10.69±0.22 10.62 (10.35-10.89)	26.41±3.1 25.43 (21.47-30.45)	10.73±0.32 10.67 (10.44-11.34)	25.1±3.1 24.98 (19.65-30.91)	M (<0.001) F (<0.001)
GDA specific activity (IU/g)	0.126±0.018 0.124 (0.102-0.161)	0.231±0.024 0.232 (0.202-0.270)	0.122±0.008 0.122 (0.114-0.132)	0.244±0.032 0.24 (0.203-0.285)	M (<0.001) F (<0.001)

Data are presented as mean±SD, median (min–max), M: male, F: female, *P-value ≤0.05 is considered to be statistically significant.

This study also revealed that there was a highly significant positive correlation between ADA and GDA in both male and female groups, as shown in Figure1. Pearson's correlations of ADA and GDA with age, HbA1c, iron, Tp, and uric acid are listed in Table 3.

Table 3: Pearson's correlations of ADA and GDA with age, HbA1c, iron, total protein and uric acid

Parameters	ADA		GDA	
	r	*P*-value	r	*P*-value
Age	0.298	0.190	-0.236	0.236
HbA1c	**0.644**	**<0.001**	**0.668**	**<0.001**
Iron	**-0.831**	**<0.001**	**-0.879**	**<0.001**
Tp	**0.443**	**0.018**	**0.685**	**<0.001**
Uric acid	-0.006	0.977	-0.086	0.647

No significant correlations were observed between ADA activity and each of age and uric acid level. This was also noticed with GDA correlations. A significant positive correlationwas appeared between HbA1c level and both ADA and GDA. This was also observedbetween Tp and each of ADA and GDA. In contrast, a highly significant negative correlation was revealed between iron level and each of ADA and GDA.

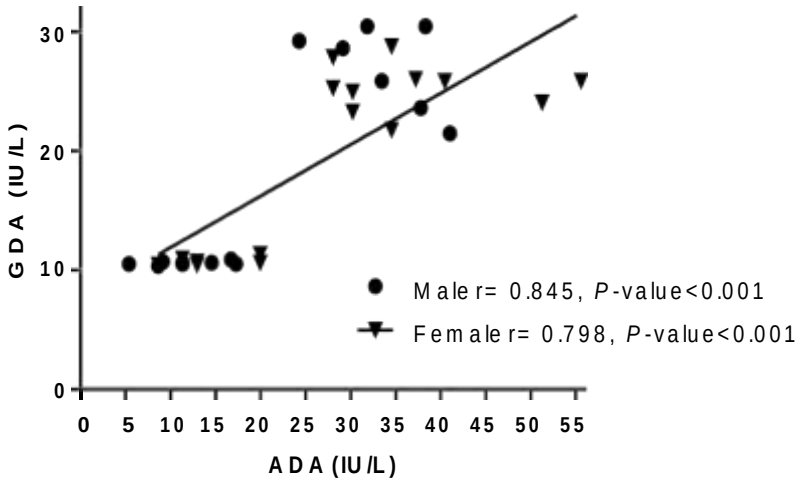

Figure 1: Pearson's correlation between ADA and GDA in male and female groups.

DFU patients were also sub-grouped according to HbA1c level to patients with moderate elevated HbA1c (HbA1c between 5.7% to 6.4%) and patients with severe elevated HbA1c (HbA1c ≥ 6.5%). It was found that ADA and GDA activities and specific activities and Tp level were significantly increased, whereas iron level was significantly decreased in patients with moderate and severe elevated HbA1c compared to control group. However, the differences in uric acid level were not significantly changed among these groups, as shown in Table 4.

Table 4: Clinical characteristics of DFU patients with moderate elevated HbA1c and severe elevated HbA1c

Parameters	Controls	Patients with moderate HbA1c	Patients with severe HbA1c	*P*-value
ADA(IU/L)	12.11±3.98	31.84±4.07	39.99±12.63	<0.001[a,b],0.168[c]
ADA(IU/g)	0.145±0.032	0.290±0.044	0.347±0.108	<0.001[a,b],0.408[c]
GDA(IU/L)	10.69±0.22	26.96±2.92	25.37±3.07	<0.001[a,b],0.354[c]
GDA(IU/g)	0.126±0.018	0.23±0.021	0.24±0.030	<0.001[a,b],0.428[c]
Iron(µmol/L)	18.98±7.8	4.20±0.28	3.18±2.74	<0.001[a,b],0.617[c]
Tp(g/dL)	8.3±1.58	11.93±0.48	10.68±2.16	0.003[a],0.001[b],0.683[c]
Uric acid(mg/dL)	4.83±0.32	4.69±0.52	3.92±1.81	0.523[a],0.427[b],0.569[c]

Data are presented as mean±SD, P-value ≤0.05 is considered to be statistically significant between a (control and moderate elevated HbA1c), b (control and severe elevated HbA1c), c (moderate and severe elevated HbA1c).

The diagnostic accuracy for ADA and GDA activities was estimated according to cut-off values of Receiver operating characteristic (ROC) curves, as shown in Figure 2.

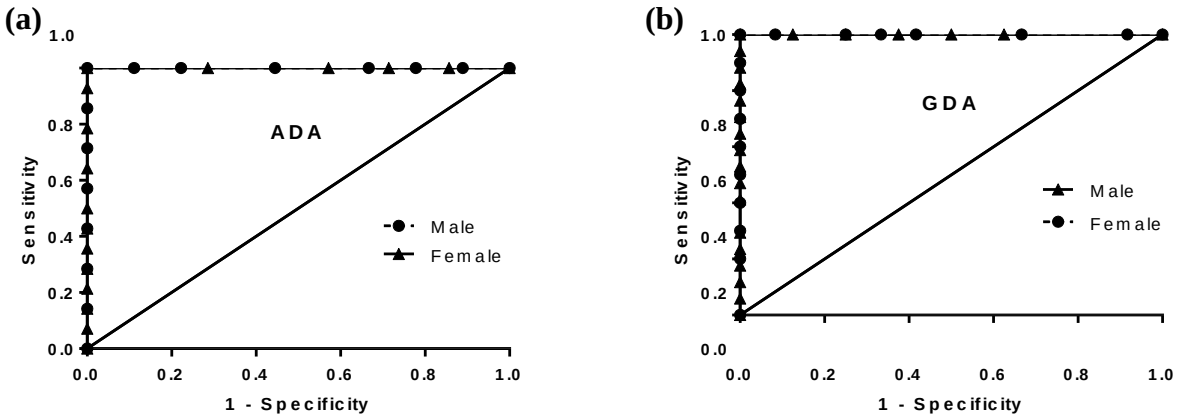

Figure 2: The ROC curve for (a) serum ADA and (b) GDA activities as diagnostic marker of DFU patients

ADA and GDA have a high diagnostic accuracy in predicting DFU with moderate and severe HbA1c (cut-off value, 20.87 IU/L and 15.91U/L respectively), as shown in Figure3.

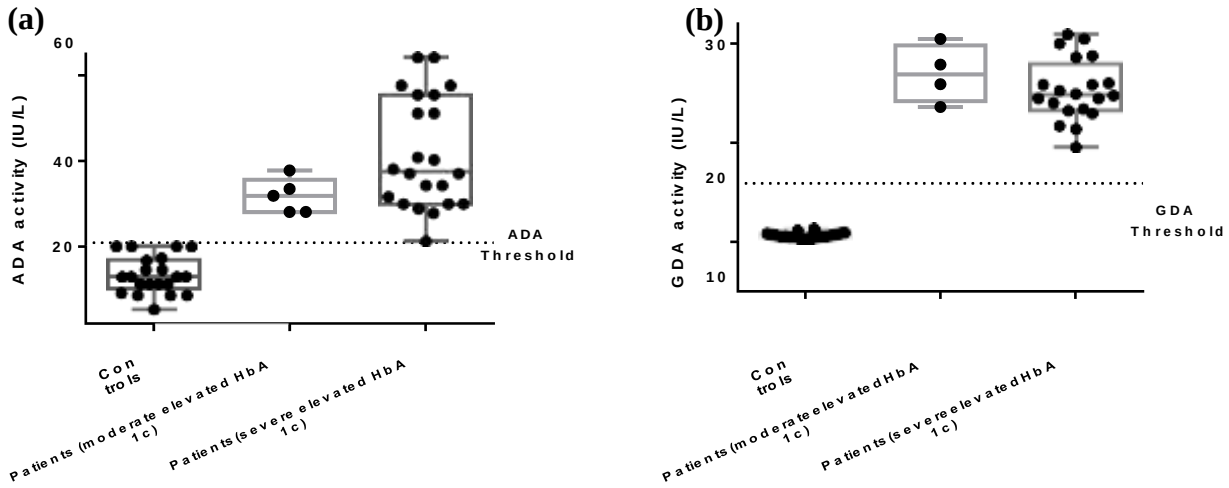

Figure 3: The level (Interquartile range) of (a) ADA and (b) GDA in DFU patients with moderate elevated HbA1c and severe elevated HbA1c.

ROC curve analysis for ADA and GDA activities shows that the percentage values of sensitivity and specificity for DFU diagnosis were 100% (AUC=1), as shown in Table 5.

Table 5: Analysis data of ROC curve shows the percentage of sensitivity and specificity at best cut-off in control and DFU discrimination and the area under curve for ADA andGDA

Parameters	ADA		GDA	
	Male	Female	Male	Female
Sensitivity%	100	100	100	100
Specificity%	100	100	100	100
AUC	1.00	1.00	1.00	1.00
Cut-off (IU/L)	>20.78	>20.78	>16.32	>15.49
P-value	0.001	<0.001	<0.001	<0.001

The kinetics of ADA and GDA were estimated using the plot of Michaelis-Menten equation. The changes in Vmax and Km of ADA and GDA between control and DFU groups are listed in Table 6.

Table 6: The Vmax and Km of ADA and GDA derived from the Michaelis-Menten plot

Parameters	ADA		GDA	
	Control	DFU	Control	DFU
Vmax(IU/L)	16.93	31.48	16.89	18.33
Km(nmol/L)	125E-9	134E-9	25.2	28.9

The values of Vmax and Km were clearly increased for both ADA and GDA in patients with DFU compared to control group.

3. Discussion

The main findings of this study were that the activities and specific activities of ADA and GDA had significantly increased in patients with DFU compared to healthy individuals. Our results are consistent with many studies which showed an elevation in ADA level associated with systemic lupus erythematosus [4], rheumatoid arthritis [5], lung diseases [6], type2 diabetes mellitus [8] and autoimmune hepatitis [9], as well as increased in GDA level in patients with liver diseases[11] and skin diseases [12]. In fact, several mechanisms could be used to explain the role of ADA and GDA in DFU occurrence. One of these mechanisms illustrated that adenosine molecules are involved in insulin mediated glucose uptake intocells. Furthermore, *in vivo* and *in vitro* observations indicated that ADA modulates thebioactivity of insulin and it is considered as a marker for insulin function [8]. In insulin tissue sensitive, the increase in ADA activity leads to decline of adenosine level which in turn decrease glucose uptake into cells and this explains the role of ADA in modulating of insulin function [24]. This mechanism may be adapted by cells as part of homeostatic cell-protecting against harmful accumulation of glucose in type2 diabetes mellitus and its complications [25].However, another mechanism indicated the critical role of ADA in immunological response
[26] and GDA in the development of neurological systems [27] may have an effect intriggering and developing the pathogenic pathway of DFU which was the main target of this study. ADA and GDA are well known markers of T-lymphocyte where there activities have increased during cell-mediated immune response [19]. It has been reported the impairment of an immune-inflammatory may negatively affect tissue homeostasis and wound healing which inducing the chronic wounds and producing a complicated clinical conditions such as DFU [20]. Since, the ADA and GDA could be involved in different stage of wound healing throughpro- and anti-inflammatory systems of DFU. Moreover, ADA and GDA are released from thedamaged cells which lead to increase their circulating levels during inflammatory stage. Finally, studies

concern by oxidative stress reported that adenosine and guanosine play as antioxidant against the bad effect of reactive oxygen species (ROS) [28]. ROS are frequently increased as a response of chronic inflammation and this usually associated by a decrease in the antioxidant activity [29, 30]. In other studies which were conducted on various antioxidantmolecules in different types of diseases, showed a significant decrease in the levels of these molecules [31-36]. It is therefore the increase in ADA and GDA activities, which involved in the decline of antioxidant activity of adenosine and guanosine, might be a part of inflammation disorders that leads to increase the ROS production. Taken together, the increase in ADA and GDA might contribute to DFU complications severity.

Furthermore, our findings showed that there was a strong positive correlation between the activities of ADA and GDA in male and female, these results support aforementioned mechanisms and explain that ADA and GDA show a similar behavior in response to DFU. Synchronized rise in ADA and GDA activities in response to viral hepatitis was previously reported by Kalkan *et al*. [37] which is consistent with our result. It has been also observed that there was a significant positive correlation between HbA1c and both ADA and GDA, this outcome is consistent with earlier study which explained that both of HbA1c and ADA are considered a good marker for predicting of glycemic status where their levels were directly correlation to severity of inflammation in chronic hyperglycemia status of type2 diabetes mellitus [8]. In contrast, the iron level showed a strong negative correlation with ADA and GDA, this result agreement with Moustafa *et al*. [38] who found that the decrease in iron level was associated with increase in ADA activity in psoriatic patients. However, the positive relationship between iron level and ADA was reported by several studies [39, 40]. The inconsistency in results might indicate that the change in iron and ADA levels occurs independently of one another. To the best of our knowledge, no studies have been conducted regarding to relationship between GDA and each of HbA1c and iron levels. The association between iron deficiency and DFU pathogenesis has been described by many studies and this deficiency may attribute to insufficient nutrition, less iron absorption through gut, and depression of bone marrow due to chronic inflammatory mechanism [41].

The ROC curve analysis was adopted in this study as a suitable method for evaluating the accuracy of ADA and GDA as diagnostic test. The best cut-off value of serum ADA and GDA showed a higher percentage of sensitivity and specificity in discriminating DFU from controls. Although elevated HbA1c was the best common feature of DFU [42], some people who had moderate HbA1c were diagnosed to be infected by DFU injury. It is therefore we suggested that ADA and GDA levels in serum could be used for predicting the progression of DFU.

A pilot experiment to determine Vmax and Km parameters was carried out using different concentrations of ADA and GDA substrates. Our study on enzyme kinetics showed that there was an increase in Vmax and Km of ADA and GDA in DFU status. These changes in enzyme kinetics may indicate that in case of DFU pathogenesis there has been a substantial change in enzyme structure and this led to decrease of ADA and GDA affinity for their substrates compared to controls. Further studies on ADA and GDA structures are required to prof the aforementioned suggestion.

4. Conclusion

The outcomes of current study demonstrated that both ADA and GDA activities were markedly increased in serum of patients with DFU compared to controls. Additionally, these enzymes displayed a strong positive correlation with high sensitivity and specificity for DFU pathogenesis. Alteration in enzyme kinetics may effect on ADA and GDA activates in case of DFU infection. Based on these results, it can be conclude that the increase in ADA and GDA levels would contribute to a deterioration of DFU state.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental, health, higher education, and scientific research ministries in Iraq.

Conflict Ofinterest

The authors declare that they have no conflict of interest.

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