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Original Article

Biochemistry Section

Estimation of Serum Adenosine Deaminase Levels as a Marker for Risk Assessment and Immunomodulation in Patients with Diabetic Foot

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ABSTRACT

Introduction: Diabetic foot ulcers are critical complications of uncontrolled diabetes, with peripheral neuropathy, peripheral arterial disease, and immune suppression as primary contributing factors. Adenosine Deaminase (ADA), an enzyme, has been identified as a promising marker for cell-mediated immunity. Its estimation may serve as a predictive marker for immunomodulation, which, in turn, leads to complications among individuals with diabetes.

Aim: To estimate serum ADA levels in diabetic patients without diabetic foot and diabetic patients with diabetic foot. Additionally, the study aimed to investigate the association of ADA serum levels with glucose, Glycated Haemoglobin (HbA1c), and the severity of wounds.

Materials and Methods: This cross-sectional study was conducted in the central laboratory Department of Biochemistry at Krishna Rajendra Hospital (KR Hospital), Mysuru Medical College and Research Institute, Mysuru, India, from August to September 2021. The study included diabetic foot cases (n=45) and Type 2 diabetes patients on regular follow-up in the Department of Medicine without diabetic foot and any other complications (n=45). Venous blood samples were analysed for ADA, glucose, HbA1c, and complete blood count. The data were statistically analysed using the Mann-Whitney U test, Kruskal-Wallis test, and Spearman rank correlation test.

Results: The present study observed a higher incidence of diabetic foot among males. It showed a statistically significant increase in serum ADA, HbA1C, and neutrophil count in patients with diabetic foot compared to patients with diabetes without diabetic foot ulcers, with mean Standard Deviation (SD) of 71.79±25.11, 11.53±3.11%, and 75.91±9.77%, respectively. Serum ADA exhibited a positive correlation with HbA1C and different grades of ulcers.

Conclusion: The present study demonstrated a significantly increased serum ADA in diabetic ulcers and a positive correlation with different grades of ulcers. This finding contributes to a better understanding of the disease's pathogenesis at different stages. Furthermore, the positive correlation between ADA and HbA1C levels may play an important role in predicting the glycaemic and immunological status of these patients.

Keywords: Blood glucose, Cell-mediated immunity, Diabetic complication, T-lymphocyte function

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a condition characterised by disrupted glucose homeostasis that has reached epidemic proportions worldwide. According to the World Health Organisation, the number of individuals diagnosed with T2DM has already reached 380 million and is expected to rise to 592 million by 2035 [1]. Chronic hyperglycaemia is a hallmark of T2DM and can lead to significant damage and failure of various organs, such as the eyes, kidneys, nerves, heart, and blood vessels [2].

Diabetic foot ulcers are one of the most serious complications of long-term uncontrolled diabetes, affecting over 15% of diabetic patients and often leading to lower limb amputations, reduced quality of life, and increased mortality [3]. T2DM is primarily caused by immunological and metabolic disturbances, with insulin resistance and insufficiency being the most significant metabolic variables. Recent research suggests that the development of insulin resistance may be linked to tissue-specific inflammatory responses, including proinflammatory cytokines, adipocytokines, and chemokines. Chronic exposure to these mediators may ultimately impair insulin signalling receptors in pancreatic islets, leading to insulin resistance and further exacerbating T2DM [4,5].

The T2DM has been associated with abnormal T-lymphocyte function and cell-mediated immunity problems [6]. ADA, also known as adenosine aminohydrolase or ADA, is a crucial enzyme involved in purine nucleoside metabolism found in all human tissues. It catalyses the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. This enzyme has been utilised as a biomarker for several immune system illnesses since it is critical for the differentiation and proliferation of lymphocytes and the monocyte-macrophage system [7].

Elevated serum ADA levels have been observed in infectious mononucleosis and disease-related changes in monocyte/macrophage activity. Adenosine, which stimulates insulin activity through several processes, including glucose transport and lipid synthesis, is regulated by serum ADA. Therefore, excessive ADA levels consequently result in lowered adenosine levels, which subsequently diminish glucose uptake into cells and increase insulin resistance. Lowering ADA activity may improve factors associated with T2DM pathophysiology, including insulin sensitivity, inflammation, and T-lymphocyte activity [8].

The available literature concerning ADA levels in diabetic patients is inconclusive, but a scientific association exists between ADA and cell-mediated immunity where pro- and anti-inflammatory processes are crucial in the different phases of wound healing. It is conceivable that disturbances of the immune system interfere with tissue homeostasis and wound healing after the manifestation of ulcers, leading to the chronic, non healing wounds that are characteristic of diabetic foot syndrome [8].

Given the surprising paucity of data on the role of systemic inflammation in diabetic foot ulcers, the present study was conducted

to estimate serum ADA levels in patients with diabetes without diabetic foot and patients with diabetic foot. Additionally, the study aimed to correlate the serum levels of ADA with glucose, HbA1C, and the severity of the wound.

MATERIALS AND METHODS

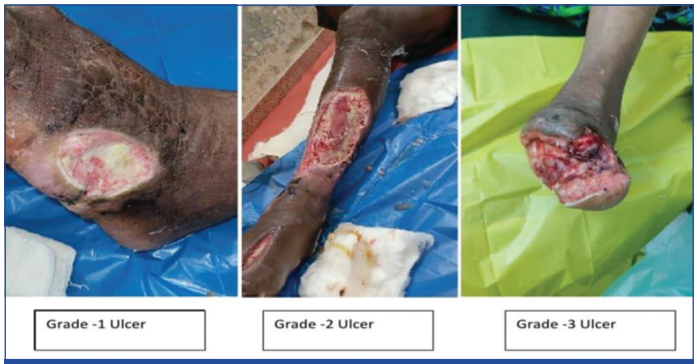
The cross-sectional study was conducted in the central laboratory department of Biochemistry at Krishna Rajendra Hospital (KR Hospital), Mysuru Medical College and Research Institute, Mysuru, Karnataka, India, for a period of two months between August and September 2021. Ethical clearance was obtained from the Institutional Time Bound Research Committee with IEC Number: (EC 155:REG:ECR/134/Inst/KA/2013/RR-16 Dated on 15.07.2021), and written informed consent was obtained from all subjects.

Inclusion criteria: The study included diabetic patients aged between 30-60 years, as most of the diabetic complications are expected to occur within this age group. The population was divided into two groups with age and gender matched. A total of 45 patients in Group-1 were selected from patients with a known history of diabetes, under control without any complications, who came for regular follow-up within the study duration. A total of 45 patients in Group-2 were selected from the Department of Surgery, admitted with diabetic foot without any other complications. The subjects were categorised into different grades based on the University of Texas Diabetic Wound Classification [9].

Exclusion criteria: Patients with a history of type 1 diabetes mellitus, gestational diabetes, cardiac, and renal diseases were excluded.

Study Procedure

Data regarding age, gender, occupation, smoking status, duration of diabetes, history of hypertension, renal, and cardiac diseases were collected in the proforma. The severity of the wound was assessed based on a grading system [Table/Fig-1] according to the University of Texas Diabetic Wound Classification [9].



[Table/Fig-1]: Showing the different grades of the ulcers.

Grades of ulcer:

0- Epithelialised wound

1- Superficial wound

2- Wound penetrates to tendon or capsule

3- Wound penetrates to bone or joints

A 3 mL fasting venous blood sample was collected from the study population in a plain vacutainer under aseptic precautions, and serum was immediately analysed for serum glucose by the Glucose Oxidase and Peroxides (GOD-POD) method. Serum ADA levels were determined using the spectrophotometric method based on different absorption spectra of adenosine and inosine at 265 nm. A 2 mL EDTA sample was used to estimate HbA1C by the latex agglutination inhibition method using a fully automated chemistry analyser Cobas 6000. The same whole blood was also used for a complete blood cell count using a 6-part hematology automated cell count instrument from Transasia. The normal reference range for fasting glucose is 70-100 mg/dL, ADA is 4-22 U/L, HbA1C is 4-6%, Neutrophils counts are 40-70%, and Lymphocytes are 20-40% [10].

STATISTICAL ANALYSIS

The results were expressed as mean±standard deviation. Statistical analysis was performed using Epi Info software version 7, and the tests used were the Mann-Whitney U test and Kruskal-Wallis test. To correlate the serum ADA with Glucose, HbA1C, and the severity of the wound, the Spearman's rank correlation test was used. A p-value <0.05 was considered statistically significant.

RESULTS

In the present study, out of 45 study subjects in each group, gender differences were found to be statistically significant between both groups. Diabetic foot was statistically significantly associated with illiteracy. There was no statistical association observed with respect to age, duration of disease, history of smoking, and history of hypertension. The present study showed a statistically significant increase in serum ADA, HbA1C, Neutrophils, and a decrease in Lymphocyte count in patients with diabetic foot ulcer compared to those without foot ulcer [Table/Fig-2].

Parameters	Diabetic patients without diabetic foot (n=45)	Diabetic patients with diabetic foot (n=45)	p-value
Age (years)	52.02±13.48	55.36±9.48	0.053
Gender	Male=28	Male=37	0.034*
	Female=17	Female=08	
Duration of diabetes (years)	5.44±6.2	6.44±5.5	0.188
Education	Literate=32	Literate=23	0.031*
	Illiterate=13	Illiterate=22	
History of smoking	Yes- 04	Yes- 10	0.081
	No=41	No=35	
History of hypertension	Yes- 14	Yes=11	0.480
	No=31	No=34	
Serum fasting glucose (mg/dL)	180.52±96.84	202.30±87.04	0.686
S. Adenosine Deaminase (ADA) (IU/L)	47.26±14.32	71.79±25.11	0.001*
HbA1c%	10.02±2.70	11.53±3.11	0.017*
Neutrophils %	59.97±8.07	75.91±9.77	0.001*
Lymphocytes%	28.63±8.17	17.01±7.99	0.001*

[Table/Fig-2]: Different parameters among diabetic patients without diabetic foot and diabetic patients with diabetic foot.
Test of significance used; Chi-square test and Mann Whitney U Test

The present study also showed a significantly positive correlation between serum ADA and HbA1C, as well as ADA with Fasting Blood Sugar (FBS), among both study groups [Table/Fig-3].

Correlation b/w	Group-1 (DM without D. Foot)		Group-2 (DM with D. foot)	
	Correlation coefficient (r)	p-value	Correlation coefficient (r)	p-value
ADA and HbA1c	0.539	<0.001	0.605	0.001
ADA and FBS	0.411	0.005	0.591	0.001

[Table/Fig-3]: Correlation between the Serum ADA levels with FBS and HbA1C among study groups.
Test of significance used; Spearman rank correlation test

[Table/Fig-4] shows the statistically significant increase in serum ADA levels with different grades of diabetic foot ulcer, with significantly higher levels of serum ADA in Grade-3 diabetic ulcers with a mean value of 88.71±25.55 U/L compared to Grade-1 ulcers with a mean value of 51.97±6.19 U/L.

The Kruskal-Wallis test showed that serum ADA did not show any significant changes with the duration of diabetes among the diabetic subjects [Table/Fig-5].

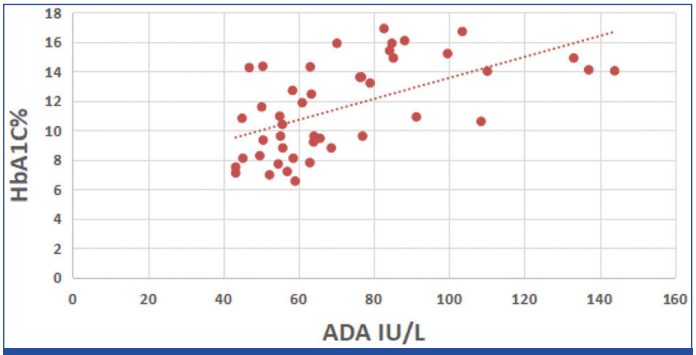
Grade	Number	Mean±SD inU/L	p-value
0	0	-	0.001
1	16	51.97±6.19	
2	8	67.03±15.28	
3	21	88.71±25.55	

[Table/Fig-4]: Mean serum ADA level in different grades of ulcers in diabetic patients with foot ulcer.
Test of significance used Kruskal-Wallis test

Duration (years)	Number	Mean±SD (U/L)	p-value
0-5	51	59.83±27.91	0.519
6-10	21	58.00±19.00	
>10	18	60.46±15.48	

[Table/Fig-5]: Mean serum level of ADA with respect to duration of diabetes (n=90).
Test of significance used Kruskal-Wallis test

The scatter diagram [Table/Fig-6] shows a positive correlation between HbA1C and serum ADA levels in Group-2, with a correlation coefficient (r value) of 0.605 and p<0.001.



[Table/Fig-6]: Scattered diagram showing the positive correlation between the HbA1C and serum ADA levels.
Correlation coefficient r value=0.605, p<0.001

DISCUSSION

The diabetic foot is one of the important complications of diabetes. The present study highlights the importance of the immune system in the development of chronic wounds in diabetic patients, particularly diabetic foot ulcers. Immune activation may precede the incidence of a diabetic foot ulcer in the same way as it precedes the manifestation of type 2 diabetes and coronary heart disease. Disturbances in the immune system prevent tissue homeostasis and wound repair, leading to chronic, non healing wounds [11,12]. In a cross-sectional research study analysing the relationship between foot ulcers and immune status in diabetes patients, blood levels of ADA were used, as there is a surprising lack of information on the immune system's function in diabetic foot ulcers.

The present study demonstrated significantly increased serum levels of ADA, HbA1C, and neutrophil count in patients with diabetic foot compared to those without diabetic foot, with mean standard deviations of 71.79±25.11 IU/L, 11.53±3.11%, and 75.91±9.77% respectively. The study did not show any significant variation in age and duration of diabetes. This is in accordance with previous studies conducted by Kaur A et al., and Niraula A et al., [13,14].

In present study population, high serum ADA activity was observed, particularly among patients with diabetic foot ulcers, and these values were significantly correlated with the severity of the ulcer. This is attributed to chronic inflammation, which results in the release of lymphocytes. These lymphocytes play various roles once they enter the inflamed tissue. Consequently, present study also revealed lymphopaenia among patients with diabetic foot, with the most notable impact on "T" cells that activate macrophages. The activity of ADA enzyme is required for phagocytosis in dealing with different pathogens. ADA has been shown to be necessary for lymphocyte proliferation and differentiation [15]. "T" lymphocytes detect this enzyme, and thus, in chronic inflammatory conditions, there may be an increase in serum ADA levels. As a result, elevated ADA levels may contribute to inflammation by reducing extracellular adenosine concentration. Adenosine serves as a significant anti-inflammatory agent [16].

Under healthy conditions, adenosine concentrations are typically low, but they increase more than 100 times compared to baseline levels when cells are under stress [17]. An elevated adenosine concentration can have anti-inflammatory and cell-protective effects by preventing macrophage activation and the synthesis of cytokines and chemokines [18]. Adenosine is a local hormone that regulates various biological activities, such as vasodilation, bradycardia, inhibition of platelet aggregation, and promotion of glucose uptake by cells. Previous studies have concluded that adenosine has a similar effect to insulin on glucose and lipid metabolism in adipose tissue [19].

The ADA activity is widely distributed in organs such as the heart, skeletal muscle, liver, and fatty tissues [20]. Increased ADA activity in insulin-sensitive tissues leads to lower adenosine levels, resulting in reduced glucose absorption by cells [21]. Serum ADA plays a crucial role in the maturation and activation of lymphocytes, and conditions involving cell-mediated immune responses have been associated with high ADA activity. Its blood levels could aid in diagnosing immunological dysfunction in type 2 diabetes [22]. Chronic hyperglycaemia promotes oxidative stress and insulin resistance through the formation of enediol radicals and superoxide ions by the Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase system, along with an increase in ADA levels [23].

Previous studies by Chang FY and Shaio MF have demonstrated a link between aberran T-lymphocyte proliferation and poor cell-mediated immunity [24]. The present study revealed a significant increase in ADA levels in patients with diabetic foot ulcers, with ADA levels increasing with the severity of ulcers, thus indicating the severity of the condition and predicting the prognosis. Studies have shown a direct correlation between the degree and intensity of inflammation and the expression and activity of ADA [25,26]. The study conducted by Lee PY et al., concluded that ADA serves as a biomarker of immunomodulation based on their study outcomes [26].

In present study, there was no correlation between serum ADA levels and the duration of diabetes. However, there was a significant positive correlation between serum ADA levels, HbA1C, and the severity of ulcers, with a p-value <0.05. This suggests that serum ADA levels may be used as a biomarker to predict glycaemic control in diabetic patients and, more importantly, as a marker of insulin resistance, aiding in predicting future complications and prognosis. These findings align with studies conducted by Yu C et al., and Lu CF et al., which concluded that serum ADA levels serve as early diagnostic markers in Diabetic Neuropathy and Diabetic Kidney Disease [27,28].

Limitation(s)

The present study had certain limitations. Firstly, the findings were not compared with other inflammatory markers or immunological markers, which could provide a more comprehensive understanding of the immune response in diabetic foot ulcers. Secondly, it is important to note that the study was a short-term student project conducted over a period of two months, and therefore, the sample size was designed accordingly.

CONCLUSION(S)

The serum ADA levels are significantly increased in patients with diabetic foot ulcers. There is a positive correlation between serum ADA levels, HbA1C, FBS, and the severity of the ulcer. These findings indicate that serum ADA levels can serve as a cost-effective biomarker for assessing disease severity and as a prognostic marker for monitoring and evaluating the effectiveness of treatment

in diabetic foot ulcers. Additionally, due to its positive correlation with HbA1C, serum ADA levels can be used as an indirect marker of glycaemic control, providing insights into the seriousness of the disease.

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