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THE STUDY OF SERUM ADENOSINE DEAMINASE ACTIVITY AS AN IMMUNOENZYME MARKER IN TYPE 2 DIABETES MELLITUS PATIENTS.

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ABSTRACT

Background: Diabetes Mellitus is metabolic disorder with physiological abnormalities like insulin resistance and impaired secretion of insulin. Immunological disturbances such as the cell mediated immune system and improper T- Lymphocyte function play important role in the pathophysiology of type 2 Diabetes Mellitus. The aim of present study was to assess the serum adenosine deaminase level as an immunoenzyme marker in type 2 Diabetes Mellitus. **Material and methods:** The present study was case-control study. Total 70 subjects were included and divided into two groups. Group A consisted 35 subjects of type 2 diabetes mellitus in the age group 40-50 years while Group B consisted of age and sex matched 35 normal healthy individuals who served as control with no history of Diabetes Mellitus. Serum levels of adenosine deaminase, fasting blood glucose and glycosylated hemoglobin were estimated in all the subjects under study. Values were expressed as mean± standard deviation. SYSTAT version 12 software was used for statistical analysis. **Results**: Serum adenosine deaminase level was increased significantly ($p<0.01$) in diabetic patients as compared with controls. Fasting blood glucose and glycated hemoglobin levels were significantly higher ($p<0.0001$) in cases compared to controls. Correlation was positive and significant when adenosine deaminase was correlated with fasting blood glucose and glycated hemoglobin. **Conclusion:** Adenosine deaminase level is elevated whenever stimulation of cell mediated immunity. Thus Adenosine deaminase may be an important immnoenzyme marker in assessing the cell mediated response in type 2 diabetic patients for better management and for developing new treatment strategies.

KEYWORDS: Type 2 diabetes mellitus, Adenosine deaminase, Glycated hemoglobin, Fasting blood glucose.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrinological disorder characterized by metabolic abnormalities and long term complication. The metabolic disorder characterized by chronic hyperglycemia. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ system that imposes a tremendous burden on the individual with diabetes and on the health care system.[1]

According to WHO, In the year 2002, the global burden of disease estimates the world wide burden of diabetes in adult to be around 173 million and it is estimated that 80 million people in India would be having diabetes by the year 2030.[2]

ADA (Adenosine Deaminase) is an enzyme involved in purine metabolism. The concentration of intracellular and extracellular adenosine is regulated by adenosine deaminase. In DM persistent hyperglycemia contributes for the formation of superoxide anion which leads to formation of hydrogen peroxide which is responsible for the activating the signaling molecules leading to inflammation, cell growth, apoptosis and fibrosis.[3]

Adenosine is selective regulation of pro and anti inflammatory cytokines released and free radical production.[4,5] ADA is an enzyme distributed in the human tissues was considered as good marker of cell mediated immunity and it converts adenosine into inosine through an irreversible deamination reaction. Increased free radical leads to increased activity of ADA. Hence DM is characterizing by increased ADA level.[6] Higher serum ADA level, Triglycrides and fasting plasma glucose level in nonobese type 2 DM patients and strong correlation between ADA and fasting plasma glucose which suggests an association between ADA and nonobese T2DM subjects.[7] M. Shiva et al have reported that increased ADA activity may be due to altered Immunity.[6]

MATERIAL AND METHOD

The present case control study was conducted in department of biochemistry PDVVPf' s Medical College Ahmednagar. The was approved by Ethics Committee of

PDVVPF's Medical College Ahmednagar with all participants providing informed consent and utmost care was taken during experimental procedure according to the declaration of Helsinki 1975.

Study type
Case- Control study.

Study Design
total 70 samples were enrolled in the present study.

Control group
35 healthy age and sex matched individuals without any evidence of type 2 DM per clinical examination were taken as control subjects.

Patients group
The study included total 35 patients between age group 40-50 years of type 2 DM.

Inclusion criteria
a) Patients with clinically proved Type 2 Diabetes Mellitus, with a known Diabetic for minimum period of 3 months between 40 - 50 years were taken. The criteria of uncontrolled Diabetic subjects was ascertained by Glycosylated Hemoglobin (>7 gm/dl)
b) Controls are healthy individuals, age and sex matched without any major illness and not on any medication

Exclusion criteria
Insulin dependent Diabetes mellitus, hypertensive, smokers and patients with other concurrent chronic disease such as cardiac disease, thrombotic stroke, tuberculosis, Rhematoid arthritis, Sarcoidosis, gout, renal failure or any additional condition which alters the ADA levels in the serum.

Collection of blood sample
About 5 ml of venous blood was drawn from subjects under aseptic precautions, using a sterile disposable syringe. Of this, 2ml of blood was taken in EDTA container for glycosylated hemoglobin estimation. 3ml of blood was utilized for estimation of fasting plasma glucose, ADA. After an hour, the samples were centrifuged at 3000 rpm for 10 minutes to separate serum and used for analysis of ADA.

METHOD

1) Determination of serum ADA activity
The ADA levels measured spectrophotometrically by (Gusti and Galanti) method based on the berthlot reaction that is formatiom of colored indophenols complexes from ammonia liberated from adenosine. Intensity of the blue coloured indophenols complex formed is directly proportional to the amount of ADA present in the serum sample.

2) Estimation of fasting blood glucose. By GOD-POD method
Glucose is oxidised to gluconic acid and H2O2 in the presence of glucose oxidase. The enzyme peroxidase catalyses the oxidation coupling of 4-aminoantipyrin with phenol to yield a coloured complex i.e. quinoneimine complex. Intensity of the pink color formed is proportional to concentration of the gluconic acid and can be measured photo metrically between 500 to 540 nm.

3) Estimation of Glycosylated Hemogliobin by ion-exchange high performance liquid chromatography.
The D-10 Hemoglobin A1c program utilizes principle of ion –exchange high performance liquid chromatography. The sample were automatically diluted on D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increased ionic strength to the cartridge, where the hemoglobin were separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbace were measured at 415 nm.

Statistical Analysis
Statistical software SYSTAT version-12 (by Cranes software, Bangalore) was used to analyze the data. The result were expressed in mean ± Standard Deviation (Mean± SD) Data was analysed by descriptive statistics as mean, SD, percentage etc. Comparisons of study group to control group by using the Students't' test. Pearson's correlation coefficient was used to find out the correlation between two variables. P – Values of <0.01 and <0.0001were considered as statistically significant.

RESULTS
As shown in the table no.1, the mean serum fasting blood sugar in type 2 DM was 175.11 ± 62.1mg/dl and in controls it was 96.54 ±8.51 mg/dl. From this table it can be inferred that there is a significant increase in mean Fasting Blood Sugar values in type2 DM patients when compared with normal healthy controls (p <0.0001) Also the mean HbA1c levels in type 2 DM was 7.89 ±2.3 % and in controls it was 4.80 ± 6. 6 % that is significantly increased in mean HbA1c values in type2 DM patients when compared with normal healthy controls (p <0.0001).

Table No.1 showed that, the mean serum ADA levels in type 2 DM was 60.11 ±2.3 and in controls it was 23.08 ± 7.1 The mean serum ADA in type 2 DM was signicantly higher in type 2 DM when compared with normal healthy controls (p<0.0001) .

Table No. 2 showed correlation between the parameters. 'r ' values were for ADA Vs fasting blood sugar 0.594 (p<0.001) and for ADA Vs glycosylated Hb was 0.912 (0.0001) in type 2 DM. this illustrates that correlation between ADA and Fasting blood sugar and Glycosylated

Hb was positively correlated and highly significant in Type 2 DM.

DISCUSSION

DM is a cluster of abnormal metabolic paradigm having a common feature of hyperglycemia. It is a metabolic disorder characterized by chronic hyperglycemia. Depending on the etiology of DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production. Chronic hyperglycemia leads to disturbances in carbohydrate, fat and protein metabolism resulting from defect in insulin secretion, insulin action or both.[1]

Persistent hyperglycemia leads to formation of advanced glycosylated end products and further complications, which include long term damage, dysfunction and failure of various organs. Immunological disturbances of cell mediated origin are believed to initiate from T-lymphocyte dysfunction.[8] ADA, an enzyme distributed in the human tissues is considered as good marker of cell mediated immunity. ADA is an enzyme that converts adenosine into inosine through an irreversible deamination reaction. Hyperglycemia causes formation of Advanced Glycation End Products (AGEs) as result of non-enzymatic reactions between intra-cellular glucose-derived dicarbonyl precursors with the amino group of both intracellular and Extracellular protein. These AGEs stimulates receptors for advance glycation end roducts(RAGE), their interaction is believed to initiate and aggravate the diabetic complications. This event leads to superoxide anion which further leads to increase generation of ROS causing oxidative stress. The consequences of oxidative stress activates several cell type like endothelial cells, mesengial cells and macrophages to release inflammatory mediators like cytokines, Tumor necrosis factor- alpha, Interleukin -1,6. The released mediator activates the T lymphocytes consequently these activated T lymphocytes undergo abnormal proliferation and differentiation.[9, 10]

An ADA is a marker of T lymphocyte proliferation and differentiation due to the above consequences. Highest activity of ADA is seen because of the abnormal T lymphocytes proliferation. Frankie B et al have suggested that improper immune response in type 2 DM is may be due to defects in action of insulin that is required for function of T-lymphocyte.[11] In view of this, the present study has been taken to know the role of ADA as an immunoenzyme marker and in development and progression of complications in type 2 DM. In the present study, the fasting blood glucose levels were significantly increased (p <0.0001) in type 2 DM compared to controls. The glycated hemoglobin levels were significantly higher (p<0.0001) in cases compared to controls. In current study, serum ADA levels was raised in type2 DM when compared to controls and it is statistically significant (P <0.0001) ADA levels is positively correlated with glycated hemoglobin (HbA1c) which were statistically significant at p <0.0001. These findings are exactly coordinated with previous studies, where significantly increased serum ADA, Serum Glycosylated Hb and fasting blood sugar level.[12, 13] Bhavita Patel et al have demonstrated that serum ADA levels increase with increase in fasting blood glucose and glycated Hb which may have an important role in dertermining the glycemic status in diabetes. Elevated ADA may be an important role in the immune-pathogenesis of type-2 DM. A positive correlation between ADA level with short and long term glycemic control suggest its role in glucose and lipid metabolic derangements seen in type 2 DM patients.[12] In hospital based case-control study, Nisha SR et al have suggested that ADA can be a marker of glycemic staus in the Diabetic population.[13]

M. Shiva Prakash et al have hypothesized that increased ADA activity may be due to altered immunity. Therefore, ADA may serve as an immunoenzyme marker in type 2 DM. In present study, high level of ADA might be due to abnormal T-lymphocyte responses or proliferation mainly altered insulin related T-lymphocyte function. 6 Ahmed Al-Shulaili et al have studied the different inflammatory mediators like interlekin-10, Tumor necrosis factor-alpha, etc in Type 2 DM, according to their study, alternation in the function of the immune system mainly occurred in Type 2 DM.[14] thus suppression of ADA activity may help improve insulin sensitivity, inflammation, cell proliferation and T- lymphocyte activity, which are associated with the pathophysiology of Type 2 DM.[15, 16]

CONCLUSION

The present study showed that increased ADA levels in type 2 DM. this finding can explain increased T-lymphocyte response because of greater formation of AGEs products. So these are the factors for development of diabetic complications. In conclusion it can be said that elevated ADA activity may be used as indicator in the immune-pathogenesis of type 2 DM. Thus ADA may be an Important immunoenzyme marker in assessing the cell mediated response in type 2 diabetic patients for better management and for developing new treatment strategies.

Table No.1 Subjects characteristics in Type 2 DM and Controls.

Variable	Type 2 DM (n= 35)	Controls (n=35)	't' values	P value	Inference
Age (in years)	42-50	40-50	-----	-----	-----
Sex (M/F)	22/13	17/18	-----	-----	-----
Fasting blood sugar (mg/dl)	175.11±62.1	96.54±8.51	9.01	<0.0001	Highly Significant

Glycosylated Hb (%)	7.89±2.31	4.80±6.6	9.09	<0.0001	Highly Significant
ADA (U/L)	60.11 ±2.3	23.08±7.1	8.99	<0.0001	Highly Significant

Table No.2 Pearson's correlation between parameters in type 2 DM

Parameters	'r' value	'p' value
ADA Vs Fasting blood sugar	0.594	<0.001
ADA Vs Glycosylated Hb	0.912	<0.0001

REFERENCES

1. Sheetz MJ, King GL. Molecular understanding of hypergycemia's adverse effects for diabetic complications. JAMA, 2002; 288: 2579-88.
2. Wild S Roglic G, Green A, et al global prevalence of diabetes: estimates for the year 2000 and projection for 2030 diabetic care, 2004; 27: 1047-53.
3. Singh PP, Mahadi F, Roy A and Sharma P. Reactive oxygen species, reactive nitrogen spiecies and anti oxidants in etiopathogenesis of diabetes mellitus type 2. Indian journal of clinical biochemistry, 2009; 24(4): 324–42.
4. HaskoG, kuhel DG, chenJF et al. Adenosine inhibits IL -12 and TNF α production via adenosine A2a receptor dependent and independent mechanism. FASEB J, 2000; 14: 2065.
5. Hosko G Szabo C, Nemeth Z H et al. Adenosine receptor agonist differently regulate IL – 10, TNF α and nitric oxide production in macrophage and in edoemic mice. J immunol, 1996; 157: 4634.
6. M Shiva Prakash, S Chennaiah, YSR Murthy, E Anjaiah, S Ananda Rao, C Suresh. Altered adenosine deaminase activity in type 2 Diabetes Mellitus. JIACM, 2006; 7 (2): 114-2
7. Vineet Kumar K, Debajit B, Arindam G, Oishimaya S, Aritri B, Sasanka C et al Raised serum adenosine deaminase level in nonobese type 2 diabetes mellitus. The Scientific world journal, 2013; 1-5.
8. Stymvoli M, Goldstecn B, Haeften TW. Type 2 diabetes: Principles of pathogenesis and therapy. Lancet, 2005; 365: 1333-45.
9. Kumar V, Abbas AK, Fausto N. The Endocrine system. Robbin's and cotran Pathologic basis of disease 7th ed; 2008; 82: 1198.
10. Goldsby RA, kindt TJ, Osborne BA, cytokinines. Koby immunology. 4th Ed. Newyork: W.H freeman and company, 2000: 320.
11. Frankie B, Abbas E. Activated T-lymphocyte in type 2 diabetes: implications from in vitro studies. Curr Drug Targets, 2003; 4: 463-503.
12. Bhavita P, Dilip T, Brahmareddy M, Mitul C, Rita S. Serum Adenosine deaminase in patients with type-2 Diabetes Mellitus and its relation with blood glucose and gycated hemoglobin levels. International Journal of Biomedical Research, 2014; 05(09): 01-03.
13. Nisha SR, Krishnamurthy N, Raghavendra PBN, Ashakiran S, Sumathi M.E, Harish R. role of adenosine deaminase to predict glycemic status in type 2 Diabetes mellitus. J Clin Biomed Sci., 2012; 2(3): 123-133.
14. Ahmed Al-Shukaili,Saif AL- Ghafri, Safia Al-Marhoobi, Said Al- Abri, Jawad Al-Lawati, Masoud Al- Maskari. Analysis of inflammatory mediators in type 2 Diabets patients. International Journal of Endocrinology. 2013;Article ID 976810,7 pages.
15. J.G. Lee, D.G. Kang, J. R. Yu et al, Changes in adenosine deaminase activity in patients with type 2 DM and effect of DPP-4 inhibitor treatment on ADA activity. Diabetes and Metabolism Journal, 2011; 35(2): 149-158.
16. J. Rutkiewicz and J. Gorski. On the role of insulin of regulation of adenosine deaminase activity in rat tissues. FEBS Letters, 1990; 271(1-2): 79-80.