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Role of Adenosine Deaminase in Dyslipidemia

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ABSTRACT

Background: Dyslipidemia is a family of lipoprotein metabolism disorders manifested by elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and reduced high density lipoprotein cholesterol (HDL-C) concentrations in the blood.Adenosine deaminase (EC 3.5.4.4) is involved in catabolism of adenosine and deoxyadenosine. Adenosine is a nucleoside that has been implicated in a wide variety of biological functions including the regulation of lipid(e.g. cholesterol, triglycerides and fatty acids) availability in the cells (Reiss and Cronstein,2012) and in the systemic circulation (Koupenova et al.,2012).

Aim: The aim of study is to show that the lipolysis and cholesterol level is inhibited by ADA which is one of the factor for causing dyslipidemia and only few studies are done to show the correlation with dyslipidemia.

Materials and Methods: A total of 50 dyslipidemia patients and 50 healthy subjects aged >20 years who were newly diagnosed patients of dyslipidemia and having no Familial hypercholesterolemia visiting to both outpatients and inpatients department of Rama Medical College, Hospital & Research Centre were included in our society. Parameters such as age, sex and biochemical indicators like ADA and Lipid profile were determined and compared with control subjects.

Result: ADA levels were reported significantly higher in dyslipidemia patients compared to healthy controls in both genders.

Conclusion: ADA can be used in daily routine laboratory assessment of most metabolic diseases especially in obese, dyslipidemia and diabetic patients. Thus, targeting ADA in the treatment of metabolic diseases would be very appropriate. Hence, ADA level may be used as risk factors for atherosclerosis.

Keywords: Dyslipidemia, Adenosine Deaminase, Adenosine, Atherosclerosis, Lipolysis

I. INTRODUCTION

Dyslipidemia is a family of lipoprotein metabolism disorders manifested by elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and reduced high density lipoprotein cholesterol (HDL-C) concentrations in the blood. [1] A combination of hypercholesterolemia and hypertriglyceridemia was classified as mixed hyperlipidemia while atherogenic dyslipidemia was a combination of HDL hypocholesterolemia, hypertriglyceridemia and LDL hypercholesterolemia.[2]

The prevalence of dyslipidemia is increasing rapidly around the globe due to westernization of dietary patterns, reduced physical activity, obesity, aging of population and many other co factors contribution.[3,4] Studies revealed that early onset of dyslipidemia is associated with the development of early atherosclerotic coronary and peripheral artery disease and increased incidence of cardiovascular disease(CVD) in adulthood.[5,6] Recently, dyslipidemia is increasingly prevalent in all age groups, and the incidence tends to be younger.[7] Indians in India have lower total cholesterol and lower HDL cholesterol and greater triglyceride levels.[8,9]

Adenosine deaminase (ADA) is also known as adenosine aminohydrolase. It is an enzyme present in most tissues of the body and involved in purine metabolism. It catalyses the irreversible deamination of adenosine to produce inosine and ammonia. Removal of endogenous adenosine by ADA resulted in an immediate rise in lipolytic activity. Consequently, elevated ADA levels in DM patients may augment hyperlipidemia by increasing lipolysis.[10]

Adenosine (Ado) acts directly to stimulate insulin activity via several processes such as glucose transport, lipid synthesis, pyruvate dehydrogenase activity, leucine oxidation and cyclic nucleotide phosphodiesterase activity.[11] The effects of adenosine are mediated by the action of

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the nucleoside on four different adenosine receptors (ARs). Lipolysis is the catabolic pathway through which triglycerides are enzymatically hydrolysed to non-esterified fatty acids (NEFAs) and glycerol, a process inhibited by A1R activation (Johansson et al., 2008; MacPherson et al., 2016) which is involved in lipolysis. A1R activation leads to reduced cAMP levels that control the lipolytic process mainly by regulating cAMP-dependent protein kinase and lipases activity (Johansson et al., 2007).[12]

II. MATERIALS AND METHODS

The present observational case control hospital based study was carried out after approval of Ethical Committee of Rama Medical Hospital and Research centre in Department of Biochemistry on dyslipidemia patients and age sex matched healthy individuals. A total of 50 newly diagnosed patients of dyslipidemia and 50 normal healthy subjects were enrolled in the study as per inclusion criteria who visited OPD and IPD. Informed consent was duly taken from each subjects during the study period.

Study Design: Case – Control study.
Study Duration: February 2020 to February 2021.
Sample Size: 100 patients
Inclusion Criteria :

i. Patients of dyslipidemia will be included in study group i.e. a total cholesterol > 200 mg/dL or an LDL cholesterol > 130 mg/dL while HDL hypocholesterolemia was at HDL cholesterol < 40 mg/dL. Hypertriglyceridemia was declared for triglyceride plasma level > 150 mg/dL.
ii. Newly diagnosed Hypothyroidism Patients
iii. Newly Diagnosed Type 2 Diabetes Mellitus Patients
iv. Normal healthy persons are taken as controls

Exclusion criteria:

- Tuberculosis patients
- Kidney disorder
- Viral hepatitis
- Chronic malnutriton
- Leprosy
- Brucellosis
- Liver cirrhosis
- Hyperammonemia
- Familial hypercholesterolemia

Sample Collection:

5ml of fasting blood sample was collected from antecubital vein into each plain vial for lipid profile and for ADA fresh serum/plasma EDTA, Citrate, heparinised or oxalate anticoagulated sample can be taken from each of the subjects under all aseptic conditions after explaining the procedure to the study subjects. Blood sample was allowed to clot at room temperature for 15 minutes and serum was obtained by centrifugation at 4000 rpm (rotation per minute) for 5 minutes in the biochemistry laboratory and stored at -20^{o}C until assayed. The supernatant serum will be used for the analysis of serum Adenosine deaminase, total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and triglycerides level.

Procedure methodology:
Serum Total Cholesterol (TC) estimation:
Serum Total Cholesterol was estimated by CHOD-PAP method on Erba CHEM-7 semi auto analyzer.
Serum Triglycerides (TG) estimation:
Serum Total Cholesterol was estimated by GPO, end point method on Erba CHEM-7 semi auto analyzer.
Serum High Density Lipoprotein-Cholesterol (HDL-C) estimation:
Serum HDL-C was estimated by Phosphotungstic acid method on ErbaCHEM-7 semi auto analyzer.
Serum Very- Low Density Lipoprotein Cholesterol (VLDL-C) estimation:

- Method : Friedwald's equation[98]
- VLDL-C = TG/5

Serum Low Density Lipoprotein Cholesterol (LDL-C) estimation:

- Method : Friedwald's equation[98]
- LDL-C = TC- (HDL-C+ TG/5)

Serum Adenosine Deaminase (ADA) estimation:
Serum ADA was estimated by Giusti and Galantimethod on ErbaCHEM-7 semi auto analyzer.

Statistical Analysis:

All the parameters of two groups were analyzed for mean and standard deviation. The results were expressed as Mean ± standard deviation. Data was analyzed by statistical software **SPSS Version 22.0.** Comparison among two groups was done by using t- Test. Pearson's correlation coefficient was used to find the correlation between ADA and Lipid profile. The differences among the means (Mean±SD) were considered significant if P<0.01 & 0.05.

III. RESULTS

The present research work included 100 Subjects (50 dyslipidemia patients and 50 healthy individuals). Gender and age distribution have been done to see the prevalence of dyslipidemia and healthy among study subjects. Clinical data is

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studied to find out the Gender and age distribution of cases and controls. The observations of the **Table 5-1** evince predominantly male population which comprises 60% in the cases, 62% control and the ratio of female over male is 1**:**1.5 and 1**:**1.63 (1**:**2 approximately) respectively**.** There is a trend toward a higher prevalence dyslipidemia in the age group 41-60 years and majority (46%) of subjects in controls are in 21-40 years age group **(Table 5-1).**

The **Table 5-2 and Graph 5-1**shows the mean values of ADA, TC, HDL-C, LDL-C and TG in cases and controls respectively**.** Higher mean ADA (59.08± 17.98 U/L) is recorded in cases compared to controls (17.01±5.86); and the variance in mean ADA among the cases and controls is found to be statistically significant with p value 0.000. Mean serum TC level is higher in cases (226.18 ± 70.64 mg/dl) than in controls (149.38 ± 28.60 mg/dl). Mean serum HDL-C level is lower in cases (42.14 ± 11.48 mg/dl) than in controls (51.56 ± 10.78 mg/dl). Mean serum LDL-C level is higher in cases (137.72 ± 69.77 mg/dl) than in controls (76.70 ± 24.22 mg/dl). Mean serum TG level is higher in cases (231,62 ± 91.14 mg/dl) than in controls (105.60 ± 26.16 mg/dl) and the Variances in mean TC, HDL-C, LDL-C and TG levels among the cases and controls are statistically significant ('p' = 0.000).

Table 5-3 evident a significant positive correlation between ADA and TC ('p'= .000), LDL-C ('p'=.000) and TG ('p'=.000) in cases indicates that an increase in the first variable would correspond to an increase in the second variable in comparedto controls.**Table 5-3** also depict a significant negative correlation between ADA and HDL-C in cases ('p'=.000) which imply an inverse relationship that is when one variable increases, second variable decreases in compared to controls.

Table 5.1: The gender and age distribution of total subjects (dyslipidemia cases and healthy individuals) included in the study.

Subjects		**Cases**		**Controls**		**Total**	
		N	%	N	%		
Gender	**Male**	30	60	31	62	61	100
	Female	20	40	19	38	39	
Age (Years)	**21-40**	14	28	23	46	37	100
	41-60	23	46	16	32	39	
	61-80	13	26	11	22	24	
Total		50		50			
		100					

Table 5.2: Analysis of variance between cases and controls

Parameters	**Cases (Mean ± SD)**	**Controls (Mean ± SD)**	**'p' value**
ADA	**59.08 ± 17.98**	**17.01 ± 5.86**	**.000**
TC	**226.18 ± 70.64**	**149.38 ± 28.60**	**.000**
HDL-C	**42.14 ± 11.48**	**51.56 ± 10.78**	**.000**
LDL-C	**137.72 ± 69.77**	**76.70 ± 24.22**	**.000**
TG	**231.62 ± 91.14**	**105.60 ± 26.16**	**.000**

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Graph 5.1: Comparison of mean of variance between cases and controls.

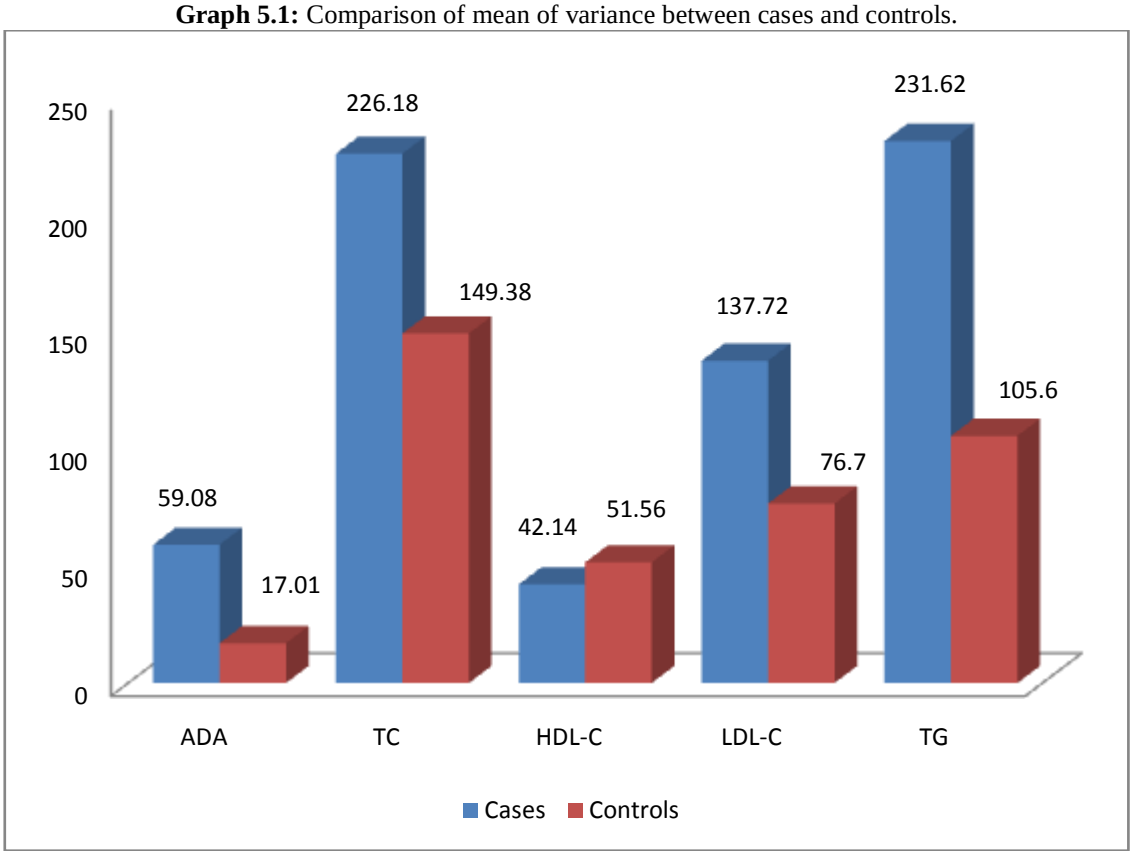

Table 5-3: Pearson correlation studied of ADA with Dyslipidemic profile in cases and controls**.**

S.N.	Parameters	Cases		Controls	
		r -value	**p -value**	**r -value**	**P -value**
1.	**TC**	**.224**	**.000**	**-.001**	**.000**
2.	**HDL-C**	**-.300***	**.000**	**-.150**	**.000**
3.	**LDL-C**	**.222**	**.000**	**.107**	**.000**
4.	**TG**	**.209**	**.000**	**-.193**	**.000**

*.Correlation is significant at the 0.05 level (2-tailed).

IV.DISCUSSION

The present study was conducted at Rama Medical College, Hospital & Research Centre, Kanpur, Uttar Pradesh, India with the objective to study role of adenosine deaminase (ADA) in dyslipidemia and compare it with matched healthy individuals in the population. Dyslipidemia is a condition in which the body suffers from a number of lipid disorders. Since functions of adenosine deaminase are involved in central role in differentiation and maturation of the lymphoid system, purine metabolism etc. More importantly purine metabolism, which deaminates adenosine and deoxyadenosine into inosine which results into lipogenesis resulting into dyslipidemia.

Dyslipidemia is a very common condition and seen more in men than in women. Although all age groups are presented with a high prevalence of dyslipidemia, our study indicates that there is a trend toward a higher prevalence of dyslipidemia in the age group 41-60 years in cases **(Table 5.1).**

This is in accordance with the earlier studies that is Combined dyslipidemia was the most common dyslipidemia pattern observed and this accounted for a third of the study population. Jayaramet al.,in a single center study in about 800 patients reported that 44.2% males and 42.97% females had combined dyslipidemia.[13] and Wube A.T et al.[14] The higher prevalence of dyslipidemia in men suggests that environmental/lifestyle imbalances or as a consequences of other diseases might be involved in the pathophysiology dyslipidemia. Diabetic dyslipidemia is extremely common in type 2 diabetes mellitus and

atherogenic dyslipidemia often observed in MetS, obesity etc.

In the present study, the Mean ± SD levels of ADA were significantly higher in cases than that of healthy controls also serum ADA shows a statistically significant between cases and controls. Similar findings were reported by Venketeshwarlu K. et al.[15], Nwankwo A. A. et al.[16], Prasad D. V. et al.[17],Oinam S. et al.[18], Karunashree et al.[19] and Dasegowda M. S. et al.[20]**[Table 5.2, 5.3 and Graph 5.1].**Adenosine Deaminase has a significant role to play in purine metabolism which deaminates adenosine to inosine. As we know adenosine play important role in regulation of lipid availability through lipolysis. Deficiency of adenosine tends to cause hyperlipidemia, which is a known risk factor for development of atherosclerotic disease. In this study, we discuss the role of adenosine deaminase in dyslipidemia patients.

From our study, it is observed that dyslipidemia patients having elevated total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) shows positive correlation with ADA and reduced high density lipoprotein (HDL-C) shows negative correlation with ADA. Our findings are in consistent with the figures mentioned in local as well as in the international literatures of authors, Venketeshwarlu K. et al.[86], Nwankwo A. A. et al.[87], Oinam S. et al.[89], Karunashree et al.[90] and Dasegowda M. S. et al.[91]. who reported positive correlation with TC, TG, and LDL-C and negative correlation with HDL-C values with adenosine deaminase in cases.

The present study found an elevated serum adenosine deaminase activity in cases of dyslipidemia when compare to age and sex matched controls. It has been shown that that in the adipose tissue most of adenosine is formed extracellularly. Extracellular adenosine is metabolised only by adenosine deaminase. Adenosine is secreted by adipocytes in which the main adenosine receptor is A1R. A1R activation leads to reduced cAMP levels that control the lipolytic process mainly by regulating cAMP-dependent protein kinase and lipases activity. In addition to regulating NEFAs in adipocytes, adenosine and ARs also regulate the content of cholesterol in monocytes/macrophages by increasing cholesterol efflux via A2AR activation and reducing inflammation via A2AR and A2BR activation, two process that are directly related with the formation of foam cell and thus with the development of atherosclerosis.

As adenosine is metabolized by Adenosine deaminase, role of adenosine as modulators of lipolysis, cholesterol efflux and inflammation is inhibited which results into lipogenesis thus increasing the level of lipid contents such as TC, LDL-C and TG in blood plasma. The increased number of TG-rich lipoproteins results in increased cholesteryl ester-transfer-protein (CETP) activity, which exchanges cholesterol esters from HDL for TG from VLDL and LDL. Moreover, lipolysis of these TG-rich HDL occurs by hepatic lipase resulting in small HDL with a reduced affinity for A-I, which leads to dissociation of apo A-I from HDL. This will ultimately lead to lower levels of HDL-C and a reduction in circulating HDL particles with impairment of reversed cholesterol transport.

Dyslipidemia on a long term causes cardiovascular complications such as CHD or arteriosclerosis. Increased activity of adenosine deaminase increases lipid contents in blood plasma. Increased levels of plasma cholesterol as well as endothelial expression of adhesion molecules such as E-selectin, P-selectin and ICAM-1, promote endothelial dysfunction, an early phenomenon in the development of atherosclerosis. Dysfunctional endothelial cells favour the attachment of monocytes that express chemokines that produce inflammatory signals, which precedes the shift of monocytes to macrophages, which are cells that uptake of LDL (C) and promote a pro-inflammatory environment to cause the formation of foam cells. The accumulation of foam cells and apoptotic foam cells in the intima space leads to the development of a fatty streak or early atheromatous lesion.

Our study is limited by the limited number of patients attending only Rama Medical College Hospital and Research Centre, Kanpur U.P and also due to limited period. Additionally, the list of potential confounders for above enzyme assay disturbances is long which need to be studied in details and requires large population to reflect the correlation correctly.

V.CONCLUSIONS

Biochemical screening for Adenosine Deaminase is of paramount importance in all dyslipidemic patients, as well as in all patients with unexpected worsening of their lipid profile or vice-versa because our data statistically suggest that the effect of adenosine deaminase is associated with lipid disorders that are characterized by increased serum Total Cholesterol, Triglycerides, LDL-C and decreased HDL-C levels.

From this study, it can be concluded that dyslipidemia is most common in middle aged subjects. So, clinicians should remain highly

suspicious in middle aged subjects with lipid profile for increase in atherogenic parameters which may enhance the risk for atherosclerosis leading to coronary artery disease.

Therefore, treatment and follow-up of dyslipidemia patients should include the monitoring of lipid profile parameters, serum ADA in order to decrease the possible effect of changing in the level of these parameters on the risk of cardiovascular diseases.ADA can be used in daily routine laboratory assessment of most metabolic diseases especially in obese and diabetic patients Thus, targeting ADA in the treatment of metabolic diseases would be very appropriatee. Hence, ADA level may be used as risk factors for atherosclerosis.

On the other hand, there is need for large studies designed to answer the questions whether as ADA is associated with increased level in dyslipidemic profile and cardiovascular diseases.

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