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## ORIGINAL ARTICLE

# The Effect Of Hyperglycaemia On Some Biochemical Parameters In Diabetes Mellitus

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### ABSTRACT

**Objective:** Diabetes Mellitus (DM) is the most common endocrinological disorder which is characterized by metabolic abnormalities and long term complications. The purpose of this study was to investigate the role of hyperglycaemia on various parameters like 1) Malondialdehyde (MDA), a parameter to study increased oxidative stress, as hyperglycaemia leads to increased lipid peroxidation 2) Adenosine Deaminase (ADA) activity which is suggested to be an important enzyme for modulating the bioactivity of insulin and 3) Free Fatty acids (FFA) to study dyslipidaemias which are associated with diabetes. **Design and Methods:** MDA, ADA and FFA levels in serum were measured spectrophotometrically in 50 patients of type 2 Diabetes Mellitus (DM) and also in 25 healthy controls. The patients were divided into three groups according to the levels of Glycosylated haemoglobin (HbA1c); Group I (with HbA1c 6-10%), Group II (with HbA1c 10-13%) and Group III (with HbA1c >13%). The results were also compared with the control group which had HbA1c of 4-6%. **Results:** All the three parameters, ADA, MDA and FFA levels were found to be significantly higher in the case subjects as compared to the controls ( $p < 0.001$ ). In addition to this correlation, the study revealed that MDA levels are positively associated with FBS and PPBS and also, that ADA is positively correlated with MDA ( $r = +0.57$ ,  $p < 0.001$ ). **Conclusion:** These findings suggest that hyperglycaemia is associated with increased levels of ADA and that it is one of the factors which lead to the increased production of oxidative stress and also the derangements of lipid metabolism which are associated with DM. Also, ADA has got a role in increasing lipid peroxidation by reactive oxygen species (ROS) generation, as reflected by increasing MDA levels.

**Key Words:** Diabetes Mellitus, Glycosylated Haemoglobin, Adenosine Deaminase, Malondialdehyde, Free Fatty Acids

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### Introduction

Diabetes is a major worldwide health problem, leading to markedly increased mortality and serious morbidity. It is a state which is characterized by chronic hyperglycaemia resulting from a diversity of aetiologies and environmental and genetic factors acting together. Hyperglycaemia itself leads to increased oxidative stress and dyslipidaemias. The long term control of DM is judged by

levels of HbA1c, which was first isolated by Allen et al. [1].

Lipid peroxidation is a chain reaction which provides a continuous supply of free radicals. MDA is a secondary product of lipid peroxidation and it is derived from lipid peroxides of polyunsaturated fatty acids with two or more double bonds. It is used to measure the degree of lipid peroxidation. Chronic hyperglycaemia has an adverse effect

on  $\beta$  cell function, which eventually leads to worse hyperglycaemia. This vicious cycle has led to the adage that 'hyperglycaemia begets hyperglycaemia'. This phenomenon has been designated as 'glucose toxicity'. Although the mechanism of glucose toxicity is unknown, recent in vitro [2],[3] studies show that ROS (oxidative stress) promotes the formation of cytotoxic lipid peroxides.

ADA (Adenosine aminohydrolase, EC 3.5.3.3) is an enzyme of purine metabolism. It acts on adenosine and several other adenosine nucleoside analogues. It was concluded that increased adenosine activity mimics the activity of insulin on glucose and lipid metabolism in adipose tissue [4]. Also, it was found to be a marker of T cell activation and a producer of ROS [5]. Free fatty acids are the unesterified, transport form of fatty acids in the plasma. These are the most active metabolic plasma lipids. Insulin regulates the formation of free fatty acids. In diabetes mellitus, insulin deficiency leads to the increased breakdown of triglycerides. So, in diabetes, there is an increased release of free fatty acids.

The present study was done to evaluate the role of hyperglycaemia in increasing the levels of ADA, lipid peroxidation, and FFA in DM and to know the role of ADA in DM as an independent marker, as well as a stimulator of lipid peroxidation.

## Materials and Methods

The study was conducted on 50 known patients of type 2 DM reporting to the Govt. Medical College and Hospital, Patiala. The patients were divided into Group I (with HbA1c 6-10%), Group II (with HbA1c 10-13%) and Group III (with HbA1c >13%). The criteria for the diagnosis of DM were the same as the one which was given by the National Diabetes Data Group:

Symptoms of diabetes plus random blood glucose conc.  $\geq$  11.1 mmol/l (200mg/dl)

Fasting plasma glucose > 7.0 mmol/l (126 mg/dl)

25 subjects of similar age, sex and socioeconomic status served as controls. The controls were free from any major ailment

which could affect the parameters under study (No clinical history or investigative results showing involvement of any organ). The exclusion criteria for the patients were Tuberculosis (TB), Infectious Mononucleolus, Breast cancer and Behcet's disease. Informed consent was obtained for venipuncture. A detailed history was taken to know duration of the disease, treatment history and any complication of the disease.

Blood was drawn in the fasting state for Fasting Blood Sugar (FBS) and two hours after meals for Post Prandial Blood Sugar (PPBS) estimation. The samples were collected in fluoridated vials. For HbA1c, the sample was collected in a heparinised vial, as whole blood was required for its estimation. The samples were collected in plain vials for the estimation of ADA, MDA and FFA. Sera were separated from the samples within half an hour and these were stored at the appropriate temperature till analysis was done. FBS and PPBS were measured by Asatoor and King's method [6].

HbA1c was estimated by the chromatographic spectrophotometer ion exchange method of Bisse and Abraham [7]. After preparing the haemolysate, where the labile fraction was eliminated, the haemoglobins were retained by a cationic exchange resin. HbA1c was specially eluted after washing away the HbA1a+b fraction and it was quantified by direct photometric reading at 415nm. ADA was estimated by the method of Giusti G. [8]. Ammonia which is liberated by the reaction of ADA on adenosine forms an intensely blue indophenol complex with sodium hypochlorite and phenol in an alkaline solution. The intensity of the colour formed was measured spectrophotometrically at 620nm.

MDA was measured by measuring the complex which was formed between MDA and Thiobarbituric Acid (TBA) with Sodiumdodecyl Sulphate (SDS) [9]. The free fatty acids from the aqueous phase in serum were transferred into chloroform, in which a copper complex of free fatty acids was formed. FFA was measured spectrophotometrically from the copper complex by using sodium diethyl dithiocarbamate[10].

**Results**

This study was an exercise to evaluate the role of hyperglycaemia on the levels of MDA(for the extent of lipid peroxidation), ADA and FFA in DM. MDA was taken as a marker of lipid peroxidation and the control of diabetes was assessed by measuring HbA1c. The study and the control groups were compared according to age, sex distribution and routine investigations, which showed non significant results, except for blood sugar levels.

[Table/Fig 1]: showing age, sex distribution and blood sugar in control & study groups.

	Control group	Study group
Number	25	50
Female/Male %	44/56	42/58
Age in Years	43.32±12.50	44.62±13.48
FBS in mg%	88.6±15.35	178.76±67.39
PPBS in mg%	120.44±6.24	235.46±58.57

The mean fasting and postprandial sugar levels were found to be significantly higher in the study group as compared to the control group (p< 0.001), thus showing poor control of blood glucose in diabetic patients as compared to the control group.

The levels of these parameters were also compared with different ranges of HbA1c to study the effect of hyperglycaemia on these parameters.

[Table/Fig 2]: showing different parameters in control and study group

Group	HbA1c(%)	No. of cases	MDA(µmol/L)	ADA(U/L)	FFA (µmol/l)
Control	4-6	25	452 ±0.87	19.08 ±1.84	550.6 ±29.48
I	6-10	8	7.17±0.56	50.62 ±11.59	771.50± 36.16
II	10-13.0	16	7.20±0.97	61.0 ±9.34	781.35 ±56.43
III	> 13.0	26	7.84 ±0.50	67.0±9.46	809.07 ±55.29

In all the groups, the levels of MDA were found to be significantly higher (p<0.001) as compared to the control group. When the levels were compared between the groups, they were found to be non significant between groups I vs. II, but were significant between groups II vs. III and groups I vs. II (p<0.05).The levels of ADA were found to be significantly higher in all the study groups as

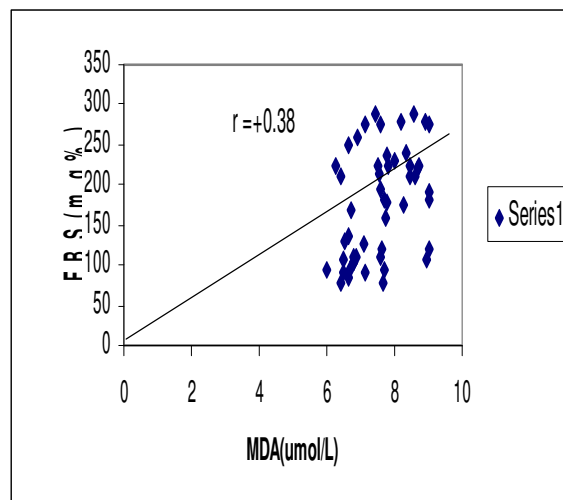
compared to the control group (p<0.001).When the levels were compared between the groups, they were found to be significant between groups I and II, between groups II and III and between groups I vs. III (p<0.001) When the levels of FFA were compared between the groups, they were found to be non significant between the three study groups (p >0.05), but were significant between the control group and all the three study groups. (p <0.001).

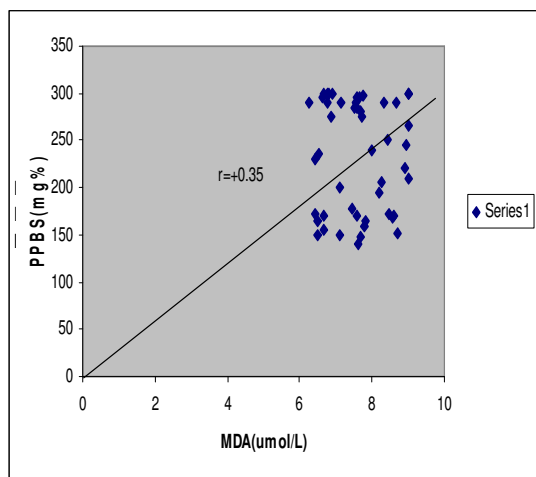
The correlation studies showed a strong positive correlation between MDA and FBS levels and also between MDA and PPBS levels.

[Table/Fig 3]: showing correlation between MDA, FBS and PPBS

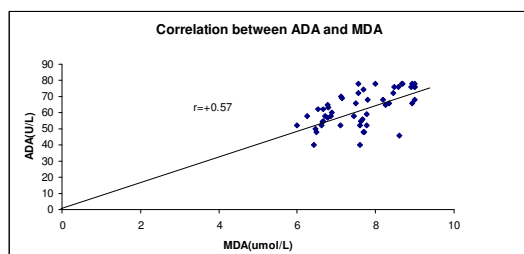
Parameter	No. of cases	Mean value± SD	Coefficient of correlation	P	S
MDA(µmol/L)	50	7.53±0.88			
FBS mg%	50	178.76± 67.39	+0.38	<0.01	S
PPBS mg%	50	235.46 ± 58.57	+0.35	<0.01	S

[Table/Fig: 4] and [5] showing correlation between MDA, FBS and PPBS





[Table/Fig 6] showing correlation between ADA and MDA

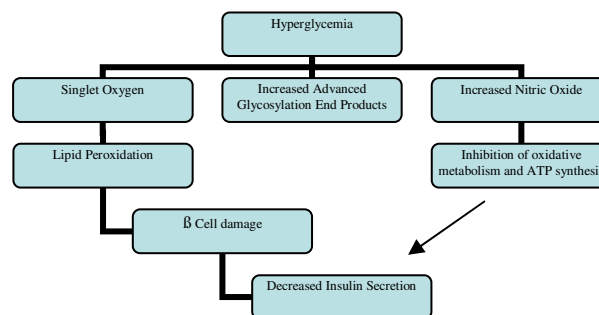


The data revealed that with the increase in ADA levels there was increase in oxidative stress.

### Discussion

The number of people affected with type 2 diabetes has reached epidemic proportions worldwide. It is estimated that by the year 2020, there will be approximately 250 million people who will be affected worldwide. Diabetes is associated with a variety of metabolic abnormalities, principal among which is hyperglycaemia, which is linked to oxidative stress. In addition to advanced glycosylation end (AGE) product formation, the most direct effect is the auto oxidation of glucose which is subjected to enediol rearrangements that result in the formation of an enediol radical ion. This species is capable of reducing molecular oxygen to form superoxide anion, which may contribute to the oxidation of lipids or to the activation of platelets. The Advanced Glycosylation End products induce cellular lipid peroxidation by interacting with their specific surface receptors [11]. Also, there is overproduction of nitric acid by inducible nitric oxide synthase in

diabetes, which further damages the beta cells [12].



The chronic hyperglycaemia status favours auto-oxidation and the glycation reaction, thereby leading to advanced glycosylation end products. Via their recognition by the cell receptors, these advanced glycosylation end products also participate in the development of oxidative stress and inflammatory status. The involvement of oxidative stress and AGE in diabetic complications is the basis of the development of adjuvant therapies with antioxidants [13],[14]. Amelioration of oxidative stress might slow down apoptosis at the same time when it repairs the existing beta cells, which could lead to the improvement of insulin secretion independently from conventional therapy [15].

Hyperglycaemia is also associated with increased levels of ADA. It was seen that adenosine in the extracellular space modulates stimulated glucose transport in striated muscle. In heart and adipocytes, adenosine potentiates insulin stimulated glucose transport. It was found that in muscles, removing adenosine with adenosine deaminase markedly reduced the responsiveness of glucose transport to stimulation by (i) insulin alone, (ii) contraction alone and (iii) by both. It was concluded that ADA treatment markedly reduced cell surface Glucose Uptake Transporter (GLUT) - 4 labelling. The results showed that adenosine potentiated insulin and contraction stimulated glucose transport in skeletal muscles by enhancing the increase in GLUT - 4 at the cell surface and raised the possibility that decreased adenosine production or action could play a causative role in insulin resistance [16]. In the present study, the values of ADA were significantly higher in the case subjects as compared to the controls. Markedly increased ADA levels showed their role in DM, maybe by causing insulin resistance and

ADA was also found to increase lipid peroxidation, as shown by our correlation study which showed a positive correlation between ADA and MDA ( $r=+0.57$ ,  $p<0.001$ ) levels. This indicated increased oxidative stress with increased levels of ADA. The proposed mechanisms for increased oxidative stress can be 1) role of ADA as a marker of T cell activation and 2) its relation to the production of ROS with the production of NO, O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> and OH, as studied by Erkilic [5]. Plasma ADA amplifies the release of toxic oxygen radicals from neutrophils through a down regulation of the inhibitory adenosine-c-AMP system [17]. MDA, ADA and xanthine oxidase levels were found to be higher in maternal and foetal plasma in preeclampsia than in normal pregnancy and also, similar findings were there in patients of acute myeloid leukaemia [18],[19].

Recent studies showed that elevated plasma levels of FFA might increase insulin resistance in the muscles and the liver. It was proposed that the earliest effect of high plasma FFA levels were 1) the inhibition of carbohydrate oxidation 2) the inhibition of glucose transport/phosphorylation and 3) the inhibition of glycogen synthesis. These studies support the hypothesis that elevated FFA levels induce insulin resistance principally at the level of glucose transport [20]. The acute elevation of plasma free fatty acids is necessary for insulin secretion. Sustained elevation however leads to the apoptosis of pancreatic beta cells and it is a major risk factor for cardiovascular disease and sudden death in patients with insulin resistance [21].

It can be concluded from the present study, that hyperglycaemia aggravates oxidative stress, as well as increased levels of adenosine deaminase in diabetes, which plays an important role in DM, which may be because of local insulin resistance in the target organs and also because of the increased production of free radicals and oxidative stress. Along with this, there are also increased levels of FFA, which also cause insulin resistance. The limitation of this study is that the exact role of adenosine deaminase in the pathogenesis of diabetes mellitus is not clear. Further studies at the molecular level are required to know the role of ADA levels in

modifying the effect of insulin and oxidative stress in DM.

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