HYPERGLYCEMIA INDUCED OXIDATIVE STRESS AND HYPOMAGNESEMIA: A DANGER FOR DIABETIC PATIENTS

KMDS Panag[1], Gitanjali Goyal[2], Anil Batta[3], Sheenam[4]

[1]*Professor and HOD, Department of Biochemistry, GGS Medical College, Faridkot.*
[2]*Associate Professor, Department of Biochemistry, GGS Medical College, Faridkot.*
[3]*Professor, Department of Biochemistry, GGS Medical College, Faridkot.*
[4]*MD Student, Department of Biochemistry, GGS Medical College, Faridkot.*

ABSTRACT

BACKGROUND
Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The morbidity and mortality associated with diabetes is a result of the myriad complications related to the disease. One of the most explored hypotheses to explain the onset of complications is hyperglycemia-induced increase in oxidative stress. Hypomagnesemia has also been correlated with both impaired glucose tolerance and insulin resistance in non-diabetic elderly patients. Aim of the study was to know the role of hyperglycemia and hypomagnesemia in increasing the oxidative stress in diabetes mellitus.

METHODOLOGY
The study was conducted on 50 known diabetic patients at Guru Gobind Singh Medical College and Hospital, Faridkot. Fasting plasma glucose (FPG), post prandial plasma glucose at 2 hours (PP$_2$PG), and magnesium levels were measured using fully autoanalyzer and compared with healthy controls. Glycosylated hemoglobin (HbA1c) was estimated by NycoCard Reader. Malondialdehyde (MDA) levels were estimated in serum. The study population was divided into three groups based on the HbA1c values, i.e., Group I (HbA1c <7% -good control), Group II (HbA1c 7-9% -fair control), Group III (HbA1c >9% -Poor control).

RESULTS AND CONCLUSION
The mean FPG and PP$_2$PG levels were significantly increased in patients as compared to control group in this study. Our study showed that MDA levels increased as the levels of glycated hemoglobin increased. Mg levels showed fall as levels of HbA1c increased. Also, there is a significant positive correlation of MDA levels with HbA1c with P value < 0.01 and negative correlation with magnesium.

To conclude, the data presented here suggests that diabetic patients are at increased risk of complications due to increased oxidative stress induced by hyperglycemia and hypomagnesemia. Magnesium deficiency may play a role in insulin resistance and development of diabetic complications independently also.

KEYWORDS
Diabetes, Hyperglycemia, Hypomagnesemia, Malonyldialdehyde, HbA1c.

INTRODUCTION
Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes causes about 5% of all deaths globally each year. The relatively ineffective insulin in this condition results in hyperglycemia. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.[1] The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and is predicted to be 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The diabetes mellitus in urban population in developing countries is projected to double between 2000 and 2030.

According to the latest World Health Organization (WHO) report, India has 31.7 million diabetic subjects and the number is expected to increase to a staggering 79.4 million by 2030.[2] Glycemic control is an important aspect in managing diabetes in order to prevent acute or chronic complications of diabetes mellitus.

Many randomized prospective clinical trials in type 1 and 2 diabetes have clearly shown that achieving glycemic control or reducing hyperglycemia significantly decreases the microvascular complications of diabetes. Each 1% reduction in hemoglobin A1c (HbA1c) was associated with a 37% decrease in risk for microvascular complications and a 21% decrease in the risk of any end point or death related to diabetes.[3,4]

The morbidity and mortality associated with diabetes is the result of the myriad complications related to the disease. One of the most explored hypotheses to explain the onset of complications is a hyperglycemia-induced increase in oxidative stress. Reactive oxygen species (ROS) are produced by oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, the uncoupling of lipoxygenases, cytochrome P450 mono-oxygenases and glucose autoxidation.[5]

Hyperglycemia can stimulate ROS production from a variety of sources. The relative importance of each source varies with cell or tissue type.

Polyunsaturated fatty acids can be non-enzymatically oxidized to produce lipid oxidation by products like lipid hydroperoxides, isoprostanes and malondialdehyde (MDA). These molecules are generally reactive and some such as malondialdehyde have been measured as markers of oxidative stress in DM (Fig. 1). Malondialdehyde is a late-stage lipid oxidation byproduct that can be formed non-enzymatically or as a byproduct of cyclo-oxygenase activity. MDA is a volatile molecule that reacts via Schiff base formation with free amine groups of proteins, lipids, and DNA.

Magnesium, the second most abundant intracellular cation has a fundamental role in carbohydrate metabolism in general and in the action of insulin in particular. A complex interplay exists between magnesium and glucose metabolism. Magnesium is an essential cofactor required for the generation of both aerobic and anaerobic energy from carbohydrate metabolism.[6]

Magnesium plays an indispensable role in glucose homeostasis. Hyperglycemia and insulin on the other hand affect plasma and erythrocyte magnesium differently. Hypomagnesemia has been correlated with both impaired glucose tolerance and insulin resistance in non-diabetic elderly patients.[7] Although poor glycemic control in diabetes is associated with magnesium deficiency, hypomagnesemia is not corrected by improvement in the metabolic control as documented by glycosylated hemoglobin.[8]

Magnesium depletion has been suggested to be related to the development of diabetic microvascular disorders and its other complications.[9,10,11,12] Magnesium deficiency has been suggested to be a state of increased oxidative stress as documented by recent studies.[13,14] Magnesium itself has been reported to posses antioxidant properties.[15,16]

Hyperglycemia and hypomagnesemia induced oxidative stress leads to increased risk of complications of Diabetes Mellitus. Besides this insulin resistance caused due to decreased magnesium levels further aggravate the problem (Fig. 2).

The present study was aimed to know the role of hyperglycemia in causing oxidative stress and hypomagnesimia in diabetes mellitus.

PROCEDURE
The study was conducted in Guru Gobind Singh Medical College and Hospital, Faridkot. A total of 50 known diabetic patients were enrolled for the study after taking informed consent. The parameters to know control of plasma glucose (FPG, PP$_2$PG), Magnesium were measured on fully autoanalyzer and were compared with 25 healthy controls. Hemoglobin A1c was estimated by NycoCard Reader. NycoCard HbA1c is a boronate affinity assay. MDA (Malondialdehyde) levels were estimated in serum for assessing lipid peroxidation. Malondialdehyde thiobarbituric acid complex was measured spectrophotometrically.[17]

The study population was divided into three groups based on the HbA1c values, i.e., Group I (HbA1c <7% - good control), Group II (HbA1c 7-9% - fair control), Group III (HbA1c >9% - poor control).

Statistical methods used: SPSS for windows was used for statistical analysis.

RESULTS
The mean age and sex distribution of study group was found to be statistically nonsignificant when compared with control group.

The mean FPG and PPPG levels were significantly increased in study group as compared to control group.

Table 1. shows that difference in FPG and PPPG, both were highly significant with P value <0.001 in the study group as compared to the control group.

Table 2. shows that glycated hemoglobin was significantly higher in study group as compared to controls indicating poor control of blood sugar. MDA levels were high in all the three study groups when compared with control. The MDA levels were highest in group III patients with HbA1c >9%, i.e., poorly controlled diabetes mellitus. The data shows that MDA levels are high in study group as compared to control group and further increase as HbA1c levels are increased.

Table 3. shows that there is a significant positive correlation of MDA levels with HbA1c with P value <0.01 (Fig. 3), whereas there is a negative correlation between MDA and Mg levels indicating association of hypomagnesemia and lipid peroxidation (Fig. 4).

DISCUSSION
Diabetes mellitus is a chronic illness that requires continuing medical care, patient education and support to prevent acute complications and to reduce the risk of long-term complications. Control of blood glucose in patients with diabetes can be assessed by several methods.

These include assessment of glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), and postprandial plasma glucose (PPPG). The gold standard for assessment of glycemic control at followup is the glycated hemoglobin level.[18]

Many in vitro studies have suggested that oxidative stress induced by hyperglycemic conditions has a pivotal role in the pathogenesis of vascular complications in diabetics. Increased oxidative stress as well as reduction in antioxidant capacity could be related to the complications in patients with diabetes such as oxidative DNA damage and insulin resistance.[19]

Due to decrease in antioxidant potential of plasma, complications of diabetes increase which include cardiovascular disease, nerve damage, blindness, and nephropathy. Thus, the increasing incidence of diabetes is a significant health concern beyond the disease itself.[20]

Similar findings were suggested by Hans CP.[13] MDA levels were significantly increased (p<0.001). Magnesium levels were also found to be negatively correlated with MDA (r=-0.648, p<0.001). In another study, magnesium levels were found to be significantly lower in diabetics (p<0.001) as compared to control.[21]

High concentrations of glucose can increase the glycation of common proteins such as HbA1c, formed through the non-enzymatic attachment of glucose to haemoglobin, which is commonly considered to reflect the integrated mean glucose level over the previous 8–12 weeks, the time period being dictated by the 120-day lifespan of the erythrocyte. The concentration of HbA1c predicts diabetes complications, because it reflects more harmful glycation sequelae of diabetes such as retinopathy and nephropathy, which are understood to be due to harmful advanced glycation end products.[22,23,24]

Increased MDA levels in plasma, serum and many others tissues has been reported in diabetic patients.[25,26] In a study, it was reported that lipid peroxidation in diabetes induced many secondary chronic complications including atherosclerosis and neural disorders.[27,28] Yang et al. observed greater serum lipid peroxidation evaluated in terms of MDA may exacerbate the occurrence of myocardial infarction through NADPH oxidase activation.[29]

The mechanism responsible for magnesium deficiency in patients with diabetes is not completely known. Osmotic diuresis clearly accounts for a portion of the magnesium loss. The renal glycosuria that accompanies the diabetic state is believed to impair renal tubular reabsorption of magnesium from the glomerular filtrate.[30]

However, other factors like diarrhea, vomiting, sodium intake and diuretics use may play a role in magnesium deficiency in diabetes mellitus. Insulin has been reported to enhance the transport of magnesium into cells. Therefore, impaired insulin action or insulin resistance may result in an intracellular magnesium deficit.[31]

Biological sequelae of hypomagnesemia in diabetic patients are not completely defined. It is possible that magnesium may be an important determinant of insulin sensitivity in non-insulin dependent diabetes mellitus. Magnesium deficiency has been linked to two most common complications of diabetes, namely retinopathy and ischemic heart disease.[9,11]

Hypomagnesemia can be a consequence of hyperglycemia and a cause of insulin resistance. In humans, insulin resistance has been implicated to impair the ability of insulin to stimulate magnesium or glucose uptake in diabetic individuals thus suggesting that low serum magnesium could be a marker of insulin resistance and hyperinsulinemia.[30]

Regression analysis considering magnesium as the independent variable and malondialdehyde as dependent variable showed a strongly significant correlation between these variables suggesting that magnesium deficiency might participate in the increased oxidative stress and decreased antioxidant potential.[32] This stressed the concept of evaluating magnesium levels in serum of diabetic patients, particularly with poor glycemic control.

CONCLUSION

To conclude, the data presented here suggests that diabetic patients are at increased risk of complications due to increased oxidative stress induced by hyperglycemia and hypomagnesemia. Magnesium deficiency may play a role in insulin resistance and development of diabetic complications independently also. Hence the diabetic patients needs to be educated regarding the importance of achieving good glycemic control (To achieve HbA1c < 7%) with and also from nutritive aspect to overcome magnesium deficiency as well as to supplement antoioxidants so as to reduce the morbidity and mortality due to various complications of diabetes mellitus.

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	Study Group	Control Group	T	P	S
Age (Years)	44.62 ± 13.48	43.32 ± 12.50	0.43	>0.05	NS
Sex (M:F)	58:42	56:44		>0.05	NS
FPG(mg%)	178.76 ± 67.39	88.16 ± 15.35	6.61		
PP$_2$PG (mg%)	235.46 ± 58.57	120.44 ± 6.24	9.75	<0.001	HS

Table 1: Showing Comparison of FPG and PP$_2$PG in Control Group and Study Group

Group	No. of Cases	GHb (%)	MDA (μmol/L)	Mg (mg%)
Control	25	4.5 ± 0.45	4.52 ± 0.87	2.01±0.17
Study-Group I (HbA1c< 7%)	10	6.5 ± 1.20	7.05 ± 0.70	1.80±0.16
Group II (HbA1c 7-9%)	25	8.85 ± 1.42	7.81 ± 0.55	1.75±0.10
Group III (HbA1c> 9%)	15	11.96 ± 2.29	8.54 ± 0.50	1.68±0.05

Table 2: Showing mean values of MDA and Magnesium levels according to Glycated Hemoglobin (GHb) in control Group and Study Group

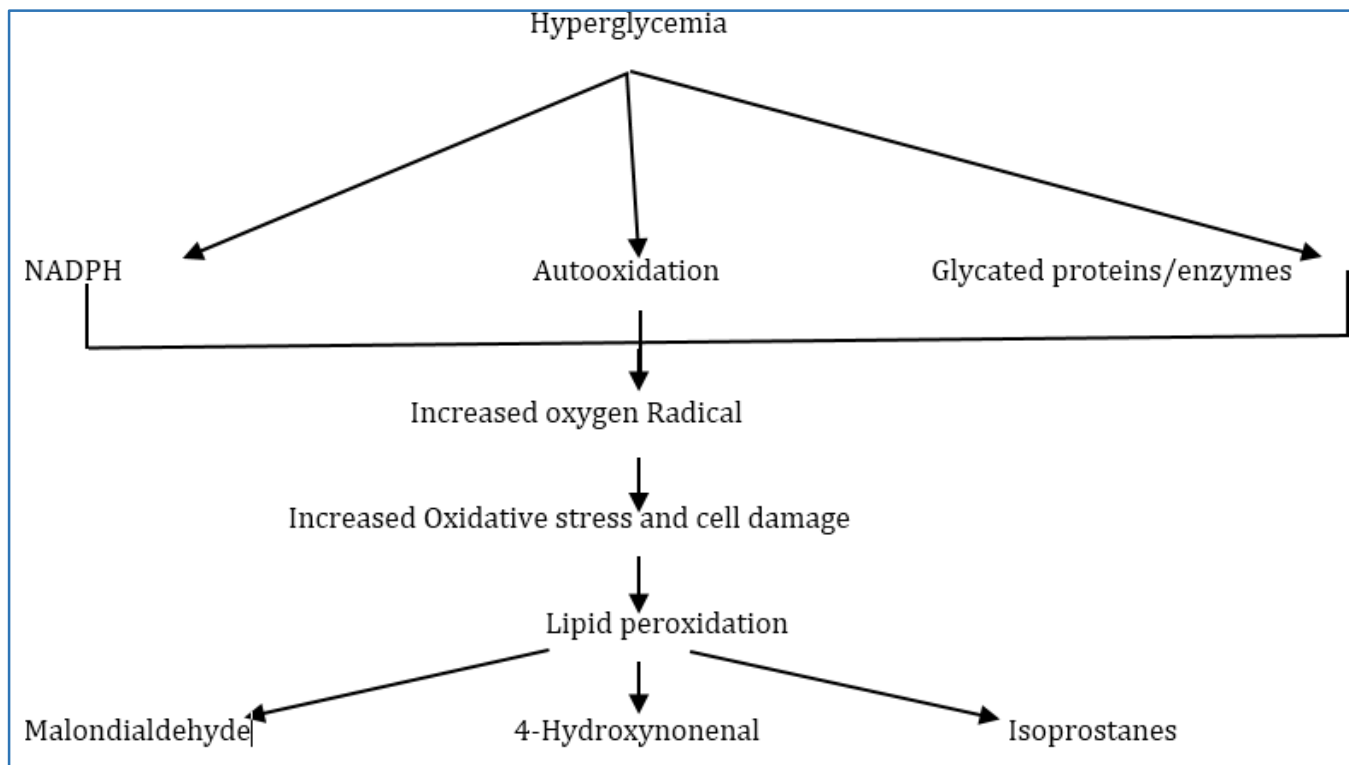| Parameters	Coefficient of Correlation	P	S
MDA (µmol/L) & HbA1c(%)	+ 0.38	< 0.01	S
MDA(µmol/L) & Mg (mg%)	- 0.57	<0.01	S
Table 3: Showing Correlation of MDA with HbA1c and Mg of Study Group			

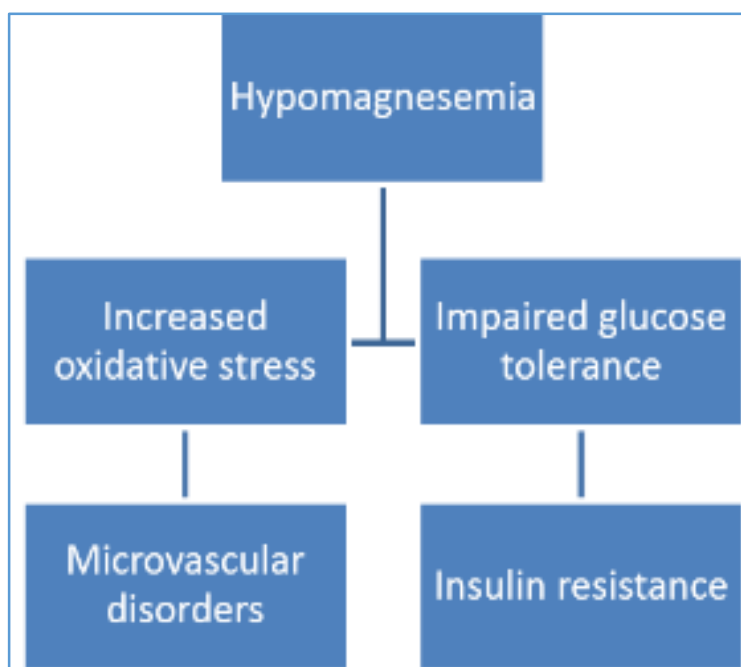*Fig. 1: Hyperglycemia leads to Increased Free Radical Production by Various Pathways*

Fig. 2: Showing Role of Decreased Magnesium Level in Causing Oxidative Stress and Insulin Resistance
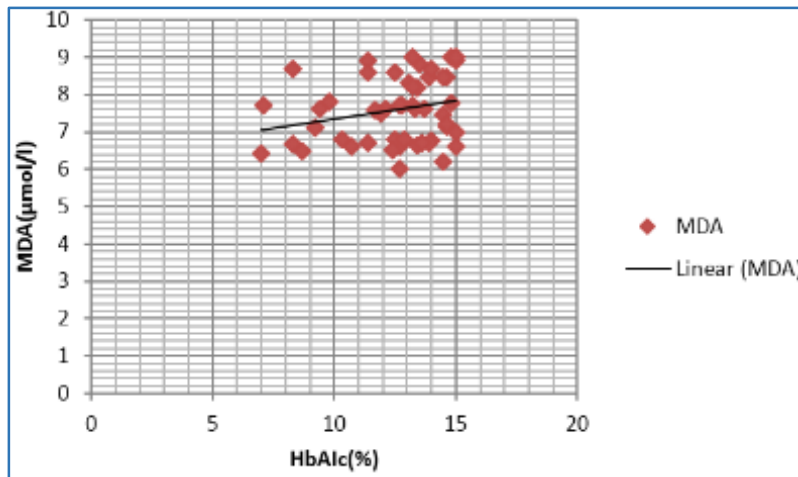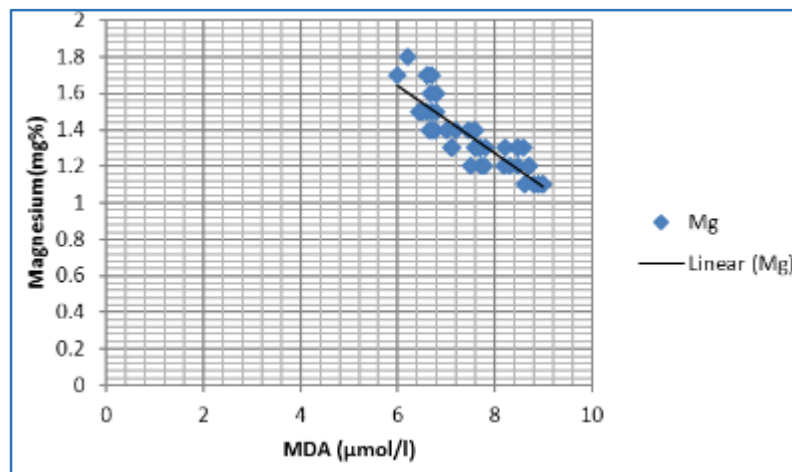
Fig. 3: Showing Positive Correlation between HbA1c and MDA

Fig. 4: Showing Negative Correlation between Magnesium and MDA