Association of Serum Magnesium with Oxidative Stress in the Pathogenesis of Diabetic Cataract

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Abstract

Magnesium deficiency enhances oxidative stress which contributes to early development of cataract formation, and also the progression in type 2 diabetes mellitus patients remains unclear. The present study was designed to evaluate the serum levels of magnesium, oxidative stress marker and antioxidant status and to find out if there is any association between them in the pathogenesis of diabetic cataract compared with non-diabetic senile cataract, diabetes without cataract and normal healthy subjects. This comparative study includes 90 type 2 diabetes mellitus patients with cataract, 90 non-diabetic senile cataract patients, 90 type 2 diabetes mellitus without cataract and 90 normal healthy individual subjects without cataract in the age group between 40 and 75 years of both genders. Serum magnesium was estimated by using a fully automated analyser. Serum malondialdehyde (MDA), an indicator of oxidative stress biomarker, was determined by spectrophotometry, and the antioxidant status such as serum reduced glutathione (GSH) and glutathione peroxidase-3(GPX-3) levels was estimated by ELISA method. The present study shows significantly decreased levels of magnesium, GSH, GPX-3 and increased level of MDA in type 2 diabetes mellitus patients with cataract when compared with non-diabetic senile cataract patients, type 2 DM without cataract and normal healthy individuals. A significant negative correlation of serum magnesium with MDA and positive correlation with GPX-3 were observed. The present findings indicate that hypomagnesaemia is a significant pathogenic factor which causes increased oxidative stress which may trigger earlier cataractogenesis in patients with type 2 diabetes mellitus.

Keywords Hypomagnesaemia · Oxidative stress · Cataract · Type 2 diabetes mellitus

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Introduction

Cataract is a major leading cause of visual impairment in diabetes mellitus patients [1]. Cataract formation occurs earlier and much faster in patients with diabetes than normal subjects [2, 3]. It is well known fact that oxidative stress plays a major role in most of the diabetic complications, including diabetic cataract [4, 5].

On the contrary, Mg deficiency (MgD) also has been suggested as a contributing factor in the pathogenesis of complications in diabetes [6]. However, the role of magnesium deficiency in the pathogenesis of cataract formation in diabetes population is still unknown.

Experimental animal studies have suggested that there is a correlation between MgD and the OS development, yet the link between these two pathogenic factors in humans is unclear [7]. In addition, several interventional studies using animal models of MgD have provided convincing evidence to show the link between magnesium, inflammation and oxidative stress [8–10].

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Furthermore, to the best of our knowledge, there is no previous study proving any relationship between magnesium and oxidative stress in the pathogenesis of diabetes-induced cataract. Therefore, we undertook this study to investigate the levels of magnesium, MDA and antioxidant status in serum, to find out the association between them in the pathogenesis of cataract development and its progression in diabetes by comparing the same with non-diabetic senile cataract subjects, type 2 DM without cataract and normal healthy subjects without cataract.

Materials and Methods

This observational comparative study was carried out in the Department of Biochemistry in collaboration with Department of Ophthalmology in a tertiary care hospital from April 2018 to May 2019. Sample size was calculated by using the formula $n \geq (Z1-\alpha/2\sigma/d)\ 2$. The average value of MDA among diabetic cataract patients was taken as 4.00 ± 0.27 from review of literature by assuming alpha = 0.05; absolute error as 6% and a sample size was calculated per group. Hence, the total sample size for each group minimum needed is 78. However, sample size was fixed as 90 per group and the total sample size for four groups is $90 \times 4 = 360$. The study population consists of four groups, where group I includes 90 type 2 DM patients with cataract, group II 90 non-diabetic senile cataract patients, group III 90 type 2 diabetes mellitus without cataract and group IV 90 normal healthy individual without cataract having both the genders in the age group between 40 and 75 years old. Among 360 subjects, 158 were males (71 cataract cases, 87 without cataract as controls) and 202 were females (109 cataract cases, 93 without cataract subjects as controls). The study was approved by the Institutional Human Ethics Committee (No. IHEC/C-P/06-A/2017), and informed consent form was obtained from all the participants in the study. The subjects were selected based on inclusion and exclusion criteria from ophthalmology OPD. All subjects underwent complete eye examination in the ophthalmology OPD, and cataract was diagnosed by using slit-lamp examination and fundus examination was done. LOCS III classification was used for grading the cataract. Only pure nuclear type of lens opacity cases before surgery were taken for the study and excluded the cortical, posterior subcapsular and mixed varieties type of cataract on the basis of examination by the consultant ophthalmologist.

Inclusion Criteria

Group I: Type 2 diabetes mellitus patients having more than 5 years of duration who are under treatment of oral hypoglycaemic drugs with cataract.

Group II: Age-related or senile cataract subjects who has no history of diabetes.

Group III: Type 2 diabetes mellitus patients having more than 5 years of duration who are under treatment of oral hypoglycaemic drugs without cataract.

Group IV: Normal healthy individuals having no history of diabetes and without cataract that were recruited from employees of our institute and diagnosed by clinical and biochemical examination by clinician in the Ophthalmology OPD were included in this study.

Exclusion Criteria

Subjects who had history of steroid intake, renal dysfunction, hepatic disease, hypo or hyperthyroidism, traumatic or toxic cataract and other systemic diseases and drugs known to affect magnesium status such as Aminoglycosides, Amphotericin B, Cetuximab, Cyclosporine, Digoxin, Diuretics (loop, thiazide, osmotic), alcohol and smoking were excluded from the study.

Sample Collection and Analysis

The fasting venous blood sample was drawn from the subjects and collected in clot activator with serum gel separator tube, EDTA and sodium fluoride-potassium oxalate anticoagulant vacutainers. The plasma and serum samples were separated by centrifuging at 3500 rpm for 15–20 min. The plasma sample was used for the estimation of glucose by hexokinase method using Beckman Coulter Olympus AU400 auto-analyser. Whole blood was used for the estimation of glycated haemoglobin (HbA1c) by HPLC method using Bio-Rad D10 HbA1c analyser. The serum sample was used for the estimation of magnesium by xylidyl blue method using Beckman Coulter Olympus AU400 auto-analyser (as per kit insert package reference range for serum magnesium in adult: 1.8–2.6 mg/dl and below 1.8 mg/dl is defined as hypomagnesaemia). The oxidative stress biomarker serum MDA was estimated by Kei Satoh method using spectrophotometer. The serum reduced glutathione (GSH) and glutathione peroxidase-3 (GPX-3) were estimated by sandwich ELISA (Bioassay Technology Lab, Shanghai, china).

Statistical Analysis The current study results were expressed as mean ± standard deviation (SD). Data was analysed using JASP 8.4. The statistical significant differences between groups were analysed using one-way analysis of variance (ANOVA) followed by a Tukey's HSD post hoc analysis. Pearson's correlation coefficient (r) was used to assess the association between the variables. Odds ratio was calculated to find the relative risk. A p value of < 0.05 was considered as statistically significant.

Table 1 Comparison of glycaemic status, serum magnesium and oxidative stress levels among groups (one-way ANOVA)

Parameters	Group I—type 2 DM with cataract N = 90 (mean ± SD)	Group II—non-diabetic senile cataract N = 90 (mean ± SD)	Group III—type 2 DM without cataract N = 90 (mean ± SD)	Group IV—normal healthy individuals N = 90 (mean ± SD)	ANOVA p value
Fasting plasma glucose (mg/dL)	153 ± 68	94 ± 15	124 ± 36	90 ± 10	< 0.001*
HbA1c (%)	8.3 ± 2.4	5.3 ± 0.5	7.0 ± 1.2	5.4 ± 0.4	< 0.001*
Serum magnesium (mg/dL)	$1.4 \pm 0.31_{abc}$†	$1.6 \pm 0.27_{e}$‡	$1.6 \pm 0.29_{f}$‡	1.8 ± 0.30	< 0.001*
Serum MDA (μmol/L)	$2.95 \pm 0.30_{abc}$†	$2.07 \pm 0.27_{d\ddagger e}$†	$2.24 \pm 0.54_{f}$†	0.85 ± 0.27	< 0.001*
Serum GSH (μmol/L)	$4.37 \pm 2.36_{abc}$†	$7.87 \pm 2.35_{e}$†	$8.32 \pm 2.80_{f}$†	5.70 ± 0.01	< 0.001*
Serum GPX (ng/ml)	$36.10 \pm 12.85_{abc}$†	$58.30 \pm 12.90_{e}$†	$60.58 \pm 10.53_{f}$†	85.78 ± 11.00	< 0.001*

*$p < 0.05$ significant; *HbA1c* glycosylated haemoglobin, *MDA* malondialdehyde, *GSH* reduced glutathione, *GPX* glutathione peroxidase

a, comparison of group 1vs group 2; b, comparison of group 1vs group 3; c, comparison of group 1vs group 4; d, comparison of group 2 vs group 3; e, comparison of group 2 vs group 4; f, comparison of group 3 vs group 4

† Tukey's post hoc test, $p < 0.001$; ‡Tukey's post hoc test, $p < 0.01$

Results

The mean age of the diabetic and non-diabetic senile cataract cases were 59.26 ± 7.78 and 59.94 ± 8.39 years and that of controls (type 2 DM without cataract and non-diabetic without cataract) were 57.40 ± 6.58 and 54.83 ± 7.28 years. A significant difference was observed for age among four groups by using one-way ANOVA, $p < 0.001$.

Table 1 shows the descriptive value of glycaemic status, magnesium and oxidative stress and antioxidant status among four groups. Four groups were compared using one-way ANOVA followed by Tukey's HSD post hoc analysis test. The mean fasting plasma glucose levels of groups I, II, III and IV were 153 ± 68, 94 ± 15, 124 ± 36 and 90 ± 10, respectively. The mean HbA1c of subjects in groups I, II, III and IV were 8.3 ± 2.4, 5.3 ± 0.5, 7.0 ± 1.2 and 5.4 ± 0.4, respectively, ($p < 0.001$). The FPG and HbA1c levels were significantly higher in group I as compared with other groups ($p < 0.001$).

On comparing the four groups using one-way ANOVA, a significant decreased levels of serum magnesium, reduced glutathione and glutathione peroxidase were observed in type 2 DM patients with cataract, i.e., group I ($p < 0.001$), as compared with the other groups. When the groups were compared in pairs for serum magnesium, reduced glutathione and glutathione peroxidase using Tukey's HSD post hoc analysis test, a significant difference were observed between all paired groups except between group II and III ($p = 0.997$), ($p = 0.499$) and ($p = 0.561$). There was significantly increased serum MDA level that was observed in group I ($p < 0.001$) as compared with the other three groups using ANOVA, and followed by Tukey's HSD post hoc test compared in pairs, a significant difference was observed between all paired groups as shown in Table 1.

Pearson's correlation analysis showed a significant negative correlation between serum magnesium and MDA (Fig. 1), and FBS, HbA1c and positive correlation with GPX-3 respectively were shown in Table 2.

The odd's ratio was calculated to find out the magnitude of association between hypomagnesaemia and risk of cataract formation. Subjects with hypomagnesaemia have 2.6 times ($p < 0.001$) higher risk of developing cataract when compared with normomagnesemia subjects as shown in Table 3.

Discussion

Magnesium deficiency (MgD) is proposed to be a potential risk factor in the pathogenesis of diabetes complications [11]. Inflammation is the other important cause of the oxidative stress which results from MgD [12]. However, the relationship between MgD and the development of oxidative stress (OS) is still unclear, and a few studies available.

As far as we know, there is a no previous study concerning the potential interaction between hypomagnesaemia and

Table 2 Association of serum magnesium with oxidative stress and glycaemic status

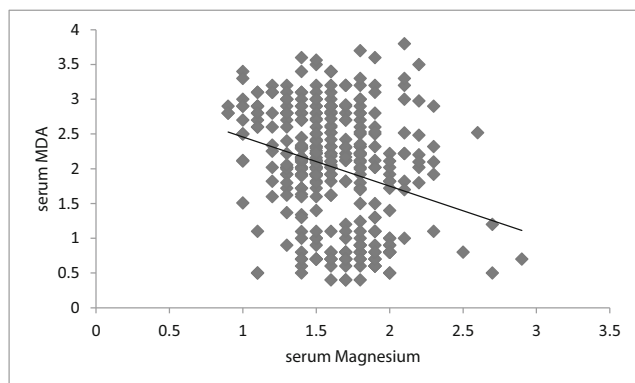| Parameters		Pearson's *correlation* coefficient (*r* value)	p value
Mg Vs	MDA	− 0.268*	< 0.001
	GSH	0.054	0.304
	GPX	0.301*	< 0.001
	FBS	− 0.159*	0.001
	HbA1c	− 0.170*	0.002

*$p < 0.05$ significant,

Fig. 1 Association between serum magnesium and MDA

oxidative stress on the pathogenesis of cataract formation in type 2 DM patients. Therefore, we aimed to analyse the levels of serum magnesium, MDA, GSH and GPX-3 and look for any association between the analytes.

In our study, we found that mean serum MDA level was significantly elevated whereas reduced glutathione; glutathione peroxidase levels were significantly decreased in type2 DM with cataract subjects compared with non-diabetic senile cataract subjects, type 2 DM without cataract and normal healthy subjects. The results suggested that there is an increased oxidative stress in subjects with diabetic cataract than non-diabetic senile cataract and type 2 DM without cataract.

Our findings are close resemblance with the study reported by Bhatia et al. where there was increased serum concentration of MDA level and decreased concentration of GSH level in diabetes cataract than senile cataract subjects [13]. Another study reported by [14] revealed that patients with diabetic cataract have shown increased plasma levels of lipid peroxidation products and decreased levels of reduced glutathione compared with non-diabetic and controls. Our results are supported by the research work of [15, 16] who have reported that there is an increase in oxidative stress in diabetic cataract.

In our study, mean serum magnesium level was significantly decreased in subjects with diabetic cataract than compared with other groups. In addition, subjects with hypomagnesaemia has 2.6 times ($p < 0.001$) higher risk of developing cataract when compared with subjects with normomagnesaemia using odd's ratio calculation which clearly indicates that hypomagnesaemia also acts as a risk factor for cataract formation in both diabetic and non-diabetic subjects. Despite this interest, no one to the best of our knowledge who measured serum magnesium levels in type 2 DM with cataract subjects compared with non-diabetics senile cataract and type 2 DM without cataract compared with healthy control.

Our reports are in accordance with the study by [17–19] where mean serum magnesium in diabetic study group was lower when compared with control group.

The reasons for lower magnesium levels remain unclear but that may include lower dietary intake, decreased absorption from intestine and increased loss of urinary magnesium along with glucose or due to reduced magnesium uptake by cells as compared with non-diabetic healthy individuals [20].

Our study revealed a significant positive correlation of serum magnesium with GPX-3 whereas there was negative correlation with MDA. These findings support our hypothesis that there is strong relationship between hypomagnesaemia and the development of oxidative stress in the pathogenesis of diabetes-induced cataract.

Conclusion Hypomagnesaemia may induce oxidative stress, which triggers cataract formation much earlier in type 2 diabetes mellitus patients. Thus, hypomagnesaemia also acts as a risk factor in the pathogenesis of diabetic cataract. So, we suggest that serum magnesium can be used as a surrogate marker for prevention and progression of diabetic cataract, and correction of impaired magnesium levels is highly recommended in diabetic cataract patients. However, our studies clearly relay that association between magnesium deficiency and oxidative stress are correlated. Therefore, further future aspects of preclinical and clinical studies are necessary to clarify these findings.

Limitation of the Study We did not assess the dietary magnesium intake in the study subjects due to variations in the food habits in the study participants.

Table 3 Odds ratio for serum magnesium

Parameters	Subjects with cataract (N = 180)	Subjects without cataract (N = 180)	Odds ratio	95% confidence interval		p value
				Lower bound	Upper bound	
Hypomagnesaemia (< 1.8 mg/dL)	141	104	2.642	1.665	4.193	< 0.001*
Normal magnesium (1.8–2.6 mg/dL)	39	76				

*p < 0.05 significant

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval The study was approved by the Institutional Human Ethics Committee (No. IHEC/C-P/06-A/2017).

Informed Consent Informed consent form was obtained from all the participants in the study group.

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