Original Article

Study of Glutathione and Ascorbic Acid in Cases of Diabetic Retinopathy

Prabhavathi K., Ashakiran S., Narendra P. Datti[1], Krishnamurthy N.
Dayanand C.D., Mamtha K. and Sumathi M.E.
Dept. of Biochemistry and Ophthalmology, Sri Devaraj Urs Medical College, Kolar - 563 101

ABSTRACT

Background : Diabetes mellitus is known to induce oxidative stress along with deranging various metabolisms; one of the late complications of diabetes mellitus is diabetic retinopathy which is a leading cause of acquired blindness. Poor glycemic control and oxidative stress has been attributed to the development of complications like diabetic retinopathy.

Objectives : To study oxidative stress parameters (GSH & Vitamin C) and HbA_{1c} in diabetic retinopathy patients, diabetic without retinopathy patients and comparing the parameters and correlating the same with the controls.

Material and Method : The study included 25 diabetic patients with retinopathy, 25 diabetic patients without retinopathy and 25 healthy controls, between 30 – 70 years of age of either sex, attending R. L. Jalappa Hospital and Research Center, Kolar. FBS, HbA_{1c} were measured by using standard methods adopted in the clinical laboratory.Glutathione in erythrocytes was assayed by colorimetric method using DTNB as a chromogen. Vitamin C was measured by colorimetric method using DNPH method.

Results : Mean FBS (mg/dl) in DM with retinopathy was180.04 ± 68.51DM, without retinopathy was 176.24 ± 59.24 and controls was 90.20 ± 12.49($p < 0.001$). The mean GSH levels, in diabetic patients with retinopathy was 6.14 ± 1.52 % in diabetic patients without retinopathy it was 6.47 ± 1.69 and in the control group it was 13.14 ± 2.48 ($p < 0.001$).The mean Vitamin C level, in diabetic patients with retinopathy was 0.69 ± 0.25 mg/dl, in diabetic patients without retinopathy it was 0.86 ± 0.32 mg/dl and in the control group it was 1.26 ± 0.25 mg/dl($p < 0.001$).

Conclusion : Estimation of HbA_{1c}, oxidative stress parameters like erythrocyte glutathione and vitamin C can be considered as a good index predicting the onset and progression of diabetic retinopathy.

Key words : Diabetic retinopathy ; HbA_{1c}, Reduced Glutathione (GSH); Vitamin C.

INTRODUCTION

Diabetes Mellitus is a complex metabolic disease, primarily characterized by hyperglycemia, caused by variable interactions between hereditary factors and environmental factors (i.e., by insulin resistance and or relative insulin deficiency). In persons with type II diabetes mellitus, complications are the major cause of morbidity and mortality. Its main features are abnormal insulin secretion & high levels of blood glucose, which are the major initiator of microvascular complications, including retinopathy, nephropathy, neuropathy and arteriosclerosis (1).These complications are predominantly seen in patients in the age group of 40 to 70 years.Chronic hyperglycemia and its associated non-enzymatic glycation play an important role in the development of microangiopathy. Intensive glycemic control as measured by serum HbA_{1c} levels have been demonstrated in randomised trials to reduce diabetic complications especially microvascular disease (2).Diabetic retinopathy is a major cause of blindness in population of working age. It is one of the leading causes of acquired blindness in adults where the chances of loosing the sight are about 25 times higher than normal population (1). Decrease in visual acuity in diabetic retinopathy is either associated with maculopathy or proliferative complications of it.There are a series of risk factors related to the development and progression of diabetic retinopathy such as duration of diabetes mellitus, poor glycemic control and oxidative stress.

Retinopathy is not only related to hyperglycemia but also related to duration of diabetes mellitus. Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules and which are generally very reactive. They are produced continuously in cells either as accidental by-products of metabolism or deliberately during phagocytosis. Cells have developed a comprehensive array of antioxidant defenses to prevent free radical formation or limit their damaging effects. Reactive free radicals formed within cells can oxidize biomolecules and lead to cell death and tissue injury. Any tissue damage resulting from an imbalance between an excessive generation of oxidant compounds and insufficient antioxidant defense mechanismis known as oxidative stress (3). Chronic hyperglycemia can influence the generation of free radicals, which may lead ultimately to increased lipid peroxidation and depletion of antioxidants, and thereby enhanced oxidative stress in subjects with type 2 diabetes mellitus. Recently there has been a focus on DNA damage due to oxidative stress and the emphasis lies on sensitive biomarkers indicating them. This study was done to know the correlation between all these risk factors in diabetic patients with retinopathy, diabetic patients without retinopathy and healthy controls.

MATERIALS AND METHODS :

Study group consists a total of 75 individuals divided into three groups.Clinically proven 25 cases of type II diabetes mellitus with retinopathy based on fundoscopic changes between 30 – 70 years of age of either sex attending at Ophthalmology OPD, R.L. Jalappa Hospital and Research Center, Kolar were included in Group I. 25 Diabetic subjects without retinopathy changes based on fundoscopic examination were included in Group II and 25 normal healthy individuals were included in Group III.

In Group-I, Non diabetic cases presenting with retinopathy and any subject with recent history of fever, infection and chronic illness like cancer, chronic obstructive lung disorders, cardiac diseases, stroke, gestational diabetic mellitus and complications related to diabetes like ulcers, neuropathy, nephropathy which are known to affect oxidative stress parameters was excluded from the study. Any subject receiving antioxidant therapies were also excluded from the study. In Group- II, individuals with type II diabetes mellitus with diabetic retinopathy or other complications & on antioxidant drugs were excluded from the study.

After obtaining informed consent, 10ml of venous blood from the study and the control group after 8 hrs of fasting was drawn from the median cubital vein under complete aseptic precautions. First sample was collected in the fluoride tube and was used for estimation of

fasting blood glucose by glucose oxidase enzymatic method by Accucare kit (4). Second sample was collected in EDTA tube for glycated hemoglobin by cation exchange resin method by Recombigen kit (5). Blood sample from the anticoagulant [EDTA] containing vacutainer was centrifuged at 3000 rpm for 10 minutes; supernatant plasma was used for ascorbic acid estimationby non-enzymatic method, using 2,4 – Dinitrophenyl hydrazine (DNPH) (6). The buffy coat was discarded. The packed cells were suspended in equal volume of cold phosphate buffer saline and re-centrifuged. The supernatant was discarded. The washing of packed cells was repeated twice; the packed cells were used for analysis of Reduced Glutathione estimation in RBC by colorimetric method using 5, 5'- Di Thiobis 2-Nitrobenzoic Acid (DTNB) as chromogen (7) .These parameters were analyzed in semi-autoanalyser and colorimetry in central Clinical Biochemistry Laboratory, R.L. Jalappa Hospital and Research Center, Kolar.

Analysis of result was done by comparison of mean and standard deviation between the groups was analysed by non-parametric test using Mann-Whitney U test and correlation of parameters by using Pearson's formula. The percentage of significance was obtained on the basis of 'r' values and 'p' values.

Table 1: FBS & Glycated hemoglobin in cases and controls.

Groups	FBS (mg/dl) Mean ±S.D	**HbA_{1c} (%) Mean ±S.D**
I - DM with retinopathy	180.04 ± 68.51*	7.86 ± 1.22*✞
II - DM without retinopathy	176.24 ± 59.24**	7.18 ± 1.05**
III - Controls	90.20 ±12.49	5.55 ± 0.68

* I Vs III - Highly Significant: $p < 0.001$, ** II Vs III - Highly Significant: $p < 0.001$,
✞ I Vs II - Significant: $p < 0.001$.

Table 2: Oxidative stress parameters in cases & controls.

Groups	Glutathione (mg/gm of Hb) Mean ±S.D	Vitamin C (mg/dl) Mean ±S.D
I- DM with retinopathy	6.14 ± 1.52*	0.69 ± 0.25*>
II - DM without retinopathy	6.47 ±1.69**	0.86 ± 0.32**
III - Controls	13.14 ±2.48	1.26 ± 0.25

* I Vs III - Highly Significant: $p < 0.001$, ** II Vs III - Highly Significant: $p < 0.001$,
> I Vs II - Significant: $p < 0.05$.

Table 3: Correlation of glutathione with other parameters

Parameter	Value	Diabetic Retionopathy	Diabetes mellitus	Controls
FBS	r	0.01	-0.14	-0.13
	p	0.95	0.49	0.55
GlyHb	r	-0.07	-0.32	-0.34
	p	0.76	0.12	0.09
Vitamin C	r	0.13	0.20	-0.13
	p	0.55	0.34	0.54

Table 4: Correlation of Vitamin C with other parameters

Parameter	Value	Diabetic Retionopathy	Diabetes mellitus	Controls
FBS	r	-0.17	0.25	0.41
	p	0.42	0.24	0.04**
GlyHb	r	0.08	0.17	0.04
	p	0.69	0.43	0.87

**Significant: $p < 0.05$

Table 3 & 4 shows the correlation between GSH and other parameters in diabetic retinopathy, diabetic without retinopathy and control groups and Vitamin C with other parameters in diabetic retinopathy, diabetic without retinopathy and control groups.

DISCUSSION:

Diabetic Retinopathy is one of the microvascular complications of diabetes mellitus, which is one of the leading causes of acquired blindness (8).It is due to microangiopathy affecting the retinal arterioles, capillaries and venules. Damage is caused by both microvascular leakage and microvascular occlusion. A series of risk factors have been related to the development and progression of retinopathy in diabetic patients

In present study, the number of cases in diabetic retinopathy group was found to be more between the age group of 61-70 yrs (56%). It was observed that diabetic retinopathy was found to be higher in males when compared to females. A cohort study done in a clinic in Chennai, CURES Eye study (9), UKPDS study (10) and the Hyderabad study (11) on diabetic retinopathy appeared to be prevalent more in the males compared to females. But in Joslin clinic patients, study reported that excess female preponderance over males.

The mean duration of diabetes mellitus in diabetic patients with retinopathy was 13.2 ± 1.66 yrs and in diabetic patients without retinopathy it was 5.84 ±1.65 yrs. The duration of diabetes mellitus was significantly higher in diabetic patients with retinopathy when compared to diabetic patients without retinopathy. Our findings are comparable with the study done by M Rema, et al (8) , (12) who have proposed that duration of diabetes mellitus is probably the strongest predictor for the development of retinopathy. Studies have also shown that for every 5 year increase in the duration of diabetes mellitus, the risk of diabetic retinopathy increases by 1.89 times (8).The above findings show that duration of diabetes mellitus is one of the important risk factors in the development of diabetic retinopathy.

In our present study, mean FBS (mg/dl) in - DM with retinopathy was180.04 ± 68.51,DM without retinopathy was176.24 ± 59.24 and controls was 90.20 ± 12.49 ($p < 0.001$). Mean HbA_{1c}(%) in DM with retinopathy was 7.86 ± 1.22, DM without retinopathy was 7.18 ± 1.05 and controls was 5.55 ± 0.68 ($p < 0.001$).Study showed there was a significant increase in the FBS levels and HbA_{1c} levels in diabetic patients with retinopathy and in diabetic patients without retinopathy when compared to the control group. There was a significant increase in the HbA_{1c} levels in diabetic patients with retinopathy when compared to the diabetic patients without retinopathy ($p < 0.001$). Similar findings of increased FBS are found in studies done in year 2000 by Zelia Maria da silvacorrea and his co workers (1).Similar findings of increased HbA_{1c} are seen in the previous studies done by Ishrat Kareem and his coworkers (13).

The mean GSH levels, in diabetic patients with retinopathy was 6.14 ± 1.52 mg/gm of Hb, in diabetic patients without retinopathy it was 6.47 ± 1.69 mg/gm of Hb and in the control group it was 13.14 ± 2.48 mg/gm of Hb ($p < 0.001$).The mean Vitamin C level, in diabetic patients with retinopathy was 0.69 ± 0.25 mg/dl, in diabetic patients without retinopathy was 0.86 ± 0.32 mg/dl and in the control group it was 1.26 ± 0.25 mg/dl($p < 0.001$). In our present study, there was a statistically significant decrease in the GSH and in Vitamin C levels in diabetic retinopathy and diabetic without retinopathy groups, when compared to the control group. There was a statistically significant decrease in Vitamin C levels in diabetic retinopathy when compared to diabetic without retinopathy group ($p < 0.05$).

We found that in our study there was no statistically significant correlation between GSH with other parameters in all three groups, but there was statistically significant correlation between Vitamin C with FBS levels in control group ($p < 0.05$).

Our findings agree with the study done in Turkey on "Antioxidant enzymes and Diabetic Retinopathy" by Zuhal Vildirim with his

coworkers, which included 25 patients with PDR (I), 25 patients with NPDR(II) & 25 non diabetic control to know the severity of DR, by measuring serum copper, zinc, nitric oxide, glutathione, advanced oxidation protein products(AOPP) levels & superoxide dismutase (SOD).The study showed no difference in levels of SOD and Zn between the groups, statistically significant differences were observed for GSH, NO and Cu levels when compared to control group. AOPP levels were statistically increased in group I when compared to control group. The study suggested that hyperglycaemia in DM is associated with accelerated non enzymatic glycation & oxidative stress (14).

Another similar study published in Clin Chim Acta in year 2004, have shown statistically significant decrease of GSH levels in diabetic retinopathy and diabetic without retinopathy groups, when compared to control group (15). Results also matches with the study done in London in the year 1998, on "The role of oxidative stress in diabetic retinopthy" by Gurle B and his associates (16). Similar study done on "Plasma MDA and antioxidant vitamins in diabetic retinopathy" by S. Kumari and co workers, who have shown statistically significant decrease of Vitamin C levels in diabetic retinopathy and diabetic without retinopathy groups, when compared to control group (17).

A study done in "The public health school, Harbin Medical University" China on "The change of oxidative stress products in Diabetes mellitus and Diabetic Retinopathy" by Hong-Zhi Pan with his coworkers, included 33 patients with Diabetes mellitus(I), 27 patients with Diabetes mellitus - DR(II) & 32 non diabetic control, by measuring MDA, conjugated diene(CD), advanced oxidation protein products(AOPP) and 8-hydroxydeoxyguanosin (8-OHdg) levels & superoxide dismutase(SOD). The study showed significant increase in MDA, 8-OHdg, CD levels & AOPP levels in DR & DM when compared to controls. It also showed significant increase in MDA, 8-OHdg,CD levels & AOPP levels in DR when compared to DM. The study suggested that Oxidative stress is an important risk factor in development of retinopathy. The levels and the type of serum oxidative stress by- products will favour in predicting the amount of retinopathy(18).

L.Sun and his coworkers, have studied the proposed theory of oxidative stress in the pathogenesis of diabetic microvascular complications.They investigated a new sensitive biomarker of oxidative DNA damage in vivo.They studied urinary levels of 8-hydroxy deoxyguanine in patient's with type II diabetes and found that they had significantly higher concentration in diabetic subjects. There correlation was significant with HbA_1C values. Hence they concluded that measuring 8-OHdG is a novel convenient method and a sensitive biomarker which is helpful for early diagnosis and treatment of patient"s with diabetic retinopathy(19).

These observations support the suggestion that chronic hyperglycaemia can influence the generation of free radicals, which may lead ultimately to increased lipid peroxidation and depletion of antioxidants and thereby enhanced oxidative stress in subjects with type 2 diabetes mellitus. Studies have suggested that increased capillary permeability, microangiopathy and retinal ischemia are probably due to the combined effects of various risk factors.

CONCLUSION:

From the results of the present study, it can be concluded that estimation of HbA_{1c}, oxidative stress parameters like erythrocyte glutathione and vitamin c can be considered as a good index predicting the onset and progression of diabetic retinopathy.

Elevated HbA_{1c} levels showsthat hyperglycemia leading to the formation of advanced glycation end products, which result in the various micro vascular complications.

GSH and Vitamin C levels were significantly altered in diabetes with complications and without complications when compared to normal, suggesting the role of uncontrolled hyperglycemia as a cause and consequence of oxidative stress.

Strict control of blood glucose as determined by fasting blood glucose and glycosylated haemoglobin is necessary to prevent the onset of complications associated with diabetes mellitus. However, with poor glycemic control, oxidative stress in diabetes can cause profound damage to the vital organs in the body. Hence determination of reduced glutathione in erythrocytes and vitamin c levels can contribute to know the extent of oxidative stress in diabetes and help in effective control and prevention of the onset and progression of complications like diabetes retinopathy.

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