Evaluation of Plasma Protein Oxidation Biomarkers in type 2 Diabetic Patients with Retinopathy

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Abstract

Objective: Retinopathy is a microvascular complication of diabetes and leading cause of blindness throughout the world. The proteins as the most chemical substances in the cells are exposed to oxidative damages of free radicals. The aim of this study was to evaluate the values of protein oxidation biomarkers in diabetic patients with retinopathy.

Materials and Methods: This study was done on 30 type 2 diabetic patients with retinopathy as cases and 30 age and sex matched type 2 diabetic patients without retinopathy as controls. We measured HbA1c by Ion exchange chromatography and fasting blood sugar (FBS), cholesterol, triglyceride, urea, creatinine, HDL and LDL, protein plasma carbonyls (PCO), advanced oxidation protein products (AOPPS), SH groups and TAC by spectrophotometry in case and control groups and compared them. Urine micro albumin was measured in both groups and compared.

Results: the mean of HbA1c, cholesterol, LDL-C, creatinine, PCO and AOPPS were significantly higher in cases (P-value<0.001). The mean of TAC and SH groups in case group were significantly less than control group (P-value<0.002).

Discussion: The oxidative stress in diabetic patients with retinopathy caused elevation of protein oxidation and increased progress of diabetic disorders especially eye disorders.

Keywords: Type 2 diabetes, Retinopathy, Protein oxidation

Introduction

Diabetes is a chronic disease and a major medical problem throughout the world. The frequency of type 2 diabetic patients is expected to be 333 million over the next 20 years (1,2). Diabetic retinopathy is one of the main diabetic complications. It causes visual impairment and finally blindness which is result of long-term accumulated damage to the small blood vessels in the retina (3). The incidence of retinopathy is rarely detected in the first few years of diabetes but the incidence increases to 50% by 10 years, and to 90% by 25 years of diabetes. The prevalence of diabetic retinopathy is increasing due to prolonged survival of diabetic patients (4).

Diabetes is associated with increased modification of proteins. In addition to the formation and accumulation of advanced glycation end products (AGEs), a family of

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oxidized protein compounds termed advanced oxidation protein products (AOPPS) has emerged as a novel class of inflammatory mediators. AOPPS are the dityrosine containing and cross linked protein products (5-7). They are supposed to be structurally similar to AGE proteins and it's biological activities as AGEs, induce pro-inflammatory cytokines and adhesive molecules (5,6). AOPPS are recognized as markers of oxidative damage to proteins, the intensity of oxidative stress and inflammation (8). Studies have already suggested that AOPPS plays a major role in the development of diabetic retinopathy (9).

Different studies showed variable results about altered values of advanced oxidation protein product (AOPPS) and plasma total antioxidant capacity (TAC) in diabetes (10-14). We tried to evaluate the values of protein oxidation biomarkers in diabetic patients with retinopathy and compared them with diabetic patients without retinopathy as control group. Furthermore we measured and compared fasting blood glucose (FBS), HbA1c, lipid profile, urea, creatinine and urine micro-albumin in the case and control groups.

Materials and Methods

This was a case and control study. The study populations were 30 type 2 diabetic patients with retinopathy who were referred to Yazd diabetic center as case group. They were matched (age and sex) with 30 type 2 diabetic patients without retinopathy as control group. The fasting blood samples of the study patients were taken and glycosylated hemoglobin was measured by Ion exchange chromatography (model VS5). Blood samples were collected in tube containing heparin. Plasma was obtained by centrifuging blood at 1800xg for 10 min at 4°C. The FBS, cholesterol, triglyceride, urea creatinine, HDL and LDL were measured by parsazmone test. Urine micro-albumin was measured by nephelometry method.

Determination of Plasma protein carbonyls (PCO)

Plasma protein carbonyls (PCO) content was measured according to procedure of Levine et al (15). PCO groups react with 2-4 dinitrophenylhydrazones (DNPH) to generate chromophoric dinitrophenylhydrazones. DNPH was dissolved in HCL, and after the DNPH reaction proteins were participiated with an equal volume of 20% (w/v) trichloroacetic acid and washed three times with 4 ml of ethanol/ethyl acetate mixture (1:1). Finally, the precipitates were dissolved in 6 M quinidine HCL solution and absorbance were measured at 370 nm.

Estimation of plasma AOPPs level

Determination of AOPPs in plasma was based on spectrophotometric detection according to Witko-Sarsat et al (7). Briefly, 200 ml of plasma (diluted 1:5 with phosphate-buffered saline (PBS) as test,200ml of chloramines- T solution (0-100 micromol/L) for calibration and 200 ml of PBS were applied.10 microliter of 1.16M potassium iodide and 20 microliter of acetic acid were added and absorbance at 340 nm was measured immediately by spectrophotometer.

Determination of Thiol groups (SH groups)

Plasma SH groups was estimated according to Kitajima's method (16), based on the ability of SH group to reduce 5,5'— dithiobis 2 nitrobenzoic acid (DTNB) and form yellow colored anionic product whose absobance is measured at 412 by spectrophotometer.

Estimation of Total antioxidant capacity (TAC)

Total antioxidant capacity of plasma was estimated by DPPH reduction assay as descibed by Janaszewks et al (17). Briefly 0.1 ml of plasma in phosphate buffered solution (10 micromolar, pH 7.4) was incubated in methanolic solution of DPPH (0.1 mM). Absorbance at 517 nm was measured after 30 min of incubation with vigorous shaking. The activity of DPPH was calculated from equation: Activity [% of DPPH reduction] = [(A-Ax)/A] x 100, where A is absorbance of

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DPPH solution with methanol, Ax is absorbance of DPPH solution with plasma.

Statistical analysis

Statistical differences were analyzed with Student's T-test and the differences were considered to be significant with *P*-value<0.001).

Results

There were no significant differences between age, BMI, blood glucose, triglyceride, HDL-C and urine micro-albumin in type 2 diabetic patients with retinopathy (case group) and without retinopathy (control) .There were Significant differences between cholesterol, LDL-C, HbA1c and creatinine in case and control groups. Also in this study the mean of PCO and AOPPS, in the type 2 diabetic patients with retinopathy were more than the type 2 diabetic patients without retinopathy (*P*-value<0.001). Mean of and plasma TAC and SH groups in diabetic patients with retinopathy were significantly less than diabetic patients without retinopathy (*P*-value<0.002).

Discussion

Oxidative stress increases in diabetic retina with hyperglycemia, causes retinal basement membrane thickening (18-20). Diabetes mellitus increases oxidative stress and induces vascular leakage and increased retinal vascular permeability, perhaps causing macular edema which correlates with vision loss in diabetic retinopathy patients (21-23). During long time hyperglycemia advanced glycation end product formation and other tissue proteins, are increased (24). So the aim of this study was evaluation of plasma protein oxidation biomarkers in type 2 diabetic patients with retinopathy.

The risk of blindness was correlated with the degree of retinal hard exudates, reducing serum lipid levels in patients with diabetic retinopathy (25). The results of our study showed that there were significant increases in mean of LDL, HbA1C, creatinine, PCO, AOPPS, TAC and decrease in SH group levels in diabetic patients with retinopathy in comparison with diabetic patients without retinopathy.

This is consistent with the study carried out by Yan et al and Faulkner et al .They showed the relationship between diabetes and increase in serum LDL and HbA1c (26,27).

Protein carbonyl content is actually the most general indicator and by far the most commonly used marker of protein oxidation.

Table 1. Demographic profile and biochemical tests of blood and urine

Parameter	Mean ± SD Control Group	Mean ± SD Case Group	*P*-value
Age (Years)	59.13 ± 0.67	59.16 ± 0.66	0.97
BMI (Kg/m^2)	26.7± 0.3	27.5± 0.3	0.133
FBS (mg/dl)	165± 37.2	156± 33.1	0.309
Cholesterol (mg/dl)	149.93± 24.38	167.67±31.01	0.016
Triglyceride (mg/dl)	160.66± 33.5	171±34.71	0.23
HDL – C (mg/dl)	33.22± 6.59	32.91±2.12	0.369
LDL-C (mg/dl)	132.22± 6.59	159.66±5.94	0.000
HbA1c (%)	6.77± 1.01	8.36±1.01	0.000
Urea (mg/dl)	39.33± 8.42	43.64±10.77	0.0807
Creatinine (mg/dl)	0.739± 0.067	1.53±0.33	0.000
Urea Microalbumin (mg/dl)	17.56± 6.1	18.67±3.11	0.296

Table 2. Protein oxidation biomarkers in control and case groups

Biomarkers	Mean ± SD Control Group	Mean ± SD Case Group	*P*-value
PCO (µmol/gm of protein)	0.88± 0.22	1.32± 0.3	0.000
AOPPs (µmol/L)	0.55± 0.94	1.65± 0.34	0.000
DPPH (µmol/l)	0.93± 0.48	0.78± 0.12	0.000
SH-group (µmol/L)	1.69± 0.28	1.44± 0.94	0.002

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Accumulation of protein carbonyls has been observed in several human diseases including diabetes (28). In this study the mean of PCO in the type 2 diabetic patients with retinopathy was significantly more than type 2 diabetic patients without retinopathy (P=0.00). Our result congregates with findings of Aparna A. Sagare et al. They observed that PCO were significantly (P<0.001) elevated in diabetes when compared with healthy subjects (29) .

AOPPS is a marker of protein damage. Increased AOPPS levels in patients with diabetic retinopathy were reported. Baskol et al found that AOPPS increased in diabetes patients. They found that plasma levels of AOPPS were significantly higher in diabetic patients with retinopathy (7). Kaushik Kar et al observed that AOPPS increase significantly in type 2 diabetes (30).

One of the antioxidant systems is the SH groups (30). Decreases and functional defects arise in the SH groups is as a result of the exposure of proteins to oxidative stress (31,32). Ceriello et al, compared the plasma total SH groups levels of patients with type 2 DM followed up by diet or receiving oral anti-diabetic treatment with those of the control group made up of healthy individuals and found that the total plasma SH groups concentration was significantly low in the diabetic group (33). Collier et al. also showed that the plasma SH groups levels in patients with type 2 DM significantly decreased (34). Our results showed that mean of SH in diabetic patients with diabetic retinopathy was less than diabetic patients without retinopathy which was significant (P=0.002). Oxidative damage plays a role in the pathogenesis of many ocular degenerative diseases (35). H.M Ucgun et al research on diabetic retinopathy patients showed that serum TAC decreased in these patients as compared to control group (36). Furthermore, Caner et al, found that the serum TAC value was significantly lower in diabetic retinopathy patients than the control group (37).

Conclusions

The results of our study indicated that serum TAC level was significantly lower in the diabetic patients with retinopathy than diabetic patients without retinopathy group (P=0.000). Our results support the idea that, oxidative stress in diabetic patients with retinopathy caused alteration of the protein oxidation biomarkers.

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