CHANGES IN GLYCOSYLATED PROTEINS IN TYPE-2 DIABETIC PATIENTS WITH AND WITHOUT COMPLICATIONS

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Background: Diabetes mellitus constitutes one of the most important problems in developing and developed countries. Increased glycosylation of various proteins in diabetic patients has been reported by many authors. The present study describes the changes in protein glycosylation in diabetic patients with and without diabetic complication. **Methods:** The study included one hundred and three subjects. Among them 21 were type 2 diabetic patients without any clinical evidence of chronic diabetic complications, 21 were type 2 diabetic patients with cardiovascular complications, 20 were type 2 diabetic patients with cataract, 20 were type 2 diabetic patients with retinopathy and 21 apparently normal, age, sex and weight matched controls. The patients were selected from Ziauddin Medical University Hospital, Karachi and Jinnah Postgraduate Medical Centre, Karachi. **Results:** Fasting plasma glucose was increased in all diabetic patients and correlated significantly with glycosylated hemoglobin, glycosylated plasma proteins and serum fructosamine concentrations. There was no significant difference in the levels of fasting plasma glucose, glycosylated plasma proteins, glycosylated hemoglobin, serum fructosamine, hexosamine or sialic acid between diabetic patients with or without chronic complications. Alpha-1 and alpha-2 globulin fraction were significantly increased in diabetic patients without complications, diabetic patients with cardiovascular complications and diabetic patients with cataract. Albumin was found to be decreased in diabetic patients with cataract while gamma globulin was increased in diabetic patients with cardiovascular complications and diabetic patients with cataract. **Conclusions:** In uncomplicated diabetic patients alpha-1 and alpha-2 glycoproteins were increased. In diabetic patients with cardiovascular complications alpha-1, alpha-2 and gamma globulin were increased while in diabetic patients with cataract alpha-1, alpha-2 and gamma globulin were increased but serum albumin was significantly decreased.

Key words: glycosylated hemoglobin, glycosylated plasma protein, serum fructosamine, sialic acid, hexosamine and total serum proteins.

INTRODUCTION

Diabetes mellitus, the most common, serious, chronic endocrine disease is characterized by hyperglycemia, metabolic abnormalities and by long-term complications involving the eyes, kidneys, nerves and blood vessels.[1] Worldwide projections suggest that more than 220 million people will have diabetes by the year 2010 and the majority of these will have type 2 diabetes.[2] Diabetes mellitus is one of the major health problem of Pakistan. Over 12% of the Pakistani population in the age group of 25 years and above suffers from this disease and about 10% from impaired glucose tolerance.[3]

It has been suggested that chronic hyperglycemia, regardless of its etiological basis carries a risk of complications and duration and severity of diabetes is correlated with chronic complications.[4,5] Mandarino[6] suggested the etiological role of hyperglycemia in early microvascular basement membrane thickening of diabetic retinopathy which is also associated with poor glycemic control. Direct metabolic tissue injury caused by hyperglycemia may also be more important to "microvascular" complications, especially neuropathy.[7] An intensive glucoregulation prevents deterioration of complications.[8] The biochemical basis of progressive diabetic complications has not been understood. Four possible mechanisms have received attention: increased polyol pathway flux, increased advanced glycation end product (AGE) formation,[9] activation of protein kinase (PKC) isoforms and increased hexosamine pathway flux.[10] From these possibilities glycosylation of proteins has been the subject of much interest. Spiro[11] first suggested that lesions occurring in diabetes are the result of some defect in the metabolism of glycoproteins or related substances. Analysis of human diabetic basement membrane showed both an absolute increase in glycoprotein and a different composition, compared with normal human basement membrane.

The knowledge concerning the involvement of glycoproteins in diabetic complications led many authors to closely observe the glycoprotein changes and studied circulating glycoproteins in diabetic patients. Nearly all plasma proteins are glycosylated except albumin which is glycosylated to a lesser extent. The protein-bound hexosamine is present in about the same concentration as glucose in the blood[12]. Kennedy et al[13] reported increased glycosylation of serum proteins in diabetic patients and levels were correlated with fasting serum glucose and $HbA1_C$. It was suggested that

glycosylated protein estimation is more useful in detecting early response to treatment. Johnson et al[14] described fructosamine determination as a screening test for the diagnosis of diabetes mellitus and to determine the control which has been found to be a useful and reliable alternative to $HbA1_C$ determination.

The understanding of pathogenesis of chronic diabetic complications is essential and its not known whether the circulating glycoproteins are deposited in the arterial wall and cause structural ad functional changes or are independently synthesized in the arterial wall of the specific tissues. The present work was undertaken to compare the changes in serum glycoprotein levels with the diabetic state without complications or with the presence of chronic diabetic complications.

MATERIAL AND METHODS

This study included one hundred and three subjects. Among them 21 were type 2 diabetic patients without any clinical evidence of chronic diabetic complications, 21 were type 2 diabetic patients with cardiovascular complications, 20 were type 2 diabetic patients with cataract and 20 were type 2 diabetic patients with retinopathy and 21 apparently normal, age, sex and weight matched control subjects, were investigated. The subject having no history of diabetes and any other major illness, like macro-vascular disease, cataract, retinopathy, tuberculosis, rheumatoid arthritis, liver disease or malignancy were selected as control subjects. All patients were over 50 years of age and were selected from Ziauddin Medical University Hospital, Karachi and Jinnah Postgraduate Medical Centre, Karachi. Duration of diabetes and its complication was recorded. Individuals were classified as having diabetes mellitus if any of the following criteria were met[15], fasting serum glucose levels ≥ 7.0 mmol/L, random glucose levels ≥ 11.1mmol/L, current use of medications prescribed to treat diabetes (e.g. insulin or drugs). Patients below fifty years of age, those with more than one complications and patients having a history of ocular trauma, uveitis or glaucoma were excluded from the study. Macrovascular disease was considered to be present if there was history of myocardial infarction, angina, stroke, intermittent claudication, vascular surgery or amputation for atherosclerotic disease or one or more absent foot pulses on examination. Patients having history of blurred vision, double vision and spots were examined by slit lamp to determine the type of cataract. Patients having cataract either in one or both eyes were included in the study. Patients with retinal micro-aneurysms, soft exudates, small intra-retinal hemorrhages, blot hemorrhages, venous bleeding, neovascularization, retinal traction or detachment, were considered to have retinopathy and were included in the study. The presence of retinopathy was determined by an ophthalmologist using direct ophthalmoscopy through dilated pupils using 0.5 % (w/v) tropicamide and conformed using a canon CR4-45 NM retinal camera in the majority of cases. Diabetic patients without any complications were also investigated.

Blood glucose was determined by glucose oxidase method. The reagents were obtained from glucose enzymatique PAP 7500 kit of bioMerieux. Glycosylated hemoglobin estimated by kit obtained from Bio Systems Reagents and lnstruments, Spain. Serum hexoamine was determined by Cessi and Pillego's method [16], total serum protein by Biuret Method of Reinhold[17], sialic acid by Natelson method[18], and glycosylated proteins by the method of Ma.[19] Serum protein electrophoresis[20] was carried out by Helena Electrophoretic System, using a kit. Titan III Cat. No. 3023 obtained from Helena Laboratories. Serum furctosamine was determined by kit method supplied by Quimica Clinica Aplicada, Spain.

RESULTS

The diabetic patients with or without complications did not differ in age and weight as compared with control subjects. Fasting plasma glucose, HbA_{1C}%, serum fructosamine, glycosylated plasma protein, serum hexosamine and serum sialic acid levels were significantly increased ($P<0.05$) in all diabetic patients with complications or without complications as compared with control subjects (Table 1). Fasting plasma glucose was increased in all diabetic patients and correlated significantly with glycosylated hemoglobin, glycosylated plasma proteins and serum fructosamine concentrations. There was no significant difference between diabetic patients with or without chronic complications in the levels of fasting plasma glucose, glycosylated plasma proteins, glycosylated hemoglobin, serum fructosamine, hexosamine and sialic acid.

On electrophoretic separation of serum proteins, it was found that alpha-1 and alpha-2 globulin fraction were significantly increased in diabetic patients without complications ($p<0.001$ and $p<0.001$ respectively), diabetic patients with cardiovascular complications ($p<0.007$ and $p<0.027$ respectively) and diabetic patients with cataract complications ($p<0.006$ and $p<0.036$ respectively) as compared with control subjects. Albumin was found to be decreased in diabetic patients with cataract ($p<0.006$) as compared with control subjects while gamma globulin was increased in diabetic patients with cardiovascular complications ($p<0.014$) and diabetic patients with cataract ($p<0.000$) as compared with control subjects.

Table-1: Physical parameters and blood analytes in Control Subjects, Diabetic Patients with and without Complications

The values are expressed as mean ± SEM. Units and numbers of cases are shown in parentheses.

Parameters	Control (21)	Diabetic Patients without complication (21)	Diabetic Patients with Cardiovascular (21)	Diabetic Patients with cataract (20)	Diabetic Patients with retinopathy (20)
Age (years)	53.81±1.18	54.71± 1.38	57.86*± 1.41	57.50*± 1.55	54.55±1.14
Weight (Kg)	64.38±1.55	64.24±1.60	68.05±1.46	67.78±1.53	64.85±1.82
Sex (F/M)	10/11	10/11	10/11	10/10	10/10
Duration of Diabetes (years)	-	9.29±0.50	10.48±0.65	9.00± 1.00	12.30±1.63
Fasting plasma glucose (mmol/L)	5.04±0.13	7.83*±0.31	11.81*±0.19	11.02*±0.14	11.39*±0.16
Glycosylated Hemoglobin (HbA_{1C}%)	4.98±0.11	11.32*±0.18	11.72*±0.14	8.31*±0.20	12.33*±0.35
Serum Fructosamine (mmol/L)	2.25±0.08	3.72*±0.17	3.67*±0.17	3.05*±0.20	3.58*±0.28
Glycosylated plasma protein (Absorbance /g of proteins	6.20±0.12	7.77*±0.30	10.21*±0.04	10.67*±0.12	8.43*±0.24
Hexosamine (mg/dl)	67.86±3.12	115.15*±3.63	118.51*±3.13	123.52*±2.37	118.25*±2.37
Sialic Acid (mg/dl)	35.36±1.34	49.66*±1.78	52.90*±1.77	50.49*±1.73	51.16*±1.73

*P<0.05-significant compared with control subjects

Table-2: Total Serum Protein and its Fractions in Control Subjects and Diabetic Patients with or without Complications

The values are expressed as mean ± s.e.m. Units and numbers of cases are shown in parentheses.

Parameters	Control (21)	Diabetic Patients without complication (21)	Diabetic Patients with Cardiovascular (21)	Diabetic Patients with cataract (20)	Diabetic Patients with retinopathy (20)
Total Serum protein (gm%)	7.32±0.12	7.94*±0.17	7.99*±0.10	7.97*±0.11	7.56±0.15
Serum Albumin (gm%)	4.01±0.10	4.03±0.11	3.95±0.12	3.57*±0.11	3.72±0.15
Alpha-1 Globulin (gm%)	0.116±0.02	0.38*±0.06	0.32*±0.05	0.36*±0.09	0.20±0.04
Alpha-2 Globulin (gm%)	0.77±0.03	0.96*±0.05	0.93*±0.07	1.00*±0.05	0.93±0.09
Beta Globulin (gm%)	1.00±0.03	0.92±0.05	1.00±0.06	1.14±0.07	1.10±0.09
Gamma Globulin (gm%)	1.48±0.07	1.67±0.09	1.76*±0.09	2.01*±0.11	1.40±0.11

*P<0.05-significant compared with control subjects

DISCUSSION

This study describes the changes of serum glycoproteins in the diabetic patients in the presence or absence of diabetic complications. It was, however, realised that the diabetic patients without complications may have developed subclinical vascular or other lesions which might influence the glycosylated protein concentration as well as its composition. Similarly, in cases of specific diabetic complications, it was not possible to obtain all cases of just one complication because any given diabetic patient might be suffering from more than one complication. However, an attempt was made to select patients with single or predominant complications but the possibility of overlapping cannot be excluded, which may influence the results. The uniform increase in fasting plasma glucose, HbA_{1C}, serum fructosamine, glycosylated plasma protein, serum hexosamine and serum sialic acid in all diabetic patients (table 1), irrespective of the complications indicates that the process of glycosylation depends upon hyperglycemia. These observations are similar to those of other workers.[21-23] Odetti et al,[24] and Mandarino,[25] suggested a connection between impaired glycemic control with protein oxidation. Glycation cascade also releases free radicals, which become responsible for further oxidative attack. The relationship among HbA_{1C}, blood glucose concentrations and late complications has been established over the last 30 years.[26] Serum fructosamine and glycosylated plasma protein concentrations have close correlation with HbA_{1C} because they reflect glycemic control with last 2 to 3 weeks and HbA_{1C} reflects glycemic control for last 4 to 6 weeks.[27] In our study serum fructosamine and glycosylated plasma proteins in diabetic patients have also close correlation with HbA_{1C}. Henricsson et al,[28] studied seventy two patients and demonstrated that one percent increase in HbA_{1C} was associated with a 1.56 fold to 1.68 fold increased risk of retinopathy. Mclellan et al,[22] in their study of twenty six type 2 diabetics with retinopathy found that the presence of retinopathy also gave positive logistic correlation with the concentration of HbA_{1C}. In the present study the diabetic patients with retinopathy had high HbA_{1C}

(table-1).The degree of glycosylation of plasma proteins, as an alternative index of control and a reflection of possible structural alterations of tissue proteins leading to complications was, associated with the diabetic state.[19]

Among the serum proteins, alpha-1 and alpha-2 globulin fraction were significantly increased in diabetic patients without complications, diabetic patients with cardiovascular complications and diabetic patients with cataract. It may be associated with a diabetic state and not with the presence of clinically manifested chronic diabetic complications[29] as the increase was found in all diabetic patients except in retinopathy (table-2). Very few studies have been reported on serum protein changes in diabetic patients.[30-33] The rise in alpha-1 and alpha-2 globulins have been quoted by Frankl[34] in diabetic patients with combined neuropathy, retinopathy and nephropathy while Berkman et al[35] cited earlier work showing an increased concentration of alpha-2 globulin and normal alpha-1 fraction in diabetic patients with glomerulo-nephritis. In present study of decrease in albumin in diabetic patients with cataract, no significant change in beta globulin and an increase in gamma globulin in diabetic patients with cardiovascular complications and diabetic patients with cataract (table-2) was observed. The study therefore, confirms that the process of glycosylation of serum proteins is increased in diabetes mellitus and is further increased with the presence of known diabetic complications. Whether these changes in glycosylation of serum proteins are etiologically involved with the development of diabetic complications is not known.

CONCLUSIONS

In uncomplicated diabetic patients alpha-1 and alpha-2 glycoproteins were increased. In diabetic patients with cardiovascular complications alpha-1, alpha-2 and gamma globulin were increased while in diabetic patients with cataract alpha-1, alpha-2 and gamma globulin were increased but serum albumin was significantly decreased.

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