Antioxidant status in diabetic and non-diabetic senile patients, with cataract or cardiovascular complications

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ABSTRACT

الأهداف: الهدف من هذه الدراسة هو تقييم الحالة المضادة للأكسدة في المرضى المصابين بالشيخوخة ومرض السكري مع الإصابة بالماء الأبيض أو مضاعفات الجهاز القلبي الوعائي أو بدون مضاعفات.

الطريقة: أجريت دراسة مقارنة على ١٨٦ مريضا مصاباً بالسكري ونماذج مجموعة التحكم في الفترة ما بين ٧ مارس ٢٠٠٤م الى ١٢ نوفمبر ٢٠٠٦م على مرضى من مستشفى ضياء الدين الجامعي بمدينة كراتشي بباكستان. كان من بينهم ٣٣ مريضاً مصاباً بالسكري وبدون أي دليل سريري على وجود مضاعفات مزمنة للسكري. ٣٢ مريضا كانوا يعانون من داء السكري مع مضاعفات في الجهاز القلبي الوعائي. ٣٠ مريضاً لم يكونوا مصابين بالسكري وكانوا يعانون من الماء الأبيض في العين و٣١ يبدون بصحة جيدة. تم التحقق من العمر ونوع الجنس والوزن المتوافق مع مجموعة التحكم. تم اختيار جميع هؤلاء المرضى بناءً على أسس سريرية.

النتائج: انخفضت الحالة الكاملة المضادة للأكسدة (نسبة الخطأ أصغر من ٠,٠٠١) لدى جميع المرضى المصابين بالسكري وبدون أي مضاعفاتٍ والمرضى غير المصابين بالسكري مع نفس المضاعفات (١٥٥ مريضاً) حسب المقارنة مع مجموعة التحكم (٣١ شخصا). ارتفع سكر الصيام (نسبة الخطأ أصغر من ٠,٠٠١) لدى جميع المرضى المصابين بالسكري مع أو بدون مضاعفات (٩٥ مريضاً) وارتباط ذلك بشكل ملحوظ مع الهيموجلوبين الجلوكوزي وتركيز مصل فروستوسامين. لم يختلف سكر الصيام والهيموجلوبين الجلوكوزي ومصل فروستوسامين لدى المرضى المصابين بداء السكري مع أو بدون مضاعفات. لم يختلف سكر الصيام والهيموجلوبين الجلوكوزي ومصل فروستوسامين ومصل البروتين الكامل لدى المرضى غير المصابين بداء السكري مع بعض المضاعفات حسب المقارنة مع مجموعة التحكم.

خاتمة: تنخفض الحالة المضادة للأكسدة لدى مرضى الشيخوخة غير مصابين بداء السكري مع نفس المضاعفات حسب المقارنة مع مجموعة التحكم. قد تكون بعض العوامل الأخرى معقولة لنقص لحالة المضادة للأكسدة.

Objective: To assess the total antioxidant status in diabetic and non-diabetic senile patients, with cataract or cardiovascular complications, and without complications.

Methods: A comparative study on 186 senile patients and control subjects was carried out from March 2004 to November 2006 on patients from Ziauddin University Hospital, Karachi, Pakistan. Among them, 33 were diabetic patients without any clinical evidence of chronic diabetic complications, 32 with cardiovascular complications, 30 non-diabetic patients with cardiovascular complications, 30 diabetic patients with cataract, 30 non-diabetic patients with cataract, and 31 apparently normal, age, gender, and weight matched control subjects were investigated. All patients were selected on clinical grounds.

Results: Total antioxidant status was significantly decreased ($p<0.001$) in all diabetic patients with and without complications, and non-diabetic patients with same complications (155 patients) as compared with control subjects (31 subjects). Fasting plasma glucose was increased ($p<0.001$) in all diabetic patients with and without complications (95 patients), and correlated significantly with glycosylated hemoglobin (HbA_{1C}) and serum fructosamine concentrations. Fasting plasma glucose, HbA_{1C}, and serum fructosamine were not different in diabetic patients with and without complications. Fasting plasma glucose, HbA_{1C}, serum fructosamine, and total serum protein were not different in non-diabetic patients with the same complications, as compared with control subjects.

Conclusion: Total antioxidant status is decreased in diabetic and non-diabetic senile patients with the same complication as compared with control subjects. Some other factors may be responsible for decrease antioxidant status.

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A common denominator in the pathogenesis of most chronic diseases is the involvement of oxidative stress. Antioxidants may be of great benefit in improving the quality of life by helping to prevent or postpone the onset of degenerative diseases.[1] There is evidence implicating them as probably being protective in the development of cancer, atherosclerotic cardiovascular disease, and cataract.[2] The antioxidant system has many components. A deficiency in any of these components can cause a reduction in overall antioxidant status of an individual. The imbalance between the rate of free radical production and the effect of protective antioxidants leads to oxidative damage, which is also known as oxidative stress.[3] Fortunately, free radical formation is controlled by a complex network of beneficial compounds known as antioxidants. Oxidative damage caused by free radicals is counteracted by a number of enzymes[4] and vitamins.[5] Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells. There is evidence suggesting that oxidative damage is increased in diabetes.[6] Possible sources of oxidative stress and damage to proteins in diabetes include free radicals generated by autoxidation reactions of sugars and sugar adducts to protein, and by autoxidation of unsaturated lipids in plasma and membrane proteins.[7] The oxidative stress may be amplified by a continuing cycle of metabolic stress, tissue damage, and cell death, leading to increased free radical production, and compromised free radical inhibitory and scavenger systems, which further exacerbate the oxidative stress. Attack by reactive oxygen intermediates, common to many kinds of cell/tissue injury, has been implicated in the development of diabetic and other vascular diseases.[8] Such oxygen-free radicals can be generated by advanced glycation end products (AGEs), which are nonenzymatically glycated and oxidized proteins. Increased glycation of proteins and build-up of tissue AGEs are believed to be responsible for the chronic complications of diabetes. Glycation and AGEs formation are accompanied by generation of free radicals via autoxidation of sugars, Amadori products, and when AGEs interact with cellular receptors.[9] Diabetes, therefore, is associated with increased oxidative stress, and a decline in antioxidant status.[10] Senile cataract, by far the most common type, is due to a process of aging and degradation. These substantial modifications of lens proteins may stimulate further glycation, oxidation, and protein aggregation leading to the formation of cataract.[11] Diabetes is associated with a high risk of coronary heart disease.[12] Free radicals can result in consumption of antioxidant defenses and enhanced susceptibility to lipid peroxidation. Oxidative modification of low density lipoprotein (LDL) may be an important step in the atherosclerotic process.[13] This study aimed to assess the total antioxidant status in diabetic, non-diabetic, and senile patients with cataract or cardiovascular complications, and without complications.

Methods. One hundred and eighty-six patients and control subjects were selected from March 2004 to November 2006, in Ziauddin University Hospital, Karachi, Pakistan. Among them, 33 were diabetic without any clinical evidence of chronic diabetic complications, 32 diabetic with cardiovascular complications, 30 non-diabetic with cardiovascular complications, 30 diabetic with cataract, 30 non-diabetic with cataract, and 31 apparently normal, age, gender, and weight matched control subjects were investigated. Gender, weight, duration of diabetes, duration of complication in diabetic and non-diabetic patients, type of diabetes, and types of treatments received were also recorded. Physical examination including measurement of blood pressure was carried out. Individuals were classified as having diabetes mellitus if any of the following criteria were met:[14] fasting serum glucose levels of 7.0 mmol/L or more, random glucose levels of more than 11.1 mmol/L, current use of medications prescribed to treat diabetes (such as insulin or drugs), or a positive response to the question "has a doctor ever told you that you had diabetes (sugar in the blood)"? Patients included in the study were over 60 years of age, and with either cataract or cardiovascular complications. Patients below the age of 60, have complications other than cataract or cardiovascular were excluded in the study. The local ethical committee approved the protocol, and informed consent from all subjects was taken. Patients were selected on clinical grounds from the National Institute of Cardiovascular Disease, and Eye Ward Jinnah Postgraduate Medical Centre, Karachi, Pakistan.

Blood samples were obtained in fasting state after a 10 hour overnight fast. Samples were withdrawn from a cubital vein into heparinized tubes, and immediately stored on ice at 4°C. Clotted blood was centrifuged at 1,500 rpm for 30 minutes, and the serum was separated and frozen at -70°C until analysis. Samples were thawed and analyzed in batches. Blood glucose was determined by the glucose oxidase method, glycosylated hemoglobin (HbA_{1C}) by kit (Bio Systems Reagents and Instruments, Spain), serum fructosamine kit (Quimica Clinia Aplicada, Spain), and total serum protein by the Biuret method of Reinhold.[15] The quantity of total antioxidant capacity was measured according to Randox kit procedure (Randox Laboratories Ltd, United Kingdom). The 2, 2'-azino-bis-3-ethylbenzthiazoline sulphonate (ABTS) is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide (H_2O_2) to produce the radical cation ABTS. This has a relatively stable, and blue-green

color which was measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree, which is proportional to their concentration.[16]

Data were estimated with the Statistical Package for Social Sciences version 10.00 program. Data were presented as mean, standard deviation, ±SEM. The statistical significance of the difference between the 2 mean of various parameters, between different groups was evaluated by analysis of variants test. The difference was regarded as highly significant if the *p* value was less than 0.001, statistically significant if the *p* value was less than 0.05, and non-significant if the *p* value was greater than 0.05.

Results. The study shows no significant difference in age, gender, and weight. Fasting plasma glucose (9.35±0.28), HbA_{1C} (9.51±0.29), and serum fructosamine (3.59±0.11) were significantly increased ($p<0.001$) in diabetic patients with cataract, cardiovascular complications (8.97±0.29, 9.26±0.26, 3.74±0.11), and diabetic patients without complications (7.64±0.24, 9.03±0.28, 3.80±0.11), as compared with non-diabetic patients with cataract (5.21±0.14, 4.92±0.10, 2.32±0.11), cardiovascular complications (5.07±0.12, 5.13±0.10, 2.11±0.10), and control subjects (5.09±0.10, 4.96±0.08, 2.33±0.06) (**Tables 1 & 2**). These parameters was not significantly different ($p>0.05$) in non-diabetic patients with cataract and cardiovascular complications, as compared with control subjects (**Tables 1 & 2**). Total antioxidant status were significantly decreased ($p<0.001$) in all diabetic patients with and without complications, and non-diabetic patients with the same complications, as compared with control subjects. Fasting plasma glucose was increased ($p<0.001$) in all diabetic patients with and without complications, and correlated significantly increased ($p<0.001$) with HbA_{1C} and serum fructosamine concentrations. Fasting plasma glucose, HbA_{1C}, and serum fructosamine were not different ($p>0.05$) in diabetic patients with and without complications. Fasting plasma glucose, HbA_{1C}, serum fructosamine, and total serum protein were not different ($p>0.05$) in non-diabetic patients, with same complications as compared with control subjects (**Tables 1 & 2**).

Discussion. Diabetes mellitus is one of the major health problems of Pakistan. 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.[17] Diabetics are prone to long-term complications, such as cataract and cardiovascular complications.[18] Previous studies have suggested that the clinical forms of late complications seem to be the same, although it has not been established whether the pathogenic factors involved in the development of the late complications are common and, if so, to what extent. Cataract is among the early

Table 1 - Age, gender, weight, and fasting plasma glucose of control subjects, diabetic patients without complication, and diabetic and non-diabetic patients with cataract and cardiovascular complications. The values are expressed as mean, standard deviation, ± standard error of mean. Units and numbers of cases are shown in parentheses.

Groups	Age (years)	Gender (F/M)	Weight (Kg)	Fasting plasma glucose (mmol/L)
Control subjects (n=31)	64.2	16/15	63.6	5.1
	3.9±0.7		6.8±1.2	0.6± 0.1
Diabetic patients without complications (n=33)	64.2	16/17	65.7	7.5*
	3.3±0.6		8.8±1.5	1.4±0.2
Diabetic patients with cataract (n=30)	64.6	16/14	64.7	9.4†
	3.5±0.6		8.1±1.5	1.6±0.3
Non-diabetic patients with cataract (n=30)	65.1	15/15	65.7	5.2
	4.6±0.8		8.1±1.5	0.8±0.1
Diabetic patients with cardiovascular complications (n=32)	66.0	14/18	66.9	9.0†
	4.5±0.8		6.0±1.1	1.7±0.3
Non-diabetic patients with cardiovascular complications (30)	65.7	15/15	63.3	5.1
	4.7±9.9		6.9±1.3	0.7±0.1

*Significant as compared with control subjects, †significant as compared with non-diabetic patients with same complications, M - male, F- female

Table 2 - Glycosylated hemoglobin, serum fructosamine, total serum proteins, and total antioxidant status of control subjects, diabetic patients without complications, and diabetic and non-diabetic patients with cataract and cardiovascular complications. The values are expressed as mean, standard deviation, ± standard error of mean. Units and numbers of cases are shown in parentheses.

Groups	Glycosylated hemoglobin (%)	Serum fructosamine (mmol/L)	Total serum protein (gm %)	Total antioxidant status (mmol/L)
Control subjects (n=31)	5.0	2.3	7.3	1.5
	0.5±0.1	0.4±0.1	0.6±0.1	0.2±0.0
Diabetic patients without complication (n=33)	9.0*	3.8*	7.6	1.1*
	1.6±0.3	0.7±0.1	0.7±0.1	0.2±0.0
Diabetic patients with cataract (n=30)	9.5†	3.6*	7.7	1.2†
	1.6±0.3	0.6±0.1	0.7±0.1	0.2±0.0
Non-diabetic patients with cataract (n=30)	5.0	2.3	7.3	1.4*
	0.6±0.1	0.6±0.1	0.9±0.1	0.1±0.0
Diabetic patients with cardiovascular complications (n=32)	9.3 †	3.7†	7.5	1.2†
	1.5±0.3	0.6±0.1	0.8±0.1	0.1±0.0
Non-diabetic patients with cardio-vascular complications (n=30)	5.1	2.1	7.5	1.4*
	0.6±0.1	0.6±0.1	0.6±0.1	0.2±0.0

*Significant as compared with control subjects, †significant as compared with non-diabetic patients with the same complications

complications of diabetes mellitus,[19] and the role of glycosylation of the lens crystalline has been suggested, which may bring conformational changes.[20]

Diabetes may influence antioxidant enzyme activity through disturbances in micronutrient status.[21] Total antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids. In the present study, beside other parameters, total antioxidant status were measured. The total antioxidant status were significantly decreased (p<0.001) in all diabetic patients with and without complication, and non-diabetic patients with the same complications as compared with control subjects indicates a high degree of oxidative stress. Results of diabetic patients with and without complications were similar to studies reported earlier, however, non-diabetic patients with the same complication data was not available.[22-24] Antioxidants, such as enzymatic and nonenzymatic defense system is necessary to prevent cellular damage.[25] High extra-cellular and intra-cellular concentrations of glucose could cause glycation, and consequently functional changes of the antioxidant enzymes.[26] Whereas hyperglycemia can result in the generation of free radicals through several biochemical pathways (nonenzymatic glycation, the polyol pathway, and glucose autoxidation),[26] the mechanisms underlying the oxidative damage in diabetes is not understood. Reports indicate that some complications of diabetes mellitus are associated with increased activity of free radicals and accumulation of lipid peroxidation products.[27] Glycation cascade also releases free radicals, which become responsible for further oxidative attacks.[26] Free radicals are defined as chemical species that possess one or more unpaired electrons. The term reactive oxygen species collectively describes free radicals such as superoxide anion, hydroxide ion, and other non-radical oxygen derivatives such as hydrogen H_2O_2 and hypochlorous acid. These reactive oxygen intermediates may participate in reactions, which give rise to free radical species. Free radicals are electrically-charged molecules that attacks your cells, tearing through cellular membranes to react and create havoc with the nucleic acids, proteins, and enzymes inside. These attacks by free radicals are collectively known as oxidative stress.[28] Defense against free radical damage, therefore, seems to be important in maintaining structural and functional integrity of the endothelium. However, providing proper antioxidant protection is a challenge similar to putting a puzzle together. All the necessary pieces must be available, and properly combined to create comprehensive, balanced protection. Increased oxidation of proteins also occurs in the presence of fructose, and this has been attributed to increased hydroxyl radical production, thus fructose could contribute towards oxidative damage in vivo.[29]

Fasting plasma glucose, HbA_{1C}, and serum fructosamine levels were significantly increased in diabetic patients, with and without complications, as compared with non-diabetic patients with the same complications, and control subjects (**Tables 1 & 2**). These observations are similar to those of other workers.[30] The uniform increase in fasting plasma glucose, HbA_{1C}, and serum fructosamine levels in diabetic patients indicates that the process of glycosylation is associated with hyperglycemia. Serum fructosamine concentrations have close correlation with HbA_{1C} as they reflect glycemic control lasting 2-3 weeks, and HbA_{1C} reflects glycemic control lasting 4-6 weeks. The HbA_{1C} has less 2,3-diphosphoglyceric acid attached, and a higher affinity for oxygen than hemoglobin A. This will result in less oxygen being released to the tissues and causes tissue hypoxia, which may be related to the development of microangiopathy.[31] Any reduction in HbA_{1C} is likely to reduce the risk of complications, with the lowest risk being in those with HbA_{1C} in the normal range.[31]

However, based on the results, relevant dysfunction of the antioxidant enzymes, and the role of any such dysfunction in the development of diabetic complications cannot be determined. A limitation of the study was the low number of patients, thus the study must be carried out on a larger population for definite conclusion. Although oxidative stress has a role in the development of late complications, it is still not clear whether it has the same importance in the different forms of diabetes and non-diabetic patients, with cataract and cardiovascular complications. Keeping all this in mind, instead of diabetes, decreased total antioxidant status may be responsible for complications in all diabetic patients with and without complication, and non-diabetic patients with the same complications. This implies that this topic needs to be studied further. More studies on a larger population are needed to determine whether these tendencies are real.

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