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SM Journal of Endocrinology and Metabolism

Research Article

Oxidative Stress, Antioxidative Enzymes and Dietary Antioxidant Intake in Patients with Diabetes Mellitus with and without Nephropathy

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Article Information

Received date: Mar 12, 2017
Accepted date: Apr 17, 2017
Published date: Apr 27, 2017

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Keywords Diabetes Mellitus; Oxidative stress; Malondialdehyde; Anti-oxidative enzyme system

Abstract

High oxidative stress due to increased free radicals generation and chronic hyperglycemia works in a vicious cycle by complementing each other, weakening the antioxidant enzyme defense system and finally may lead to diabetes mellitus. Increased oxidative stress is also suggested to be playing a key role in development of micro complication of diabetes. The present study has compared the anti-oxidative status and dietary pattern of Type-2 Diabetes subjects without complication (n=33), with complication (diabetes nephropathy: n=15) and age, gender, Body mass index matched control subjects (n=32). Indicator of oxidative stress and anti-oxidative enzyme levels were estimated. Present study has assessed oxidative stress in terms of Plasma malondialdehyde levels, which were found to be significantly high in subjects from both the diabetic groups as compared to normal subjects. All anti-oxidative enzymes were found to be lower in diabetic subjects compared to controls. Current daily consumption of dietary antioxidants (Beta carotene and vitamin C) was significantly low in all diabetic subjects compared to control whereas, amongst the two diabetic groups, it was significantly low in diabetes nephropathy group. In conclusion, increased generation of free radicals, weakened antioxidant enzyme defense and lower consumption of dietary antioxidant may increase oxidative stress and may contribute to progress of diabetic complications.

Introduction

The global burden of diabetes was reported to be 415 million in 2015 and the number is expected to rise to more than 642 million by 2040. Majority of them aged between 40 and 59 years and 80% of them belong to low and middle income countries. In India, 65.1 million People aging between 20-79 years were reported to be diabetic in 2013 [1].

Diabetes is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Chronic hyperglycemia manifests into macro and micro vascular complications of diabetes leading to mortality [2].

Management of diabetes is mainly based on maintaining the blood sugar level as close to normal through management of medicinal, dietary carbohydrate and physical activity level. But the cause related management of diabetes and its complication has not been getting addressed effectively.

Oxidative stress is now suggested to be associated with pathogenesis of diabetes and its vascular complications [3]. Oxidative stress is defined as a state in which oxidation exceeds antioxidant system in the body [4]. In the biological system, various reactive oxygen species are generated during the process of utilizing oxygen which is essential for life. These reactive oxygen species, having unpaired electron, are highly reactive and unstable than the original oxygen molecule. In physiologic condition, we have integrated antioxidant systems comprised of enzymatic and non-enzymatic antioxidants that are effective in neutralizing the harmful effect of free radicals such as superoxide, hydrogen peroxide, hydroxyl radicals, and peroxynitrite [5].

Enzymatic antioxidant system in vivo consists of superoxide dismutase, catalase and glutathione peroxidase counteracts and regulates the level of free radicals and maintains physiological homeostasis. Superoxide dismutase, a metalloenzyme, catalyzes superoxide to molecular oxygen and peroxide and protects the cell against negative impact of aerobic respiration. It neutralizes hydrogen peroxide and protects the cells against lipid peroxidation. Catalase causes oxidation of hydrogen donors and neutralizes the free radicals [6].

Non-enzymatic system includes vitamin E, C and beta carotene from dietary source, act on free radicals and breaks the chain reaction of free radical formation. Vitamin C scavenges free radicals. αTocopherol, most potent form of Vitamin E donates electron to peroxyl radical produced during lipid peroxidation. Beta carotene reacts with peroxyl, hydroxyl and superoxide radicals. Therefore intake of sufficient dietary antioxidants is expected to strengthen the anti-oxidative system of the body and improve the effective management of diabetes.

High blood glucose level in diabetic condition has shown to increase the generation of reactive oxygen species through various mechanisms. Nishikawa T (2000) reported that, high glucose level may lead to autoxidation of glucose, enhanced non-enzymatic-glycation and activation of diacylglycerol protein kinase C pathway and activation of Polyol pathway. In the process, an abnormally high level of free radicals and simultaneous decline in antioxidant enzymes leads to enhanced lipid peroxidation, which may lead to development of complications of diabetes [7].

Present study aims to assess and compare oxidative stress and antioxidative enzyme erythrocyte superoxide dismutase, catalase and glutathione peroxidase in Type-2 diabetes patients with and without complication of nephropathy and control subjects. Current Diet pattern and dietary antioxidant intake of subjects was assessed and compared among the three groups.

Materials and Methods

The research proposal including the details of methodology was cleared by Registered Medical ethics committee.

Subject profile

The study was conducted at outpatient department of Endocrinology at Bai Yamuna Anand Nair Charitable Hospital, Mumbai, India, where people mainly from low socioeconomic group usually get the treatment. The study group consisted of Type-2 Diabetes subjects without micro vascular complication (n=33) and with complication like diabetic nephropathy (n=15) along with age, gender, Body mass index matched controls (n=32).The patients were identified as diabetic subjects, based on American diabetes association guidelines. Diabetes nephropathy was diagnosed based on proteinuria and elevated blood urea nitrogen and Creatinine level. The subjects were enrolled using inclusion criteria of participants treated with Oral hypoglycemic drugs; insulin therapy or a combination of both along with Medical nutrition therapy. Subject with glycosylated haemoglobin less than 8.5 percent were enrolled to avoid extreme deviations in oxidative stress. Participants with morbid obesity (Body Mass Index >35 Kg/m^2), consuming multivitamins or mineral supplements, pregnant, lactating mothers were excluded. All participants were non-smokers, non-alcoholic and free of established diabetes macrovascular complications like cardio-vascular, cerebrovascular or peripheral vascular disease, etc. The subject profile is given in detail in table 1. Informed consent was obtained from all the subjects after their willingness to participate in the study (Table 1).

Research tools

Questionnaire: A pretested questionnaire was used for interviewing participants about demographic profile. Diabetes related information, including duration of diabetes; present glycemic control status, type of treatment. Waist circumference, Waist to hip ratio and Body Mass Index (BMI) were assessed.

Dietary assessment:A two day 24 hour recall was recorded to assess the intake of calories (kcal), macronutrients such as carbohydrates (gm), Protein (gm), fat (gm) and micronutrients beta carotene (ug) and vitamin C (mg). Food frequency questionnaire was used to assess the frequency of consumption of foods rich in calorically dense foods as fat and starch rich and dietary antioxidants beta carotene and vitamin C intake was assessed.

Antioxidant status assessment: a) Sample collection:10ml blood was drawn from each participant after an overnight fast of 12hours by venipuncture using a disposable needle and syringe under aseptic conditions. Out of which 5ml blood was collected in each EDTA tube (Labtech disposal) for the estimation of erythrocyte superoxide dismutase, erythrocyte catalase, and erythrocyte glutathione peroxidase and plasma malondialdehyde.

b. **Sample preparation:** The samples were centrifuged at 2000rpm for 10mins to separate the plasma. The buffy coat was washed three times with cold saline, and was haemolysed by adding ice cold ultrapure water to yield a 50% hemolysate. Aliquots of hemolysate were stored at -70˚C till analysis.

c. **Blood tests:** Fasting blood glucose, post prandial blood glucose, glycosylated hemoglobin lipid profile and renal function tests was done in diabetic subjects.

d. **Antioxidative status analysis:** Estimation of erythrocyte superoxide dismutase was measured by using McCord J. M. et al method [8]. Glutathione peroxidase and catalase activity of the hemolysate was estimated using method by Beutler E et al [9]. The preparation of hemolysate for catalase activity was done using method by Beutler et al [9]. Plasma malondialdehyde level was measured using method standardized by Stock J et al [10].

Statistical analysis

Statistical analysis of the data was done using Prisampad graphics by applying unpaired T test of independent variables, one way ANOVA, and Bon ferroni test. Pearson correlation test was used to assess correlation between various parameters and considered significant where p value was less than 0.05.

Results and Discussion

Demographic profile and medical history

The duration of disease since identification, was found to be significantly longer in subjects with diabetes nephropathy. Majority of the subjects with diabetes without complication were treated with oral hypoglycemic agents. Exclusive insulin therapy was the treatment modality in higher proportion of subjects with nephropathy and was given with oral hypoglycemic agent in 40% subjects (Table 2).

Hypertension was the most common co-morbidity in both the groups and it was found in 100% of the enrolled subjects with diabetes nephropathy. Blood pressure was under control in both the groups with antihypertensive medications.

Anthropometric profile

Presence of central obesity is more common in Asian Indians, having more total abdominal and visceral fat for any given BMI [11]. In the present study, mean waist circumference was recorded to be higher in diabetic subjects without complication.

Among the three groups, subjects with diabetes nephropathy were noted to have lowest waist circumference with mean value near cut off points (82.69±14.59). This may be attributed to restricted consumption of calories and macronutrients due to azotemia and

Table1: Demographic detail of subjects.

	Diabetic subjects (n =33)	Diabetic subjects with complication (Nephropathy) (n =15)	Control subjects (32)
Gender(Male : female)	24:9	8:7	18:14
Central Obesity waist circumference (cm)	87.93±9.24	82.69±14.59	85.43±10.06
Duration of diabetes	3.42±3.26	11.08±7.02	--

Table 2: Medical profile of the subjects.

Treatment	Diabetes without complication(n=33)% (n)	Diabetes Nephropathy(n=15)% (n)
Monotherapy (OHA)	33% (11)	6.67% (1)
Combination therapy (OHA)	57.58% (19)	-
Insulin therapy	3.03% (1)	60% (9)
Insulin and OHA	6.06% (2)	33.33% (5)

higher metabolic stress. Presence of protein urea may further cause weight loss along with increased cautiousness among subjects to delay further progression of nephropathy (Table 1).

Biochemical assessment

Fasting and post prandial blood sugars were higher than normal range in both the groups. However, significant difference was not noted between two groups.Better glycemic control in diabetes nephropathy group can be related to altered clearance and degradation of insulin from under functioning kidneys. Kidneys play a key role in clearance and degradation of insulin. Hence, decline in kidney function leads to increased half life of circulating insulin. This often results in management of blood glucose with lower dose of insulin [12].

As reported in table-3, diabetes is often accompanied with dyslipidemia. Studies have indicated altered lipid metabolism and lipid induced renal impairment in diabetes nephropathy subjects [13]. Similarly, studies have reported association of total and LDL cholesterol with presence of hard exudates in diabetes retinopathy [14]. Among the components of lipid profile, borderline high triglyceride, low density lipoprotein and lower high density lipoprotein were noted in all the three groups. However, the difference between groups was not statistically significant (Table 3). This indicates presence of typical features observed in diabetic dyslipidemia.

Excretion of nitrogenous waste like blood urea nitrogen, creatinine and maintaining fluid and electrolytes balance is one of the major functions of kidneys. Progressive decline in glomerular filtration rate in diabetes nephropathy results in alterations in kidney function.

As expected, blood urea nitrogen, creatinine levels and serum potassium level were significantly high (p=0.001) in diabetes nephropathy subjects compared to other groups (Table 4). No significant difference was noted in diabetes without complication and diabetes retinopathy (p>0.05). This indicates retinopathy and nephropathy may be stand alone diabetic complication at the initial stages. Tissues which are not dependent on Insulin for glucose transport like retina or medulla of kidney may not get affected by diabetes at the same time. Long standing uncontrolled diabetes may affect both.

Antioxidative profile

The marker of three months average plasma glucose concentration, Glycosylated haemoglobin level was used for the inclusion criterion as less than 8.5 percent to avoid uncontrolled diabetic subjects. It was found to be significantly higher (P<0.001) in subjects with diabetes with and without nephropathy, as compared to control subjects (Table 5). Oxidative stress as reflected by plasma malondialdehyde level was also found to be significantly higher (P<0.001) in diabetic subjects as compared to controls. Among the diabetic groups, subjects with diabetes nephropathy had significantly elevated plasma malondialdehyde (P<0.001) indicating higher oxidative stress in subjects with nephropathy compared to diabetic subjects without complications.

Table 3: Biochemical parameters across thegroups.

Parameter	Diabetes without complications(n=33) (Mean±SD)(Range)	Diabetes nephropathy(n=15) (Mean±SD)(Range)	Diabetes retinopathy(n=15) (Mean±SD)(Range)	Normal range	P value
Fasting blood glucose(mg/dl)	143.75±39.97 (82-229)	136.26±36.46 (80-220)	146.73±52.23 (80-239)	< 80-140 mg/dl	0.77
Post prandial blood glucose (mg/dl)	195.84±50.55 (99-313)	179.46±71.73 (85-383)	184.67±55.85 (90-295)	<140 mg/dl	0.68
Total cholesterol(mg/dl)	189.48±28.84 (130-267)	202.86±35.21 (140-267)	205.93±27.3 (168-261)	<200 mg/dl	0.14
LDL cholesterol(mg/dl)	118.93±32.88 (66-151)	126.26±20.47 (96-174)	132.6±13.56 (102-153)	<100 mg/dl	0.25
HDL cholesterol(mg/dl)	40.87±4.95 (30-53)	40.33±4.18 (32-45)	43.06±3.17 (35-47)	Low risk 40 mg/dl Desirable 60 mg/dl or higher	0.19
Serum Triglycerides(mg/dl)	161.03±29.58 (84-233)	177.73±36.86 (129-233)	171.26±30.12 (135-267)	<150 mg/dl	0.12

Table 4: Renal function test across the groups.

Parameter	Diabetes without complications(n=33) (Mean±SD)(Range)	Diabetes nephropathy(n=15)(Mean±SD) (Range)	Diabetes retinopathy (n=15) (Mean±SD)(Range)	Normal range	Pvalue
Blood urea nitrogen (mg/dl)	13.81±3.40 (5-24)	43.13±18.42 (32-45)	16.2±5.47 (10-26)	8-25 mg/dl	0.001
Serum creatinine (mg/dl)	0.93±0.22 (0.5-1.5)	3.56±0.9 (2.3-5.3)	1.00±0.23 (0.7-1.6)	0.6-1.3 mg/dl	0.001
Serum sodium (mEq/L)	134.87±22.76 (130-146)	138.8±3.68 (135-146)	140.2 ±3.14 (136-145)	135-145 mEq/L	0.001
Serum potassium (mEq/L)	4.18±0.46 (3.4-5.3)	5.23±0.42 (2.3-5.3)	4.13±0.31 (3.6-4.5)	3.5-5.0 mEq/L	0.001

Table 5: Oxidative stress and antioxidative status.

	HbA1c (%)	MDA (nano-moles/ml)	SOD (Unit/g Hb)	Catalase (Unit/g Hb)	Glutathione peroxidase (Unit/gHb)
Control(n=32)	5.46±0.09	1.49±0.09	1.5±0.03	1.68±0.06	25.45±1.85
Diabetes without complications(n= 33)	7.57±0.09	1.92±0.09	1.11±0.05	0.86±0.04	13.13±0.49
Diabetes nephropathy(n=15)	6.91±0.32	2.45±0.23	1.24±0.07	1.0±0.11	19.43±3.05
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

The antioxidative enzymes like erythrocyte superoxide dismutase, glutathione peroxidase and catalase in diabetic group with and without nephropathy were found to be significantly low (P<0.001) compared to controls. This confirms weakened antioxidant defense system in diabetic subjects. When compared among the diabetic groups, erythrocyte levels of all the antioxidative enzymes were found to be marginally high in all subjects with nephropathy compared to diabetic subjects without nephropathy. Although the differences were not statistically significant, the trend of higher anti-oxidative enzyme levels in all subjects with nephropathy may be explained as compensatory increase in antioxidative enzymes in severe condition of diabetes or diabetic nephropathy group (Table 5), with better control of blood glucose level as indicated by HbA1c within 8.5.

Dietary assessment

Diet control is one of the main stay for the management of diabetes. The current study wants to assess the present dietary intake levels of antioxidants by different group enrolled for the study The mean energy intake of subjects in diabetes nephropathy group was significantly lower than control and diabetic subjects without nephropathy (Table 6). However the difference between controls and diabetic subjects without nephropathy was found to be statistically insignificant. The frequency of consumption of empty calories was reported to be similar (1 to 2 days per week) in all three groups. However the mean calorie consumption of energy dense, fat and carbohydrate based food items was 2 times higher in control and diabetic subjects without nephropathy, as compared to diabetic nephropathy group. The mean carbohydrate, protein and fat consumption was significantly lower (P<0.001) in subjects with diabetes nephropathy compared to other 2 groups. The difference observed in diabetes with nephropathy as compare to diabetes without complication and normal subjects can explained due to strict regulation of diet in subjects with diabetes with related morbidity like nephropathy.

It was observed that antioxidant rich fruits and vegetable consumption was lower in diabetic patients (Table 6). This is where management of disease condition needed special attention. Patients with diabetes cut down their consumption of sweet tasting food including fruits. In fact these fruits are the treasure bank of antioxidant which is essential for restricting the progression of disease. But Patients with diabetes nephropathy needs to stop fruits and advised low potassium vegetables as they have persistent hyperkalemia. Therefore dietary management has to be case specific, depending on the progression of the disease.

Beta carotene and vitamin C intake: The dietary intake of antioxidants like Beta carotene and vitamin C intake was compared among the three groups. The mean consumption of beta carotene and

Table 6: Dietary assessment of subjects.

	Control (n=32)	Diabetic without complication (n=33)	Diabetes with Nephropathy (n=15)	P-value
Energy (Kcal)	2014±80.62	2158±78.59	1694.26±300.44	0.003*
Empty calories (Kcal)	405±164	402±224	177±124	0.004*
Carbohydrates (gm)	301.5±16.61	339.9±15.26	251.98±61.58	0.0058*
Protein (gm)	45.52±2.271	50.50±1.014	39.96±10.68	0.0041*
Fat (gm)	58.26±2.819	62.26±2.806	47.44±8.04	0.0024*
Beta-carotene (ug)	2656±158.5	1872±92.10	1147.63±262.36	<0.0001*
Vitamin C (mg)	87.64±7.267	77.92±4.426	65.53±13.08	0.0956
Number of serving of fruits per week	4.23±3.00	2.27±2.09	0.6±0.72	<0.0001*
Number of serving of vegetables per day	10.50±6.36	10.95±6.84	10.26±2.68	0.9232*

Note: * P<0.05.

vitamin C was significantly high (P<0.001) in control subjects and lowest (P<0.001) among subjects with diabetes.

Discussion

Oxidative stress is defined as shift in balance between oxidants and antioxidants in favour of oxidants. The stress plays a critical role in development of insulin resistance and β- cell dysfunction which are the major mechanisms in the pathogenesis of Type-2 diabetes mellitus [15]. Oxidative stress is enhanced in response to chronic hyperglycemia in vascular tissues of patients with diabetes mellitus leading to peroxidation of cellular membrane lipid and increased oxidative modification of amino acids and DNA [16]. Enhanced oxidative stress may play a crucial role in development of microvascular complications of diabetes.

The role of anti-oxidative enzymes to hyperglycemia induced activation of various pathways such as glucose oxidation, polyol pathway [17], Protein kinase C (PKC) activation, formation of advanced glycation end products (AGEs) etc can be discussed as follows [18].

Glucose in its enediol form is oxidised in a transitional metal dependant reaction to an enediol radical anion that is converted to superoxide anion radicals. This superoxide anion radical may undergo dismutation to hydrogen peroxide and if not degraded by catalase or glutathione peroxidise, it may lead to production of reactive hydroxyl radicals [19].

In hyperglycemia, increased intracellular glucose leads to activation of polyol pathways leading to its increased enzymatic conversion in sorbitol utilizing nicotinic acid adenine dinucleotide phosphate (NADPH). With concomitant decrease in NADPH level, the regeneration of reduced glutathione impairs the antioxidant mechanism. Since glutathione is a scavenger of reactive oxygen species, this can induce or exacerbate oxidative stress [20].

Inoguchi T, 2000 also suggested that Hyperglycemia stimulates generation of reactive oxygen species though PKC dependant activation of NAD(P)H oxidase [21] Chronic hyperglycemia increases non enzymatic glycation characterised by binding of amino groups of proteins leading to synthesis of Advanced Glycation End products (AGE). Glycation related oxidative stress is referred as glycoxidation. Each step of glycoxidation leads to generation of reactive oxygen species. Proteins modified by AGE precursors also induce intracellular oxidative stress by activating NADPH oxidase [22].

Overproduction of free radicals through all the above mentioned pathways is accompanied with dysfunctional antioxidant enzyme activity and increase in overall oxidative stress, supporting the results obtained in the present study.

Diabetic Nephropathy (DN) is one of the most common microvascular complications of diabetes and a major cause of end stage renal disease [23]. Higher oxidative stress was observed in diabetes nephropathy subjects across all the groups. Shaker O G, 2013 reported similar findings in diabetes nephropathy subjects as compared to diabetic group, without complication. Higher oxidative stress due to chronic hyperglycemia may play a critical role in development of microvascular complications of diabetes [24].

Higher chronic oxidative stress in diabetes leads to activation of protein kinase, nicotinamide dinucleotide phosphate oxidases (NOX), stimulating vascular endothelial growth factor and increased generation of Mitogen activated protein kinase [25]. This leads to increase free radical production and increased mesangial expansion, basement membrane thickening renal hypertrophy, glomerular sclerosis and tubule interstitial fibrosis in diabetic kidney, altered vascular permeability to albumin and renal damage [26]. Cvetkoviae T, 2009 also reported that hyperglycemia induced reactive oxygen species increases lipid peroxidation and oxidation of protein yielding protein carbonyl derivatives producing high levels of plasma malondialdehyde in subjects with diabetic nephropathy [27].

Plasma malondialdehyde levels were found to be higher in all the diabetic subjects compared to controls indicating higher oxidative stress in diabetes patients. The present study records a decline in activity of superoxide dismutase, the first line of defense against free radicals, in all diabetic subjects. This loss of enzyme activity results increased products of lipid peroxidation and oxidants like hydrogen peroxidation. Hyper-glycemia induced glycation of enzyme-protein decreases (50%) the enzyme activity. Similar trend was observed by Song et al and Bikkadet al [28,29].

Decreased erythrocyte catalase activity as observed in the present study in all diabetic subjects may be attributed to inactivation of enzyme catalase due to glycation and combination of other sugars with simultaneous increase in superoxide and peroxide radicals. Similar findings were observed by El-Bab MF et al in diabetes retinopathy subjects [30].

The reduction in erythrocyte glutathione peroxidase activity in subjects with diabetes group without complications compared to controls in the present study may be attributed to metabolism of excessive glucose by polyol pathway. This pathway utilizes NADPH as a hydrogen donor and decreases the NADPH/ NADP+ ratio. Increased sorbitol pathway utilizes the NADPH leading to decreased regeneration of reduced glutathione (GSH). Failure in regeneration of glutathione (GSH) weakens the antioxidant defense by glutathione peroxidase and decreases its activity. The decrease in SOD activity may lead to increase level of superoxide radicals which will cause the inactivation of GPx, increasing free radical damage [31]. Similar trend was observed by Blum et al and Komosinska-Vassev K [32,33].

Antioxidative status of diabetic subjects with and without nephropathy

Marginal increase in activity of all the three antioxidative enzymes in diabetes nephropathy subjects compared to without complication diabetic group may be due to over expression of these enzymes to compensate for higher oxidative stress in the nephropathy group.

Bhatia et al, Varma et al, Dave et al, Kesavalu et al reported lower levels of all antioxidative enzymes in diabetic subjects with nephropathy group than without complication group [34,45,36]. However, all the studies have enrolled uncontrolled hyperglycemic subjects (HbA1c ranging between 9.2 to 10.51%) compared to 6.91±0.32% in the present study. In the present study group, mean glycosylated haemoglobin levels were higher in all the diabetic subjects compared to controls. However significantly lower (P<0.05) glycosylated haemoglobin in diabetes nephropathy subjects compared to without nephropathy subjects may be attributed to increased

awareness, better dietary compliance with longer duration of diabetes and guided management of blood glucose with insulin therapy to delay further progression of nephropathy.

This is again proving the importance of controlling the blood sugar level around the normal level, as considered in the present study as the inclusion criterion in the present study.

Dietary assessment

Medical nutrition therapy is a fundamental element in diabetes management and self-management education. Medical nutrition therapy prescribed by Registered Dietitian has shown to reduce A1C by 0.5-2% in Type-2 diabetes patients [38,39]. As compared to simple diabetic subjects, nutrition management of nephropathy involves stringent fluid and electrolyte balance and protein restriction, besides glycemic control.

As indicated in the table 6, lower caloric intake in subjects with nephropathy may be attributed to decreased consumption of calories from energy dense fat and starch based foods to delay the progression of the disease through better compliance to medical nutrition therapy which was reflected in better glycemic control (6.91±0.32%) and lower waist circumference (82.69±14.59cm) in this group. Higher intake of total calories and increased consumption of fat and starch based food items as reported by the subjects may be responsible for increased glycosylated hemoglobin (7.573±0.09%) and waist circumference in early stage of diabetic condition.

American diabetic association recommends protein intake of 0.8gm/Kg body weight per day for patients with diabetic kidney diseases [40]. In the present study, daily protein intake of diabetic subjects without complication was similar as 0.79gm/Kg body weight. But diabetic nephropathy group was found to consume significant lower protein intake (0.6 g/Kg body weight), may be due to total low calorie intake and excessive protein restriction for compromised kidney function. The total dietary fat intake was estimated to contribute to 25% of total calories in all three groups.

Vitamin C is the most potent hydrophilic antioxidant which acts synergistically with vitamin E against lipid peroxidation in solution, membranes and lipoproteins. Daily vitamin C intake was reported to be higher than the recommended dietary allowance (RDA) (40mg) in all the groups. Vegetables were reported to be the main sources of vitamin C compared to fresh fruits as reported in table 4. Vitamin C content of food is lost when it is cooked at high temperature for longer time [41]. Therefore availability of vitamin C from cooked vegetable was lower compared to fresh fruits. Thus, higher proportion of vitamin C was available to controls, followed by diabetic subjects without complication and lowest in diabetes nephropathy group based on weekly servings of fruits consumed.

Beta carotene is alipophilic antioxidant present at the interior membranes or lipoproteins and scavenges free radicals. In the present study weekly serving of fruits was inversely related to plasma malondialdehyde in diabetes nephropathy and diabetic subjects without complications. Similar findings were reported by Asgard et al [42]. In the present study, beta carotene intake is only 24% of RDA (4800ug/day) in diabetes nephropathy group followed by 39% of RDA in diabetic group without complication and 55% of RDA in controls. The diabetic population enrolled for the present study is from low and middle socioeconomic class and has a poor intake level of antioxidants through diet.

Thus, lower dietary antioxidant intake in diabetic groups compared to controls may lead to impaired defense against lipid peroxidation. This may further increase the oxidative stress as reflected by raised plasma malondialdehyde.

Conclusion

The duration of diabetes increases chronic hyperglycemia leading to increased oxidative stress that may contribute in development of microvascular complications in diabetes nephropathy subjects as compared to diabetic subjects without complications. All the antioxidative enzymes are lower in diabetic subjects without complication compared to controls, indicating low antioxidative capacity in diabetic subjects. In the present study, in subjects with nephropathy, all the enzymes are marginally elevated which may indicate compensatory increase to combat increased oxidative stress. Central obesity was prevalent in diabetic subjects without complications as compared to nephropathy group. Present dietary antioxidant consumption was lowest in subjects with diabetic complications among the three groups. Thus, hyperglycemia induced higher oxidative stress along with weak in vivo antioxidant system and poor dietary antioxidant intake such as fruits and vegetables may have a role in development of diabetic complication like nephropathy. Subjects with diabetes nephropathy, when maintain their blood sugar level as shown by controlled HbA1c level, may cause over expression of anti-oxidative enzymes. Electrolyte imbalance associated in diabetes nephropathy may further reduce dietary antioxidant intake and contribute to higher oxidative stress. Therefore along withkeeping the blood sugar level as close to normal value through medication and dietary control, anti-oxidative system needs to be supported through the modifiable factor of dietary antioxidant supply.

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