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Biochemical Evaluation of the Antioxidant Nutrients on Blood Rheology among Prediabetics

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: Oxidative stress is involved in the developing of several diseases including diabetes followed by the majority of severe acute illness and/or contra complications. Current studies indicated increasing plasma viscosity with progressing microangiopathy among diabetic cases.
Objectives: To evaluate whether antioxidant nutrients biomarkers of selenium, vitamin A and vitamin E are associated with alterations of blood viscosity in pre-diabetes (PD).
Materials and Methods: Whole blood samples were collected from (20) PD non obese subjects with blood HbA c1 (5.7-6.4%) and (20) control healthy subjects. Different biomolecules were assessed including selenium concentration in both plasma and red blood cells (RBCs), glutathione peroxidase activity in RBCs, plasma lipids, vitamin A and vitamin E plasma concentration, and the blood viscosity.
Results: Comparing to control healthy subjects, blood viscosity was significantly increased, selenium was not altered in the plasma of PD, subjects but it was markedly decreased in RBCs and negative correlation with the elastic component of whole blood viscosity. Mean RBCs, glutathione activity was reduced. Vitamin A and vitamin E plasma levels were correlated with blood viscosity. Plasma viscosity correlated strongly with increased cholesterol level (r=0.67, P 0.002) and triglycerides concentration (r=0.66 , P 0.005).

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Conclusion: Blood viscosity increased in PD and this may be contributed to reduced nutritional antioxidants.

Keywords: Prediabetes; selenium; glutathione peroxidase; vitamin E; vitamin A; blood viscosity.

ABBREVIATIONS

(PD) pre-diabetes; (RBCs) red blood cells; (OS) oxidative stress; (GPx) glutathione peroxidases; (HbA1c) glycosylated hemoglobin; (WBV) whole blood viscosity .

1. INTRODUCTION

By the end of 2030, diabetes mellitus will be the seventh leading cause of death [1]. The PD is a triggering stage between normal health and diabetes in which fasting plasma glucose is increased with impaired glucose tolerance. PD populations are willing to get diabetes mellitus within 10 years or less [2].

Current researchers investigated increasing oxidative stress (OS) among hypoglycemic populations. In diabetes mellitus, OS plays a part acute endothelial dysfunction and activation of inflammation in the blood vessels of diabetic patients. It takes part in the development of different microvascular complications [3] but there are no data for early stages of the disease. In turn, it causes overproduction superoxides in mitochondria within endothelial cells of different blood vessels. It increases the biosynthesis of glycated end products, expression of their receptors, activation of protein kinase C and the overabundance of hexosamine pathways [4]. The OS stimulates intravascular blood coagulation in diabetes-associated atherosclerotic complications by increasing fibrin deposits that in turn proliferates plasma viscosity [5,6]. It passes reactive oxygen derivatives in small amounts that possess free radical properties mediated lipid peroxidation with increasing antioxidant enzyme levels [7]. It takes part in decreasing plasma levels of vitamins A, C and E that in turn points decreasing antioxidative barrier in patients with different metabolic syndromes [8].

Increasing reactive oxygen products affect plasma antioxidant activity and disturbed oxidation-reduction balance and lead to severe alterations of cellular function with subsequent cell damage [9]. Mainly, glutathione peroxidases (GPx) play an antioxidant to protect of erythrocyte against peroxides from oxidative damages by increasing the level of reduced glutathione. GPx is involved in the detoxification of hydrogen and lipid peroxides. Low levels of GPx have been correlated with free radical related disorders that in turn damage DNA, proteins, and lipids of the cell. Accordingly, it leads to cell dysfunction in different tissues, causing pathogenesis of vascular complications [10,11]. As the deficiency of antioxidants may increase the risk of late complications in diabetics [8]. Nutrients with the antioxidant action, such as selenium, vitamin A and vitamin E have been suggested to exist protective effects in diabetes mellitus.

Diabetes, OS obesity, inflammation, aging, smoking and cancer are important factors for getting deterioration of blood rheology [12]. Mainly, impairment of blood rheological parameters affects both viscosity and RBCs elasticity which subsequently diminishes both blood flow rate and tissue oxygenation. In addition, RBCs elasticity reflects their blood aggregability and deformability [13,14]. Little information about blood rheology in the early stages before getting diabetes and/or PD cases are known. Consequently, monitoring of blood rheology is urgently useful for those PD populations.

Therefore, the main aim of the present study was to examine the state of antioxidant nutrients like selenium together with selenium-dependent GSH, vitamin A, and vitamin E in PD cases and to establish whether they are associated with alterations of blood viscosity.

2. MATERIALS AND METHODS

Whole venous blood samples were collected from 20 PD. All were none obese (BMI < 30) and none smokers. In addition, 20 healthy persons matched for sex, age, and body mass index was considered as (control group). All participants were recruited from the outpatient clinic of the Suez Canal University Hospital for the study. PD was considered with HbA1c (5.7-6.5)%. Both PD and control groups were subjected to the same

laboratory measurements. Applicants were asked to fast for at least 8hs earlier morning blood collection. Aliquots of all samples were centrifuged at 3000 rpm then collecting plasma to keep at -70C till biochemical analysis. The study protocol was confirmed to the ethical guidelines of the declaration of Helsinki (Sixth revision, 2008). The laboratory investigations were done for the followings:

2.1 Glycosylated Hemoglobin (HbA1c)

Blood glucose and plasma lipids were measured via enzyme immunoassay technique (ELISA). Fasting serum glucose was measured by the glucose oxidase method (Roche Diagnostics GmbH, Germany) [15]. Glucose was measured by the glucose oxidase method using the YSI analyzer (Yellow Springs, OH, USA). HbA1c was measured by HPLC (Biorad, Hercules, CA, USA). Numerous of biochemical measurements were performed on fasting plasma collected. Plasma fasting glucose, HDL, cholesterol and triglycerides levels were measured by marketable test kit methods using the automatic clinical chemistry analyzer (Architect CI8200, Abbott Diagnostics.

2.2 Selenium

Selenium concentrations in plasma and RBCs were measured with hydride generation atomic absorption spectrophotometry (Perkin-Elmer 3030, Perkin-Elmer Corp., Norwalk, CT) [16]. Mainly, the whole blood –anticoagulant with EDTA-was centrifuged, then plasma and buffy coat were removed. The RBCs washed three times with isotonic saline solution; 1 ml of packed RBCs. Plasma and/or RBCs samples were diluted 1:4 with a triton-X (Sigma Chemical, St. Louis, MO) and nitric acid solution (Fisher Scientific, Pittsburgh, PA) and incubated at room temperature for 10 min, then adding 70% perchloric acid with boiling 3 mins, then adding v/v 20 mmol/l HC1 using HPLC deionized water then treated with 30% sodium borohydride, and 15% sodium hydroxide reagent. Treated plasma and RBCs Se levels absorbance were measured at 196 nm.

2.3 Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activities in RBCs were measured enzymatically based on Pagalia and Valantine method [17] using GPx assay (RANSEL) assay kit, Randox Laboratories Ltd, Crumlin, BT29 4QY, UK. Packed washed cells were lysed with 1 ml of demineralized water, diluted 1:1 with double strength Drapkin reagent and used for both the determination of GPx activity and hemoglobin. Samples were frozen at – 40 C until determination of GPx activity. The reaction solution was: 0.2 mM NADPH, 4 mM GSH, 4 mM EDTA, 4 mM NaN3, 1 I.U glutathione reductase, H202, and the sample in 100 mM phosphate buffer PH 7.0. The oxidation of NADPH was followed at 37 Co using a 340 nm filter with a spectrophotometer (Hitachi U –2000). Enzymatic activity is expressed as ug Hb/L. Hb in the RBCs lysate was measured by the cyanmethemoglobin method [18]. The coefficient of variation (between-run) for GPx activity ranged from 2.2% to 7.5%. The reproducibility was 6.5%.

2.4 Antioxidative Vitamins

Plasma concentrations of vitamin A (all-trans-retinol) and vitamin E (a –tocopherol) were determined by using high-performance liquid chromatography [19]. The coefficient of variation of the method was 2.5% and 2.2% for retinol and a-tocopherol, respectively.

2.5 Whole Blood Viscosity

Whole blood viscosity (WBC) was determined in EDTA anticoagulant blood with a capillary viscometer (OCR-O Anton Paar Cokg, Graz, Austria) at a constant oscillation frequency of 2 Hz for shear rates between 1 S to 100 S. Blood samples were kept in the rotation until estimation to avoid sedimentation. Experiments were done within 1 – 2 h after blood collection at a constant temperature of 37 + 0.1 Co with blood samples adjusted to the hematocrit of 45 + 1% [20].

2.6 Statistical Analysis

Data are presented in terms of mean, standard deviation (SD) of the mean, and percentages. Statistical analysis was carried out by a computer program (SPSS Ver. 20). Student t-test, Chi-square, the Mann-Whitney U test, and correlation tests were used to evaluate the results. Data are presented as mean + SD. Statistical significance in figures is denoted with P value less 0.05.

2.7 Ethical Consideration

Before the initiation of the study, informed consent was obtained from all individuals selected for the study. The aim and the value of the work were explained to them in a simplified manner. There was no harm being inflicted on

them. On the contrary, all would have the benefits of follow-up and the results of the study.

3. RESULTS

Twenty patients with PD, 60% were males with average age 46.7±5.2 years old, were studied. Glycated hemoglobin (Hb A1c) was 6.1+1.1%. No significant difference between patients and control group regarding the age and sex distribution.

Table 1 shows that both the patient and the control groups were well matched regarding age, sex, body mass index and blood pressure. There was a significant difference as regards the Hb A1c level between the both groups. Regarding the lipid profile, there was a significant difference between the patient and control groups as regard total cholesterol level and triglycerides level. Regarding the blood viscosity, there was a significantly increased in the patient group (4.23+0.68 vs 3.51+0.6, P< 0.001).

Table 2 shows that the mean plasma selenium concentration in the patient group was not different from that in the control groups. In contrast, the concentration of selenium in RBCs of the patient group was significantly reduced compared with control groups. Selenium-dependent glutathione peroxidase activity (GPx) was slightly but significantly reduced in the patient group. Vitamin A and E concentrations differed slightly, but significantly between the groups.

In the Table 3, significant correlations were found between whole blood viscosity and RBCs selenium, vitamin A, vitamin E and GPx values and in addition to both hematocrit and HbA1c. On the other hand, no correlations were recorded with lipids plasma selenium.

4. DISCUSSION

Intensive organization for PD are required based on relevant evidence to help physicians in selecting the best possible management strategies for PD, especially those suffering from specific conditions. The PD is monitored by the level of hyperglycemia and associated with impaired fasting glucose and/or impaired glucose tolerance. Increases in HbA1c levels are related to an increasing evidence point to a progression of PD to frank diabetes [21,22].

Recent research studies in this area of PD support a central role for reactive oxygen species in both the formation of AGEs and in AGE-induced pathologic alterations in gene expression. AGEs alter matrix-matrix, matrix cell, and cell-cell interaction with subsequent changes in plasma viscosity and severity of PD [23].

Table 1. Clinical and biochemical PD of both patient and control groups

Pattern	PD (n=20)	Control (n=20)	*P*
Age (years)	46.7±5.2	41.5±7.4	n.s
Sex F/M	8/12	9/11	n.s
Body mass index (BMI) %	24.2±0.6	23.5±0.8	n.s
Systolic B.P mm Hg	122.4±13.4	121.9±6.7	n.s
Diastolic B.P mm Hg	81.4±4.2	79.9±4.7	n.s
Hb A_{1c} %	6.1±1.1	5.1±0.1	<0.001
Plasma protein gm %	7.1±0.4	7.5±0.4	n.s
Haematocrit %	44.8±3.7	45.3±2.4	n.s
Total cholesterol mg %	216.7±12.5	198.4±16.5	<0.01
Triglyceride mg %	185.4±18.9	145.8±17.9	<0.01
Whole blood viscosity cP	4.23±0.68	3.51±0.6	<0.001

n.s: non significant

Table 2. Antioxidant nutrients in both PD and control groups

Antioxidant nutrients	PD (n=20)	Control (n=20)	*P*
Plasma selenium umolL^{-1}	1.29±0.3	1.31±0.7	n.s
RBCs selenium umolL^{-1}	0.79±0.3	1.35±0.3	<0.01
GPx ugHb^{-1}	6.77±0.4	8.2±0.9	<0.05
Vitamin A umolL^{-1}	1.88±0.6	1.99±0.7	<0.05
Vitamin E umolL^{-1}	29.1±6.9	31.9±1.2	<0.05

n.s: non significant

Table 3. Correlations between the severity of antioxidant nutrient, blood chemistry and blood viscosity in PD group

Prameters	Whole blood viscosity	
	r	*P*
Plasma selenium	0.31	n.s
RBCs selenium	-0.59	<0.05
GPx	-0.63	<0.05
Vitamin A	-0.71	<0.01
Vitamin E	-0.68	<0.05
Haematocrit	+0.72	<0.05
HbA_{1c}	0.41	<0.05
Cholesterol	0.39	n.s
triglyceride	0.41	n.s
Plasma protein	0.46	n.s

n.s: non significant

Many studies using a fluorescence spectrophotometer techniques informed reduced membrane fluidity for RBCs in type 2 diabetes mellitus. These data, documents the represented data in which oxidative deregulation is represented with decreased membrane fluidity of submitochondrial particles and increased mitochondrial dysfunction with diabetes mellitus type 2 [24]. Therefore, the problem, whether nutritional antioxidants have a similar possible role in RBCs membrane fluidity and blood viscosity in PD and the correlation with plasma lipids. The present data considering measurements done on whole blood, approve concomitant increased viscous components in the PD group. Possible mechanisms for the reduction of membrane fluidity include increased cholesterol/phospholipids ratio, or enzymatic changes such as reduction of Na+, K+ ATPase activity [25-27].

However, the current results suggest that the selenium concentration in RBCs may also contribute to the fluidity of the blood. The cause of significant reduction of selenium concentration in RBCs of PD group in our study is questionable. Dietary deficiency of selenium can be excluded as there was no detectable difference in plasma selenium, a marker for nutritional intake relative to the control.

Significant reduction in GPx activity was recorded in the current study. Only a borderline significant correlation between GPx activity and selenium in RBCs in PD group was seen, probably due to the reduced selenium concentration in RBCs. Plasma selenium levels drop independently of selenium level during the acute phase response and GPx. The resulted data conclude that changed concentrations of selenium in PD with critical illness may due to the effects of the OS. The place of reduced GPx activity in the development of PD changes in blood rheology must be defined by further studies.

There was a significantly increased blood viscosity in the PD group in comparison with the control group. From this evidence, it can also hypothesize that PD patients with higher blood viscosity may prolong insulin resistance with advances to frank diabetes. It is in agreement with Zhao et al. [28] study. However, elevated whole blood viscosity is attended with a prevalence of insulin resistance in middle-aged and elderly Chinese population [29]. Therefore, further studies are recommended for studying the relationship between insulin sensitivity and blood viscosity among PD cases.

Borderline differences were found in vitamin A and vitamin E between PD patient and control group. Regarding vitamin A, a different result was recorded by Godala et al. [30]. They recorded decreased level of vitamins A and E points to the weakening of the antioxidative barrier in patients with the metabolic syndrome. In addition, García et al. [31] reported the association of low concentrations of vitamins A and E in children with lipids, inflammation, and insulin resistance and attributed these observations to a reduced mobilization of vitamin A from the liver by an unknown mechanism.

A significant negative correlation was found between vitamin A, vitamin E and whole blood viscosity. This observation may be attributed to the hypothesis that vitamin A and vitamin E are highly effective as an antioxidant for suppressing the peroxidative lysis of liposomes of PD erythrocyte lipids as in selenium and GPx activity.

5. CONCLUSION

The contribution of increasing blood viscosity based different biochemical parameters in developing PD cases to be a question of debate. The present data show that whole blood viscosity was increased in PD and affected by the RBCs concentrations of nutrient antioxidants, selenium, vitamin A, and vitamin E. Increased levels of the OS may motivate some of the increased risks of getting the development of increased whole blood viscosity in PD. Interventions to decrease

levels of the OS by methods such as antioxidants therapy, glycemic and lipid control are indicated. Whether or not nutritional antioxidant replacement improves the concentration of selenium, vitamin A, and vitamin E whole blood viscosity with a subsequent improvement in insulin resistance in the next future among prediabetics. Such studies are required to prevent progression of PD to frank diabetes mellitus.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. World Health Organization, Global Status Report on Noncommunicable Diseases 2010, WHO, Geneva, Switzerland; 2011.
2. Buysschaert M, Medina JL, Buysschaert B, Bergman M. Definitions (and Current Controversies) of diabetes and prediabetes. Curr Diabetes Rev; 2015.
3. Wang JS, Lin SD, Lee WJ, Su SL, Lee IT, Tu ST, et al. Effects of acarbose versus glibenclamide on glycemic excursion and oxidative stress in type 2 diabetic patients inadequately controlled by metformin: A 24-week, randomized, open-label, parallel-group comparison. Clin Ther. 2011;33(12): 1932-42
4. Farabi SS, Carley DW, Smith D, Quinn L. Impact of exercise on diurnal and nocturnal markers of glycaemic variability and oxidative stress in obese individuals with type 2 diabetes or impaired glucose tolerance. Diab Vasc Dis Res; 2015.
5. Lipinski B. Pathophysiology of oxidative stress in diabetes mellitus. J Diabetes Complications. 2001;15(4):203-10.
6. Otto C, Richter WO, Schwandt P. Contribution of fibrinogen and lipoproteins to plasma viscosity in hyper-cholesterolemia and hypertriglyceridemia: Evaluation by selective depletion of low-density lipoproteins or fibrinogen. Metabolism. 2000;49(6):810-3.
7. Likidlilid A, Patchanans N, Peerapatdit T, Sriratanasathavorn C. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients. J Med Assoc Thai. 2010;93(6):682-93.
8. Godala M, Materek-Kuśmierkiewicz I, Moczulski D, Rutkowski M, Szatko F, Gaszyńska E, et al. Estimation of plasma vitamin A, C and E levels in patients with metabolic syndrome. Pol Merkur Lekarski. 2014;36(215):320-3.
9. Eshaq RS, Wright WS, Harris NR. Oxygen delivery, consumption, and conversion to reactive oxygen species in experimental models of diabetic retinopathy. Redox Biol. 2014;2:661-6
10. Yu KJ, Zhang MJ, Li Y, Wang RT. Increased whole blood viscosity associated with arterial stiffness in patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol. 2014;29(3):540-4.
11. Zhao HY, Li J, Xu M, Wang TG, Sun WW, Chen Y, et al. Elevated whole blood viscosity is associated with insulin resistance and non-alcoholic fatty liver. Clin Endocrinol (Oxf); 2015.
12. Hitsumoto T. Factors affecting impairment of blood rheology in obese subjects. J Cardiol. 2012;60(5):401-6.
13. Li G, He H, Yan H, Zhao Q, Yin D. Does carbonyl stress cause increased blood viscosity during storage? Clin Hemorheol Microcirc. 2010;44(2):145-54.
14. Simmonds MJ, Meiselman HJ, Marshall-Gradisnik SM, Pyne M, Kakanis M, Keane J, et al. Assessment of oxidant susceptibility of red blood cells in various species based on cell deformability. Biorheology. 2011;48:293–304
15. Gabbay KH, Hasty K, Breslow JL, Ellison RC, Bunn HF, Gallop PM. Glycosylated hemoglobins and long-term blood glucose control in diabetes mellitus. J Clin Endocrinol Metab. 1977;44(5):859-64.
16. Aansson L, Petterson J, Eriksson L, Olin A. Atomic absorption spectrophotometric determination of selenium in human blood components. Clin Chem. 1989;35:537–542. Rosella LC, Lebenbaum M, Fitzpatrick T, Zuk A, Booth GL. The prevalence of undiagnosed and prediabetes diabetes in Canada (2007-

2011) According to Fasting Plasma Glucose and HbA1c Screening Criteria. Diabetes Care; 2015.
17. Paglia DE, Valentine WN. Studies characterization of erythrocyte glutathione peroxidase. J lab Clin Med 1967;70:158–169.
18. Von Kampen EJ, Zijlstra WG. Standardaization of hemoglobinometry. The hemoglobincyanide method. Clin Chem Acta. 1961;6:538–544. Tsai CJ, Hsieh CJ, Tung SC, et al. Acute blood glucose fluctuations can decrease blood glutathione and adiponectin levels in patients with type 2 diabetes. Diabetes Res Clin Pract. 2012;98:257–263.
19. Bieri JG, Tolliver TJ, Citignami GL. Simultaneous determination of a – Tocopherol and retinol in plasma or red cells by high performance liquid chromatography. Am J Clin Nutr. 1979;32: 2143–2149.
20. Schneditz D, Ribitsch V, Kenner T. Rheological discrimination between nature, rigid and aggregated red blood cells in oscillatory flow. Biorheol. 1985;22:209–219.
21. Gradinaru D, Borsa C, Ionescu C, Margina D. Advanced oxidative and glycoxidative protein damage markers in the elderly with type 2 diabetes. J Proteomics. 2013;92: 313-22
22. Huang M, Liang Q, Li P, Xia J, Wang Y, Hu P, et al. Biomarkers for early diagnosis of type 2 diabetic nephropathy: A study based on an integrated biomarker system. Mol Biosyst. 2013;9(8):2134-41.
23. Kellow NJ, Coughlan MT, Savige GS, Reid CM. Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: A study protocol for a double-blind placebo-controlled randomised crossover clinical trial. BMC Endocr Disord. 2014;14:55
24. Rodríguez-Carrizalez AD, Castellanos-González JA, Martínez-Romero EC, Miller-Arrevillaga G, Villa-Hernández D, Hernández-Godínez PP, et al. Oxidants, antioxidants and mitochondrial function in non-proliferative diabetic retinopathy. J Diabetes. 2014;6(2):167-75.
25. Likidlilid A, Patchanans N, Peerapatdit T, Sriratanasathavorn C. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients. J Med Assoc Thai. 2010;93(6):682-93.
26. Jacobs S, Schiller K, Jansen E, Fritsche A, Weikert C, di Giuseppe R, et al. Association between erythrocyte membrane fatty acids and biomarkers of dyslipidemia in the EPIC-Potsdam study. Eur J Clin Nutr. 2014;68(4):517-25.
27. Kröger J, Jacobs S, Jansen EH, Fritsche A, Boeing H, Schulze MB. Erythrocyte membrane fatty acid fluidity and risk of type 2 diabetes in the EPIC-Potsdam study. Diabetologia. 2015;58(2):282-9.
28. Zhao HY, Li J, Xu M, Wang TG, Sun WW, Chen Y, Bi YF, Wang WQ, Ning G. Elevated whole blood viscosity is associated with insulin resistance and non-alcoholic fatty liver. Clin Endocrinol (Oxf); 2015.
29. Vayá A, Hernández-Mijares A, Suescun M, Solá E, Cámara R, Romagnoli M, et al. Metabolic alterations in morbid obesity. Influence on the haemorheological profile. Clin Hemorheol Microcirc. 2011;48(4):247-55.
30. Godala M, Materek-Kuśmierkiewicz I, Moczulski D, Rutkowski M, Szatko F, Gaszyńska E, et al. Estimation of plasma vitamin A, C and E levels in patients with metabolic syndrom. Pol Merkur Lekarski. 2014;36(215):320-3.
31. García OP, Ronquillo D, del Carmen Caamaño M, Martínez G, Camacho M, López V, et al. Zinc, iron and vitamins A, C and E are associated with obesity, inflammation, lipid profile and insulin resistance in Mexican school-aged children. Nutrients. 2013;5(12):5012-30.

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