INVERSE RELATIONSHIP BETWEEN ADHERENCE TO THE MEDITERRANEAN DIET AND SERUM CYSTATIN C LEVELS

Natalia G. Vallianou[1], Ekavi Georgousopoulou[2], Angelos A. Evangelopoulos[1], Vassiliki Bountziouka[2], Maria S. Bonou[1], Evangelos D. Vogiatzakis[1], Petros C. Avgerinos[1], John Barbetseas[1], Demosthenes B. Panagiotakos[2]
[1]Evangelismos General Hospital, Athens, Greece
[2]Department of Nutrition and Dietetics, Harokopio University of Athens, Athens, Greece

SUMMARY

Objective: The aim of the present study was to examine serum cystatin C levels in association with the Mediterranean diet in a healthy Greek population.

Methods: Cystatin C together with basic clinical chemistry tests was measured in a total of 490 adults (46 ± 16 years, 40% of males), who underwent an annual health check. Demographic, anthropometric and lifestyle characteristics were recorded, while adherence to the Mediterranean diet was evaluated through the MedDietScore (0–55).

Results: The mean level of serum cystatin C was 0.84 mg/L, while men had increased serum cystatin C levels compared to women (0.86 mg/L vs. 0.83 mg/L, respectively, 0.017). After adjusting for age, gender, body mass index, smoking status, hypertension, diabetes, hypercholesterolemia, estimated glomerular filtration rate (eGFR), albumin and ferritin levels, each unit increase in MedDietScore led to 0.002 mg/dL drop off in cystatin C serum levels.

Conclusions: We have demonstrated an inverse relationship between the MedDietScore and serum cystatin C levels. Our finding that increases in MedDietScore are associated with decreases in serum cystatin C levels could imply that adherence to the Mediterranean diet may reduce the cardiovascular risk, as assessed by cystatin C, a prognostic marker of the cardiometabolic risk. This notion could have a great impact on public health.

Key words: cystatin C, MedDietScore, Mediterranean diet, cardiovascular risk

Address for correspondence: N. Vallianou, Evangelismos General Hospital, 45-47 Ipsilantou str 10676, Athens, Greece. E-mail: natalia.vallianou@hotmail.com

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INTRODUCTION

Cystatin C is a non-glycosylated, low molecular weight (13.250 Da), basic protein that is a member of the cystatin superfamily of cysteine protease inhibitors (1–3). It consists of 120 amino acids, it is produced by all nucleated cells at a constant rate, and it is excreted by the kidneys by free glomerular filtration and complete tubular reabsorption and degradation (4–6). Besides its usefulness as a marker of renal function, serum cystatin C appears to be a prognostic marker of cardiovascular events and death among elderly persons without chronic kidney disease (7, 8). The association of serum cystatin C with chronic low-grade inflammation and atherosclerosis has been proposed as the causal link between increased cystatin C concentrations and adverse cardiovascular outcomes (9).

As widely known, dietary habits play a critical role in the pathogenesis of atherosclerosis. The present study aimed to explore whether there is an association between the Mediterranean diet and the serum levels of cystatin C, as a marker of increased cardiovascular risk.

MATERIALS AND METHODS

Subjects

Between April 2009 and January 2010, a total of 490 consecutive adults (46 ± 16 years, 40% of men) who had visited the Polyclinic of General Hospital for a health check-up, agreed to participate in the study (85% of participation rate). The retrieved data were confidential and the study followed the ethical considerations provided by the World Medical Association (52nd WMA General Assembly, Edinburgh, Scotland, October 2000). Moreover, the Institutional Review Board approved the design, procedures and aims of the study (GA 23/14.05.2009). All participants were informed about the procedures of the study and agreed to participate providing written informed consent.

Lifestyle and Anthropometric Measures

All the participants were Caucasians. Information on age (years), gender (males vs. females), level of education (years of school) and family status (singles, i.e. single, divorced, widowed, vs. married) were recorded in a self-administrated questionnaire.

With respect to lifestyle characteristics, participants were asked to fill in a 10-grade scale range regarding their physical activity status (grade of scale used: 1–10, where 1 denotes sedentary lifestyle and 10 daily hard activity of at least 30 minutes). Participants with score ≤ 7 were classified as of low/moderate activity, while those with score >7 were considered as highly active. Current smoking was defined as smoking at least 1 cigarette/day for the past year. The number of cigarettes per day and the total years of smoking were also recorded. A 76-item valid semi-quantitative Food Frequency Questionnaire (FFQ) was used to record participants' dietary habits (10). Alcohol consumption was then assessed as the number of drinks per day measured in equivalences of ethanol intake. Furthermore, overall assessment of dietary habits in terms of adherence to the Mediterranean dietary pattern was evaluated through the MedDietScore (range 0–55) (11). Higher values of the score indicate greater adherence to this pattern and, consequently, healthier dietary habits. More specifically, one unit increase in MedDietScore could reflect small dietary modifications such as consumption of 1 more fish serving per week, 3 more fruits per week, or consumption of 1 less portion of red meat per week. The top score of MedDietScore (i.e. 55) depicts the strongest adherence to the Mediterranean diet which consists of more than 4 portions of non-refined cereals per day, more than 5 potatoes a week, 3 fruit and 4 vegetables portions a day, more than 6 fish and legumes servings per week, and everyday use of olive oil in cooking. Moreover, the highest score includes moderate alcohol intake, modest consumption of full-fat dairy (less than 10 servings per week) and poultry (maximum 3 portions per week), while red meat intake should not exceed 1 serving per week.

Waist circumference and height were measured to the nearest 0.5 cm, without shoes, and weight was measured with a lever balance to the nearest 100 g without shoes in light undergarments. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in metres. Participants were classified as overweight if BMI was between 25–29.9 kg/m^2, while obesity was defined as BMI greater than 29.9 kg/m^2.

Systolic and diastolic blood pressure was measured twice in all participants by the same physician using a standard mercury sphygmomanometer on the right arm of the seated subject. The mean value for blood pressure measurements was adopted. Hypertension was thus defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg or the use of anti-hypertensive drugs. Coronary heart disease, heart failure, cerebrovascular disease, and peripheral arterial disease were assessed by self-report and medication use. Diabetes was assessed by self-report, medication use or a positive diagnosis by fasting blood glucose level (≥ 126 mg/dL) (12). Hypercholesterolemia was defined as a serum cholesterol ≥ 200 mg/dL or by the use of cholesterol-lowering drugs.

Laboratory Parameters

Venipuncture was performed for each participant early in the morning (between 7 a.m. to 11 a.m.), after a 12-hour fasting period by applying a natural latex rubber strap and using a 20 mL syringe. Reproducibility in the lab has been determined using human samples and controls in an internal protocol. For the above mentioned tests, within run and between day coefficients of variation (CV) were less than 7%. Accuracy of results was further supported by participation in suitable external quality assurance programmes (ESEAP). All measurements were performed on a Roche/Modular Analytics analyzer. Reagents, calibrators, controls, and consumables were purchased from the same supplier (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis

Continuous variables are presented as mean values ± standard deviation under the normal distribution hypothesis and median (1st, 3rd quartile) for not normally distributed continuous variables. Categorical variables are presented as frequencies (relative frequencies). Independent samples t-test, or the non-parametric Mann-Whitney U-test were used in the unadjusted analyses (where appropriate). Multiple regression analyses were performed in order to test the associations between the MedDietScore and serum levels of Cystatin C, after controlling for several confounders in three nested models. Results are presented as Beta coefficients. All reported p-values are based on two-sided tests and compared to a significance level of 5%. SPSS 18.0 software (SPSS Inc. 2004, Chicago, Illinois, USA) was used for all statistical calculations.

RESULTS

The mean level of serum cystatin C was 0.84 mg/L, while men had increased serum cystatin C levels compared to women (0.86 mg/L vs. 0.83 mg/L, respectively, 0.017). The mean level of adherence to the Mediterranean diet was moderate (i.e. 30/55 as assessed using the MedDietScore), but it was at the same level for both genders ($p=0.329$). The socio-demographic and clinical characteristics of the participants are presented in Table 1, in respect to their gender.

As presented in Table 1, men had significantly higher Body mass index than women, higher waist circumference, higher systolic and diastolic blood pressure, higher serum glucose levels, higher serum triglycerides levels, higher cystatin C levels, higher serum albumin levels, and higher ferritin levels, while they had lower HDL-cholesterol levels and lower glomerular filtration rate (GFR) than women. In order to better evaluate the effect of adherence to the Mediterranean Diet on cystatin C serum levels three nested models adjusted for several socio-demographic and clinical characteristics were performed (Table 2).

After adjusting for age, gender, body mass index, and smoking status, each unit increase in the MedDietScore led to 0.003 mg/dL reduction of cystatin C levels (Model 1). After further adjustment for the presence of hypertension, diabetes and hypercholesterolemia, each unit increase in the MedDietScore led to a 0.003 mg/dL decrease of cystatin C levels (Model 2). In the final model (Model 3), three more confounding factors were taken into account (i.e. GFR, albumin and ferritin levels). Even in this model, each unit increase in the MedDietScore led to 0.002 mg/dL drop off in cystatin C serum levels (Model 3).

Table 1. *Socio-demographic and clinical characteristics of the participants in respect to their gender (N = 490)*

Characteristic	Men (n = 296)	Women (n = 194)	p
Age (years)	47 ± 16	46 ± 16	0.342
MedDietScore (0–55)	30 ± 4	30 ± 4	0.329
Body Mass Index (kg/m^2)	28 ± 4	26 ± 5	< 0.001
Waist circumference (cm)	99 ± 12	87 ± 13	< 0.001
Systolic blood pressure (mmHg)	123 ± 17	116 ± 19	< 0.001
Diastolic blood pressure (mmHg)	77 ± 11	72 ± 11	< 0.001
Glucose (mg/dL)	101 ± 18	94 ± 16	< 0.001
Total cholesterol (mg/dL)	200 ± 43	200 ± 38	0.612
HDL-cholesterol (mg/dL)	43 ± 11	55 ± 14	< 0.001
LDL-cholesterol (mg/dL)	130 ± 39	127 ± 34	0.257
Triglycerides (mg/dL)	118 (82, 161)	80 (59,116)	< 0.001*
C-reactive protein (mg/dL)	0.10 (0.10, 0.20)	0.10 (0.00, 0.28)	0.755*
Cystatin C (mg/dL)	0.86 ± 0.16	0.83 ± 0.16	0.017
Glomerular Filtration Rate (mL/min)	102 ± 18	107 ± 20	0.016
Albumin (g/dL)	4.8 ± 0.28	4.6 ± 0.28	< 0.001
Ferritin (ng/dL)	128 ± 90	65 ± 80	< 0.001

Continuous variables are presented as mean ± standard deviation when normally distributed (and median (1st ,3rd quartile)); p-values obtained from independent samples; t-test under the normal distribution hypothesis (and Mann-Whitney non parametric test if normality was not satisfied)

Table 2. *Nested multivariate linear regression models evaluating the role of adherence to the Mediterranean Diet in serum levels of Cystatin C*

Characteristic	Beta (95% CI)		
	Model 1	Model 2	Model 3
Age (years)	0.005 (0.004,0.006)	0.005 (0.004,0.006)	0.001 (0.000,0.002)
Gender (male vs. female)	0.023 (−0.004,0.049)	0.024 (−0.003,0.050)	0.005 (−0.013,0.022)
MedDietScore (per 1 unit out of 55)	−0.003 (−0.007,0.000)	−0.003 (−0.006,0.000)	−0.002 (−0.004,0.000)
Body Mass Index (per 1 kg/m^2)	0.001 (−0.002,0.004)	0.001 (−0.002,0.004)	0.000 (−0.002,0.002)
Smoking (yes vs. no)	0.040 (0.013,0.068)	0.043 (0.016,0.071)	−0.012 (−0.028,0.005)
Hypertension (yes vs. no)	-	0.037 (0.004,0.071)	0.004 (−0.015,0.023)
Diabetes (yes vs. no)	-	−0.020 (−0.071,0.031)	0.004 (−0.025,0.033)
Hypercholesterolemia (yes vs. no)	-	-0.034 (−0.061,-0.006)	−0.012 (−0.029,0.005)
Glomerular filtration rate (per 1 mL/min)	-	-	−0.007 (−0.007,−0.006)
Albumin (per 1 g/dL)	-	-	−0.023 (−0.055,0.010)
Ferritin (per 1 ng/dL)	-	-	0.000 (0.000,0.000)

Betas estimated through linear regression models

DISCUSSION

In this study, we observed an inverse relationship between adherence to the Mediterranean diet and serum cystatin C levels after adjustment for age, gender, body mass index, smoking habits, hypertension, diabetes, and hypercholesterolemia. In other words, adherence to the Mediterranean diet was associated with lower serum cystatin C levels.

Cystatin C is produced by all nucleated cells at a constant rate, and it is excreted by the kidneys by free glomerular filtration and complete tubular reabsorption and degradation (13, 14). Therefore, serum concentration levels of cystatin C are almost totally dependent on the glomerular filtration rate and even a slight reduction in glomerular filtration rate causes a rise in serum cystatin C (15, 16). Besides its usefulness as a marker of renal function, serum cystatin C appears to be a prognostic marker of cardiovascular events and death among elderly persons without chronic kidney disease (17, 18). In recent years, cystatin C has emerged as a potential marker for cardiovascular risk (18, 19). A plausible link between increased cystatin C concentrations and adverse cardiovascular outcome apart from renal failure could be chronic inflammation such as atherosclerosis. Indeed, high serum cystatin C concentrations have been documented to be associated with high concentrations of hs-CRP and monocytes count in blood (20, 21). In fact, the monocyte count in blood has been found to be a better cross-sectional marker of plaque presence than hs-CRP, interleukin-6 and WBC (22). Monocyte count has recently been demonstrated to be an independent predictor of future plaque formation (23). There is evidence that elastolytic cysteine proteases and their inhibitors, an important one being cystatin C, are involved in the pathogenesis of atherosclerosis. Inflammatory cytokines associated with atherosclerosis stimulate the production of lysosomal cathepsins, and increased plasma concentrations of cystatin C, a cathepsin inhibitor, may reflect an attempt to counterbalance a potentially damaging increased elastolytic activity. Studies have reported that human cathepsins are expressed in endothelial cells, smooth muscle cells, and macrophages, and that they are involved in the composition, the progression and the rupture of atherosclerotic plaques (19, 24).

On the other hand, the MedDietScore (0–55) is a means to evaluate adherence to the Mediterranean diet. Higher values on the score indicate greater adherence to this pattern and, consequently, healthier dietary habits (11). Diet is considered an essential perquisite of prevention, as its impact on the development of coronary heart disease has been demonstrated by many studies so far (25, 26). Moreover, the athero-protective role of the Mediterranean diet is not questioned (27, 28).

Our finding that increases in the MedDietScore are associated with decreases in serum cystatin C levels could imply that adherence to the Mediterranean diet may reduce the cardiovascular risk, as assessed by cystatin C, a prognostic marker of the cardiometabolic risk. This inverse relationship which remains significant even after adjustment for age, gender, body mass index, smoking habits, hypertension, diabetes, hypercholesterolemia, GFR, serum albumin and serum ferritin levels could have a great impact on public health. The notion that adherence to the Mediterranean diet could help to ameliorate public health in respect of cardiovascular risk, is of the utmost importance. It seems likely that we can do more to decrease our cardiometabolic risk than we have ever imagined.

CONCLUSION

In conclusion, we have demonstrated an inverse relationship regarding the MedDietScore and serum cystatin C levels. In other words, adherence to the Mediterranean Diet could be a simple and inexpensive method to decrease the cardiovascular risk estimated by serum cystatin C levels. Further large-scale studies are needed to confirm this inverse relationship which could promote public health.

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Conflict of Interests

None declared

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