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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

CARDIOVASCULAR RISK FACTORS IN TURKISH IMMIGRANTS WITH TYPE 2 DIABETES LIVING IN THE NETHERLANDS

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PUBLIC HEALTH

by

Shiryn D. Sukhram

2012

To: Interim Dean Michele Ciccazzo
Robert Stempel College of Public Health and Social Work

This dissertation, written by Shiryn D. Sukhram, and entitled Cardiovascular risk factors in Turkish immigrants with type 2 diabetes living in The Netherlands, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: March 23, 2012

The dissertation of Shiryn D. Sukhram is approved.

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Interim Dean Michele Ciccazzo
Robert Stempel College of Public Health and Social Work

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Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2012

DEDICATION

I dedicate this dissertation to my parents. I owe my deepest gratitude to my father, who never stopped believing in me, gave me all means necessary to succeed in life, and always supported me in all my endeavors. My mother's unconditional love and confidence has helped me overcome many barriers in life. I could not be where I am today without her support and sacrifice.

I also dedicate this dissertation to my brother who endlessly inspires me to persevere to succeed – his influence has immensely impacted and directed my life.

****

"The world steps aside and all gates are open to the person who knows where he or she is going to" R.R. Sukhram

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ABSTRACT OF THE DISSERTATION

CARDIOVASCULAR RISK FACTORS IN TURKISH IMMIGRANTS WITH TYPE 2 DIABETES LIVING IN THE NETHERLANDS.

by

Shiryn D. Sukhram

Florida International University, 2012

Miami, Florida

Professor Fatma Huffman, Major Professor

The cross sectional study investigated the association of tobacco smoke, vitamin D status, anthropometric parameters, and kidney function in Turkish immigrants with type 2 diabetes (T2D) living in the Netherlands. Study sample included a total of 110 participants aged 30 years and older (males= 46; females= 64). Serum cotinine, a biomarker for smoke exposure, was measured with a solid-phase competitive chemiluminescent immunoassay. Serum 25-hydroxyvitamin D [25(OH)D] was determined by electrochemiluminescence immunoassay (ECLIA). Measures of obesity including: body weight, body mass index (BMI), waist circumference (WC), and hip circumference (HC) were measured. Waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated. Urine albumin was measured by immunoturbidimetric assay. Urine creatinine was determined using the Jaffe method. All statistical analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA). Independent samples t-test, chi-squared tests, multiple linear regression and logistic regression analysis were used.

Cotinine levels were positively associated with cholesterol to HDL ratio and atherosclerosis-index. Serum 25(OH)D levels were negatively associated with diastolic blood pressure. Gender-specific associations between anthropometric measures and high sensitivity C-reactive protein (hs-CRP) levels were observed. Hs-CRP was positively associated with WC and WHR in males and WHtR in females. Microalbuminuria (MAU), as determined by albumin-to-creatinine ratio, was present in 21% of the Turkish immigrants with T2D. Participants with hypertension were 6.58 times more likely (adjusted odds ratio) to have positive MAU as compared to normotensive participants. Our findings indicate that serum cotinine, 25(OH)D, hs-CRP, and MAU may be assessed as a standard of care for T2D management in the Turkish immigrant population. Further research should be conducted following cohorts to determine the effects of these biomarkers on CVD morbidity and mortality.

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1. INTRODUCTION

Type 2 diabetes (T2D) is highly prevalent among ethnic minorities living in Western societies. In the Netherlands (52˚N), Turkish immigrants form the largest ethnic minority group with 388,967 inhabitants according to 2011 census. The Hague, a city that is part of the Randstad metropolitan area, is vastly urbanized with a large population of non-Western immigrants. Research indicates that the onset of diabetes occurs one decade earlier among the Turkish when compared to the indigenous Dutch. Determinants of T2D in high-risk groups require further research. Mortality from coronary heart disease in people with T2D is higher than in the general population. Common risk factors for cardiovascular disease (CVD) include ethnicity, tobacco use, abnormal blood lipid levels, obesity, and hypertension (HTN). Newer risk factors for CVD include low levels of vitamin D and elevated levels of C-reactive protein (CRP). Given the scarcity of studies available among Turkish immigrants living in the Netherlands, it is important to examine indicators of modifiable health risks within this population.

Lower levels of vitamin D are associated with several CVD risk factors and the prevalence of CVD (Martins et al., 2007; Kendrick, Targher, Smits, & Chonchol, 2009). Tuorkey and Abdul-Aziza (2010) indicate that low blood levels of vitamin D interfere with the proper functioning of insulin-producing beta-cells thereby suggesting a strong inverse association between T2D and vitamin D (Tuorkey & Abdul-Aziza, 2010). Facchini et al. (1992) have shown that chronic tobacco smokers are insulin resistant, hyperinsulinaemic, and dyslipidaemic when compared to nonsmokers consequently increasing CVD risk among smokers. Smoking and increased blood pressure (BP) levels also increase CVD risk accounting for more than 20% of the global premature death

(Nakamura et al., 2008; WHO, 2002). Pickup (2004) suggests T2D to be a state of low-grade inflammation. Various studies report elevated serum C-reactive protein (CRP) levels to be a reliable predictor of CVD and CVD risk factors (Soinio et al., 2006; Ujcic-Voortman, Baan, Verhoeff, Krol, & Seidell, 2011; Onat, Sansoy, Yildirim, Keleş, Uysal, & Hergenç, 2001; Onat, Can, & Hergenç, 2008). Chronic kidney disease is also a significant risk factor for cardiovascular morbidity and death (Eknoyan, 2004). Microalbuminuria (MAU) is a widely known predictor of diabetic nephropathy, HTN, and CVD (Keane et al., 2003; Ibsen, Olsen, Wachtell, Borch-Johnsen, Lindholm, & Mogensen, 2008).

1.1 Specific Aims and Hypothesis

Research indicates a higher prevalence of type 2 diabetes (T2D) and cardiovascular disease (CVD) among the Turkish living in the Netherlands as compared to the native Dutch. Thus, we have sufficient evidence providing the rationale for our testable hypothesis that T2D in Turkish immigrants significantly contribute to the increased prevalence of CVD risk. Examining the factors reported that affect CVD risk for ethnicities with a high prevalence of T2D would provide important insights into their diabetes care. Given the limited available studies in this subject for Turkish immigrants with T2D living in The Netherlands, the proposed study was of an exploratory nature. Therefore, the main objective of this research was to investigate the risk factors for CVD in immigrants with T2D.

To test our central hypothesis, the following inter-related specific aims were formulated:

Specific Aim 1: To determine the association between smoke exposure, serum lipids and blood pressure in Turkish immigrants with T2D.

Hypothesis 1a: Smoke exposure will be positively associated with symptoms of atherosclerosis; total cholesterol, LDL, and triglycerides in Turkish immigrants with T2D.

Rationale: Atherogenic ratios, including the ratio of total cholesterol to HDL, demonstrate that the risk for developing atherosclerosis is significantly higher among smokers than non-smokers (Venkatesan, Hemalatha, Bobby, Selvaraj, & Sathiyapriya, 2006). Gossett, et al. (2009) reported that the greater the number of cigarettes smoked daily the higher the levels of total cholesterol, low-density lipoprotein, and triglycerides.

Methodology: Smoke exposure was determined by measuring serum cotinine with a solid-phase competitive chemiluminescent immunoassay. Serum lipids were determined by enzymatic assay and included total cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG). Total cholesterol to HDL ratio (CHOL:HDL) and atherosclerosis-index (AI) were calculated.

Hypothesis 1b: Smoke exposure will be positively associated with blood pressure in Turkish immigrants with T2D.

Rationale: Several studies report an association between smoking and increased blood pressure (John, Meyer, Hanke, Volzke, & Schumann, 2006; Virdis, Giannarelli, Neves, Taddei, & Ghiadoni, 2010). Therefore it was essential to see if this held true in Turkish immigrants with T2D.

Methodology: Hypothesis 1a details methodology for smoke exposure. Blood pressure was determined by measuring systolic blood pressure (SBP) and diastolic blood pressure (DBP) using an automated office blood pressure equipment.

Specific Aim 2: To determine the association between vitamin D status, serum lipids and blood pressure in Turkish immigrants with T2D.

Hypothesis 2a: Serum vitamin D will be positively associated with triglycerides, HDL, and LDL in Turkish immigrants with T2D.

Rationale: Findings indicate that there was a significant increase in serum CHOL, HDL and LDL, and a significant decrease in serum LDL to HDL ratio across increasing serum 25(OH)D quartiles (Jorde, Figenschau, Hutchinson, Emaus, & Grimnes, 2010).

Methodology: Hypothesis 1a details methodology for serum lipids. Hypothesis 1b details methodology for blood pressure. Vitamin D status was determined by measuring serum 25-hydroxyvitamin D by electrochemiluminescence immunoassay (ECLIA).

Hypothesis 2b: Serum vitamin D will be negatively associated with blood pressure in Turkish immigrants with T2D.

Rationale: Findings indicate that higher vitamin D status is associated with lower blood pressure and reduced incidence of hypertension (Martini & Wood, 2008).

Methodology: Hypothesis 1a details methodology for serum lipids. Hypothesis 2a details methodology for vitamin D status.

Hypothesis 2c: Serum vitamin D will be negatively associated with smoke exposure in Turkish immigrants with T2D.

Rationale: Tobacco smoking has a significant negative effect on vitamin D metabolism. The greater the smoking the lower the serum levels of 25(OH)D (Brot, Jorgensen, Sorensen, 1999).

Methodology: Hypothesis 1a details methodology for smoke exposure. Hypothesis 2a details methodology for vitamin D status.

Specific Aim 3: To determine the association between measures of obesity and hs-CRP levels in Turkish immigrants with T2D.

Hypothesis 3a: Turkish females with T2D will have higher abdominal obesity and higher hs-CRP levels as compared to Turkish males with T2D.

Rationale: Abdominal obesity was an indicator of elevated CRP levels in Turkish middle-aged females (Onat, 2001; Shen, Farrell, Penedo, Schneiderman, & Orth-Gomer, 2010). Other studies have also established a relationship between obesity and CRP levels.

Methodology: Height and weight were determined by using a wall-mounted stadiometer and a digital electronic scale. Waist circumference (WC) and hip circumference (HC) were measured with a non-stretchable measuring tape. Body mass index (weight divided by height), waist-to-hip ratio (WC divided by HC) and waist-to-height ratio (WC in kg divided by height in m^2) were calculated. Serum hs-CRP levels were measured with immunoturbidimetric assay.

Specific Aim 4: To determine the association between kidney function and hypertension in Turkish immigrants with T2D.

Hypothesis 4a: Microalbumineria (MAU) will be positively associated with hypertension in immigrants with T2D.

Rationale: Turkish individuals with hypertension, diabetes, or a combination of the two conditions have an increased prevalence of MAU (Col, Ocaktan, Ozdemir, Yalcin, & Tuncbilek, 200). Therefore this was examined for Turkish immigrants.

Methodology: Fresh, single-voided, first morning urine samples were collected from each participant to determine MAU by immunoturbidimetric assay. Urinary creatinine was measured by a modified Jaffe rate calorimetry method. Hypertension was defined as: SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg or use of hypertension medications. The albumin to creatinine ratio (ACR) was calculated to determine the presence of MAU and defined as ACR $\geq$ 3.5 mg/mmol for females or ACR $\geq$ 2.5 mg/mmol for males (Jerums & MacIsaac, 2002).

1.2 Significance of the Proposed Research

This research provides important insights into the possible role of successful diabetes management that could contribute to the overall reduction in the number of comorbid conditions and deaths due to complications of this disorder. The right treatment decisions based on clinical evidence mean, appropriate successful long-term management of T2D among the general population. Positive results may help identify Turkish immigrants at high-risk for CVD and could add to the information important for novel therapeutic options. This study will add to our understanding of T2D as well as other major preventable public health concerns in a vulnerable population.

2. REVIEW OF LITERATURE

2.1 Serum cotinine, vitamin D and cardiovascular disease

Cardiovascular disease (CVD) is a significant health problem worldwide, causing over 17 million deaths in 2008 (World Health Organization, 2012). Among the many risk factors for CVD, smoking and vitamin D deficiency (defined as serum levels less than 25 nmol/L) have arisen as significant risk factors. In an attempt to better understand how to mitigate the risk for CVD, research has focused on the impact of both of these risk factors on serum lipids and blood pressure (BP). Studies have examined the link between smoking and serum levels of various lipids, including total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Other studies have examined the association between these lipids and vitamin D, measured by serum 25(OH)D, with much less encouraging findings. Smoking and vitamin D may also be related to changes in BP, both systolic blood pressure (SBP) and diastolic blood pressure (DBP). Such changes are important to understand due to the relationship between hypertension (HTN) and CVD.

Biochemical markers of tobacco exposure can provide reliable information pertaining to the smoking status of an individual. Cotinine, a by-product of nicotine and a well-known marker for smoking status, has demonstrated efficacy in distinguishing smokers from non-smokers based on serum concentrations of this marker. Another biomarker, 25(OH)D, is useful in assessing the circulating levels of vitamin D in serum, which may be obtained through supplementation or sunlight exposure. The following literature review discusses the relationships among smoking, vitamin D levels, serum

lipids, and BP as determined in part by measurements of these biomarkers in study subjects.

2.1.1 Serum Cotinine and Serum Lipids

Cigarette smoking is associated with a variety of CVD. These diseases include stroke, aortic aneurysm, atherosclerosis, and heart failure and are the result of damage to the heart and blood vessels. Active smoking may result in anatomical and biochemical changes to the coronary arteries and the myocardium, thus leading to disease. Changes to the cardiovascular system components may be assessed by studying a variety of diagnostic markers, one of which is cotinine. This marker, a metabolite of nicotine, is a specific and highly representative parameter for exposure to tobacco smoke. The chronic effects of cotinine on the cardiovascular system include changes to both HDL and LDL levels, changes in BP, and increased platelet adhesiveness, all of which play a role in the initiation of CVD (Leone, 2005).

Research suggests that an association exists between smoking and serum lipid concentrations. In one study of healthy smokers and nonsmokers, researchers assessed the differences between these two groups with regards to levels of serum CHOL, TG, HDL, LDL, VLDL, and non-HDL cholesterol. Results indicated that although no statistically significant differences existed in the lipid profiles of smokers versus non-smokers with regards to TG and HDL, smokers demonstrated significantly higher levels of CHOL, LDL, and non-HDL cholesterol. Atherogenic ratios based on these results, including the ratio of CHOL to HDL (CHOL:HDL) and the ratio of non-HDL to HDL, demonstrate that the risk for developing atherosclerosis is significantly higher among

smokers than non-smokers (Venkatesan, Hemalatha, Bobby, Selvaraj, & Sathiyapriya, 2006).

Other studies have demonstrated a link between smoking and TG, HDL, and other serum lipids. In contrast to the Venkatesan, et al. (2006) study, which demonstrated no link between smoking and TG or HDL levels, Gossett, et al. (2009) reported that the greater the number of cigarettes smoked daily, the higher the levels of CHOL, TG, and LDL. Additionally, higher levels of carbon monoxide, a byproduct of cigarette smoke, were significantly associated with lower levels of HDL. Although these two studies reported conflicting results with regards to the relationship between smoking and TG and HDL levels, it should be noted that the small sample size used in the Venkatesan, et al. (2006) study may translate into questionable validity of the results. However, Gossett, et al. (2009) utilized over 1,500 subjects, and therefore their results may be more valid and generalizable.

The association between smoking and serum lipids is particularly significant among the Turkish adult population, as up to 73% and 42% of Turkish males and females, respectively, smoke cigarettes. Authors of the landmark study, the Turkish Heart Study, reported that levels of some serum lipids, lipoproteins, and apolipoproteins raised important health concerns regarding the potential risk of premature coronary artery atherosclerosis. Generally, this population of individuals possessed CHOL levels that were not significantly higher than those of other populations; however, levels of TG were notably higher. In addition, levels of HDL were low when compared to other populations. This, in combination with elevated TG, contributes to an increased risk for CVD among the Turkish population (Mahley, et al., 1995).

Cigarette smoking significantly correlated with the concentration of several serum lipids in the Turkish Heart study. Researchers reported a relationship between the number of cigarettes smoked daily among men and increased CHOL, TG, and LDL levels. Furthermore, the heaviest male smokers demonstrated the lowest levels of HDL. Among women smokers, particularly those smoking 10 to 20 cigarettes daily, CHOL and LDL levels were higher and HDL levels lower than in non-smoking Turkish women. Taken together, these results suggest that smoking modulates lipid values and serves as a risk factor for CVD (Mahley, et al., 1995).

Research suggests that these detrimental results of cigarette smoking, and correspondingly, cotinine levels, may be reversible on smoking cessation. Lee, et al. (2011) investigated the impact of smoking abstinence through both self-report and cotinine levels on changes in blood glucose and lipid profiles. Smoking cessation resulted in a significant decrease in LDL levels. However, no effects were observed on CHOL, TG, or HDL.

2.1.2 Serum Cotinine and Blood Pressure

Smoking and HTN both serve as independent risk factors for CVD. Research indicates that statistically significant associations exist between SBP and DBP levels and the incidence of mortality due to CVD. Similarly, smoking increases mortality due to CVD (Ellekjaer, Holmen, & Vatten, 2001). One potential link between these two separate factors is their impact on the aortic wall distensibility (AD). Research indicates that both HTN and smoking significantly correlate with reduced AD, or a more rigid aortic wall. This characteristic, in conjunction with aortic wall thickness, may be related to increased

risk for CVD (Malayeri, et al., 2008). However, in spite of the impact of both smoking and HTN on CVD risk, the effects of smoking on BP remain unclear.

Although there is a link between smoking and CVD, as well as between BP and CVD, there is a lack of strong evidence to support a significant relationship between smoking and increased BP. Some studies report an association between the two variables (John, Meyer, Hanke, Volzke, & Schumann, 2006; Virdis, Giannarelli, Neves, Taddei, & Ghiadoni, 2010). A variety of other studies have attempted to further elucidate this link among different adult populations. For example, Li, Tong, Wang, Lin, and Zhang (2010) investigated the effects of smoking on SBP and DBP in the adult Chinese population. Results indicated that both SBP and DBP were significantly lower among those who smoked between 10 to 19 cigarettes daily than in nonsmokers. No statistically significant differences in BP were detected between nonsmokers and those who smoked greater than 20 cigarettes daily. Among the adult Turkish population, smoking is not associated with increased SBP. In fact, individuals within this population who also smoke actually possess lower SBP than nonsmokers. One possible reason for this finding is the ability of nicotine to stimulate angiogenesis, or the formation of new blood vessels (Can, Schwandt, Onat, Hergenc, & Haas, 2009).

Similar results were found among smokers in the Indian population. Kusuma, Babu, and Naidu (2008) investigated the relationship between BP and smoking. After adjusting the data for the variables of age and anthropometric measurements, the researchers found that while women generally possessed higher levels of both DBP and SBP, male smokers had lower SBP levels than nonsmokers. However, any associations noted between smoking and increased BP were not statistically significant.

While smoking may not result in increased BP, smoking cessation might. Researchers from one longitudinal study of females who smoked, quit smoking, or never smoked investigated the differences in BP at baseline and after nine years. The increases in both SBP and DBP were greater among those who quit smoking than in nonsmokers or those who continued to smoke. Furthermore, among the three groups, the incidence of HTN was greatest among those who quit smoking (Janzon, Hedblad, Berglund, & Engstrom, 2004).

Limited research exists regarding the impact of smoking on lipid levels and its role in CVD. Studies have reported that smoking is associated with increased CHOL, TG, LDL, and non-HDL cholesterol as well as decreased HDL. This holds true for the adult Turkish population, which has a high percentage of smokers. Furthermore, research supports the association between inhaled tobacco smoke and atherosclerosis. However, a firm link between smoking and HTN as they relate to CVD is not yet established. Some research studies have reported lower BP levels among smokers than non-smokers, while others have reported no significant correlation between the two variables.

2.1.3 Serum Vitamin D and Serum Lipids

Vitamin D, a collection of fat-soluble steroids, may be acquired through sunlight exposure or supplementation and is associated with cardiovascular health. A deficiency in vitamin D is associated with CVD. Low levels of vitamin D may be responsible for the development of atherosclerosis and the dysfunction of endothelial cells, which are the cells that line the body's blood vessels (McGreevy & Williams, 2011). Low levels of circulating 25(OH)D, or vitamin D, have been implicated in several risk factors for

atherosclerosis, including type 2 diabetes (T2D), obesity, HTN, and dyslipidemia (De Boer, et al., 2009). Supplementation with vitamin D is associated with an overall decrease in CVD risk among men, but not women (Sun, et al., 2011).

Research appears unclear as to the exact nature of the relationship between vitamin D and lipids as they relate to CVD risk. In one longitudinal study of vitamin D and calcium supplementation and its role in lipid concentrations, Rajpathak, et al. (2011) determined any changes in CHOL, TG, LDL, HDL, non-HDL cholesterol, and lipoprotein(a) among over 1,200 postmenopausal women. Results indicated that supplementation with vitamin D and calcium was associated with small and non-significant changes in each of the lipids. Similarly, another study investigating the effects of vitamin D supplementation on lipid concentrations, among men, found no association between the two. Using vitamin D3 fortified milk as the means for vitamin intake, researchers reported no statistically significant differences between the experimental and control groups after supplementation with regards to CHOL, TG, HDL, or LDL (Daily & Nowson, 2009).

Similar results hold true for ethnic groups other than Caucasians. Andersen, et al. (2009) examined the impact of vitamin D supplementation on the lipid profiles of Pakistani immigrants. Results failed to indicate any significant changes in CHOL, HDL, or LDL after supplementation with 10ug or 20ug vitamin D for one year. Lipid profiles and levels of 25(OH)D obtained from 380 Malaysian men and women revealed a slight but significant relationship between low vitamin D levels and increased TG. However, no significant association was found between vitamin D levels and HDL (Moy & Bulgiba, 2011).

On the other hand some studies reported a link between vitamin D and lipid levels. For example, Rejnmark, Vestergaard, Heickendorff, and Mosekilde (2010) conducted a randomized, controlled trial to study the effects of vitamin D status on women treated with simvastatin, a drug designed to increase bone mineral density. Lipid profiles from these subjects indicated that an association existed between increased vitamin D levels and lower levels of TG as well as small increases in HDL levels. A second cross-sectional study of over 10,000 Norwegian men and women compared vitamin D levels to lipid profiles. Results from this study indicated that as serum levels of vitamin D increased, corresponding increases were noted in serum levels of TG, HDL, and LDL. Significant decreases were also observed in the ratio between LDL and HDL. The authors speculated that these findings, which accumulated over the course of 14 years, could explain the increased mortality rate among individuals with low levels of vitamin D (Jorde, Figenschau, Hutchinson, Emaus, & Grimnes, 2010).

2.1.4 Serum Vitamin D and Blood Pressure

While the evidence supporting a link between vitamin D and lipid concentrations is conflicting, most studies indicate a link between vitamin D and BP. Daily and Nowson (2009) failed to find a link between vitamin D supplementation and BP changes in adult males. Other research speaks to the contrary. Epidemiological studies indicate that vitamin D concentration is associated with lower BP and reduced incidence of HTN. Analysis of over 12,000 individuals reported that mean SBP and DBP were lowest among individuals categorized into the highest quintile of vitamin D level. This is significant in that even a slight decrease in BP, as little as 2 mmHg, is associated with up to a 15%

decline in CVD risk. Research suggests that the amount of vitamin D needed to achieve this decrease in BP is an increase in serum vitamin D from 20 to 100 nmol/L (Martini & Wood, 2008).

In contrast to the benefits of increasing vitamin D levels, poor vitamin D status may be related to increased risk of HTN. Research suggests that the prevalence of HTN is highest among individuals with the lowest serum vitamin D concentrations. Furthermore, after adjusting for confounding factors such as family history of HTN, smoking status, and intake of vitamin D, researchers in another study found that vitamin D deficient men experienced a six-fold increase in the risk of developing HTN over men with adequate vitamin D levels (Martini & Wood, 2008).

Another large cross-sectional study, based on data obtained from the National Health and Nutrition Examination Survey (NHANES), attempted to further characterize the impact of vitamin D on BP. Blood pressure was classified to six categories, ranging from normotensive, with a SBP less than 110 mmHg, to stage 2 HTN, with a SBP exceeding 160 mmHg. Researchers then determined the serum vitamin D levels of individuals in the study and ranked them according to BP level. Results indicated that a statistically significant and inverse relationship existed between the two variables in both men and women. Individuals with deficient or insufficient vitamin D levels experienced a significantly greater increase in SBP over those who had sufficient levels of vitamin D. The researchers in this study concluded that although the evidence pointed to a notable association between 25(OH)D and SBP, determining the exact nature of the relationship may prove difficult, as vitamin D deficiencies are very common in the United States (Judd, Nanes, Ziegler, Wilson, & Tangpricha, 2008).

Evidence for this association also arises from a systematic review of the literature pertaining to vitamin D and the risk for cardiometabolic outcomes. Pittas, et al. (2010) conducted a review of 13 observational studies and 18 clinical trials. Findings from this review revealed that lower 25(OH)D concentrations were associated with an increased risk of HTN, with an adjusted odds ratio of 1.8. Analysis of a group of 10 clinical trials revealed that vitamin D supplementation reduced systolic blood pressure by 1.9 mmHg, an amount close to that observed to reduce the risk of CVD by as much as 15%. Diastolic blood pressure demonstrated no significant effect due to vitamin D supplementation. Finally, the authors reported that a lower serum concentration of vitamin D was associated with an increased risk of CVD in five of seven analyses performed.

The volume of data pertaining to the impact of vitamin D levels on serum lipids is limited. Various studies report that different types of lipids are impacted, and some studies report no association at all between vitamin D and serum lipids. For these reasons, further research is warranted to better understand the role of 25(OH)D in blood lipid concentrations in different populations. The research evidence pertaining to the link between vitamin D and BP, particularly SBP, is strong. Most notably, deficiencies in this nutrient are associated with increased BP and increased risk for CVD. Environmental influences including smoking and vitamin D need to be further investigated especially among minorities. The early detection of low 25(OH)D levels among the Turkish population is fundamental in preventing and treating diabetes complications and cardiovascular outcomes. Adding cotinine and 25(OH)D testing to standard diabetes care may be used to determine the potential risks for CVD and may direct management in the primary prevention of CVD.

2.2 C-Reactive protein

Elevated levels of C-reactive protein (CRP) is a response mechanism to low-grade systemic inflammation, by major trauma, or infection. This member of acute phase reactants is produced in the liver as well as in the coronary vessels and seems to play a role in determining the incidence and predictability of CVD. CRP levels differ between those individuals with lean body mass and athleticism, and those with risk factors such as smoking, obesity without exercise, high CHOL levels or HTN (Folsom, Golden, Boland, & Szklo, 2005). CRP is determined by a simple blood test, as measured by high-sensitivity CRP. Elevated CRP levels have been determinants to possible CVD incidence in even apparently healthy individuals, as well as those with T2D, HTN and other CVD risk factors (CRP Health.com, 2011).

CRP has been found to be a vital pathogenic mechanism in the development and progression of atherosclerosis and is exacerbated by obesity, a CVD risk factor (Straczek, et al., 2010; Stenholm, Rantanen, Heliovaara, & Koskinen, 2008). Additional research needs to expand the various populations studied to achieve a more complete picture of all available data with regard to ethnic differences.

2.2.1 Epidemiological studies

Several epidemiological studies found a correlation between CVD risk factors and elevated CRP. There are significant associations between obesity and increased circulating serum CRP levels in persons with obesity-related diseases (Onat, Sansoy, Yildirim, Keleş, Uysal & Hergenç, 2001; Huffman, Gomez, & Zarini, 2009; Huffman, Whisner, Zarini, & Nath, 2010). There is a higher prevalence of T2D, obesity, and

elevated serum CRP levels among Turkish immigrants living in the Netherlands when compared to the indigenous Dutch (Uitewaal, Goudswaard, Ubink-Veltmaat, Bruijnzeels, Hoes, & Thomas, 2004(a); Ujcic-Voortman et al., 2011; Uitewaal, Manna, Bruijnzeels, Hoes, & Thomas, 2004(b); Kriegsman, van Langen, Valk, Stalman, & Boeke, 2003; van Leest, van Dis, & Verschuren, 2002). Studies conducted globally have correlated obesity, attitudinal preceptions, environmental, social and cultural factors in the prediction of CVD. Among these studies are ones that have focused on both the Turkish general population and those that have used CRP as predictors of the development of CVD among Turkish children and adolescents (Kurtoglu et al., 2010). Other studies have been conducted to determine the effect of elevated CRP levels on the obese, those with low grade chronic inflammation, differences between genders, aging populations and other groups at risk for CVD (Erdogan et al., 2009; Hoth et al., 2008; Thomson et al., 2009). These studies have all contributed to the assumption that CRP is a direct indicator of CVD and may be used to diagnose coronary events in both healthy individuals as well as those manifesting CVD risk factors.

Altan Onat (2001) conducted an extensive study on CVD risk factors on Turkish adults and concluded that the "predictors of coronary events and death [SBP, CHOL:HDL, followed by diabetes and (central) obesity] are related to the metabolic syndrome …" (p. 1). He further indicated that obesity and CRP were inextricably interlinked and affective towards predicting CVD events.

2.2.2 Gender differences

Gender differences between males and females presenting CVD were discussed and studied by several researchers. Females tend to have a higher percentage of body fat, than do males, regardless of equivalent amounts of liver and intra-abdominal fat (Westerbacka et al., 2004). There is a higher prevalence of obesity among Turkish females as compared to their male counterparts (Uitewaal et al., 2004(b); van Leest et al., 2002). A prospective cohort of the Turkish Adult Risk Factor (TARF) study, which included 1046 Turkish adults, found CRP levels to be greater for females than for males (Onat et al., 2001). Deterioration of women's health increased their susceptibility to elevated CRP and CVD events (Kim et al., 2006). Gültekin, Akin, and Ozer (2005) compared gender differences that would later affect health patterns in adult life. Researchers showed that at a young age, there is clear evidence of sexual dimorphism in fat patterning with girls having greater subcutaneous adiposity. Greenfield et al. (2004) determined that despite genetic influences, CRP was "strongly related to total and central abdominal obesity, BP, and lipid levels," and were contributory factors to associating both CRP and T2D.

2.2.3 C-Reactive protein and anthropometric measures

Various mechanisms have been suggested as to which indicator of obesity best predicts CVD risk and the best method to identify CRP has yet to be determined. Connelly, Hanley, Harris, Hegele, and Zinman (2003) showed that body mass index (BMI) was an independent determinant of CRP level in females, whereas waist circumference (WC) was an independent determinant of CRP level in males. Mojiminiyi,

Al Mulla, and Abdella (2009) found that waist-to-hip ratio (WHR) showed the weakest correlations with CRP levels, and reported that BMI was most strongly associated with CRP levels in individuals with T2D. Can et al. (2009) suggest that the inclusion of either hip circumference (HC) or height into WC may offer additional information on CVD risk than WC alone. Other studies indicate that waist-to-height ratio (WHtR) is a better predictor of CVD than the more widely used BMI (Can et al., 2010; Schneider et al., 2010). Schneider et al. (2010) and de Koning, Merchant, Pogue, and Anand (2007) suggest that anthropometric measures of abdominal obesity are a better indicator of CVD than BMI.

Waist circumference (WC) in the central abdominal region is an indicator of elevated CRP as well as prospective CVD events. Folsom, Golden, Boland, and Szklo (2005) found increased adipose tissue was associated with increased CRP levels and fibrinogen. Onat (2001) found that WC and fibrinogen raised CRP levels in women and was a risk factor in CVD and coronary events. The correlation observed between fibrinogen, CRP and CVD was reiterated in the study conducted by Kim et al. (2006).

Findings indicate a strong association between BMI and CRP levels in individuals with T2D (Mojiminiyi et al., 2009). Body Mass Index (BMI) was the focal point of a cross-sectional study done on Turkish children. Findings indicate that unlike children from lower socio-economic groups in other countries, Turkish children were prone to be more obese as a result of a higher economic level (Discigil, Tekin, & Soylemez, 2008). Interestingly, in Turkey, being larger as a child is perceived by Turkish mothers as being advantageous (Esenay, Yigit, & Erdogan, 2010).

Hip circumference (HC) is an indicator of obesity and CRP levels. Gentile et al., (2010) found that Mediterranean women were highly susceptible to being overweight, consumed many carbohydrates and their HC reflected both their genetic propensity and dietary habits predisposing them to obesity and elevated CRP. As a result, this increased their risk of CVD and coronary events.

Waist-to-hip ratio (WHR) is linked to elevated CRP and insulin resistance (Gillum, 2003). Gillum (2003) indicated WHR to be significant in 11% of the study population and showed significant positive correlations with serum CRP. In children this ratio was indicative of future CVD and other health issues such as diabetes and insulin resistance. The low-grade inflammation associated with CRP could introduce other health issues in the future. However, other studies found WHR to show the weakest correlation with CRP level in patients with T2D. Researchers found measures of total body fat to be more strongly correlated with CRP levels than measures of fat distribution including the WHR (Connelly et al., 2003).

Waist-to-height ratio (WHtR) was studied and analyzed by Gillum (2003) as well as Knowles, et al., (2011). These studies found that in both children and adults, presence of obesity and metabolic syndrome were major risk factors for CVD and that WHtR was predictive of CVD in obese individuals. Waist-to-height ratios were one of the best predictors, among Peruvian adults, of metabolic syndrome and elevated CRP levels (Knowles, et al., 2011). Results from the Turkish Heart Study suggest that WHtR is a better predictor of CVD risk as compared to BMI, WC, and WHR (Can et al., 2009). Schneider et al. (2010) observed similar findings, where WHtR was the strongest predictor of CVD risk and mortality and discouraged the use of BMI.

2.2.4 Summary

Within the wide range of studies reviewed, there seems to be a consensus that obesity is a key determinant of CRP levels. The dichotomy that still exists is that there is a lack of specific evidence as to which indicator of obesity best predicts CVD risk. Along with inconsistencies of appropriate obesity measurements in association with CRP levels, there are limited studies available concerning the association of CVD risk factors with CRP levels in a Turkish immigrant population with T2D living in the Netherlands (Ujcic-Voortman et al., 2011). Childhood obesity indicates that the propensity for adult obesity is present while genetic indicators, BMI, disease related inflammation caused by T2D or even various endo-or-ectomorphic body types are all contributory factors. Further studies and analyses are needed to determine the exact correlation between CRP and obesity. As it stands, such conclusions are regarded with skepticism, in relation to the correlation between adiposity measurements, various waist ratios and broad measures of epidemiological studies. Populations with different demographic attributes and nutritional requirements need to be examined to verify if these findings are appropriate measures for different population groups.

2.3 Microalbuminuria

Microalbuminuria (MAU) plays a key role in the link between diabetes and CVD, although research has yet to clarify the exact mechanism underlying its influence. Microalbuminuria is an important early indicator for diabetic nephropathy and a significant risk factor for progression to proteinuria, end stage renal disease (ESRD), and mortality. Additionally, MAU is a predictor of cardiovascular mortality, as research

indicates that individuals with MAU are at four-fold increase of death from CVD, particularly among the hypertensive population. Among individuals with both T2D and HTN, the presence of MAU is associated with increased prevalence of CVD (Basi, Fesler, Mimran, & Lewis, 2008). Yang, et al. (2007) proposed one pathway aimed at explaining the relationships among MAU, HTN, diabetes, and CVD. In this pathway, hyperglycemia and HTN lead to albuminuria, which in turn results in hyperlipidemia, a significant risk factor for CVD. Albuminuria also leads to the deterioration of renal function, impacting the relationship between HDL and CVD. A factor identified in other research as associated with CVD and diabetic nephropathy is glycosylated hemoglobin (A1C), a protein implicated in glycemic control. Among individuals with diabetes, variability in A1C levels is a significant predictor of MAU, progression of renal disease, and CVD events (Waden, et al., 2009).

2.3.1 Detection of microalbuminuria

The American Diabetes Association (ADA) recommends testing for MAU in individuals with T2D on initial diagnosis of the disease and every year afterwards. Albumin levels, which are typically low due to retention of the protein in the bloodstream by the kidneys, can be detected using albumin-specific urine dipsticks. This method is a convenient and accurate screening tool (Derhaschnig, et al., 2002). However, some variation in urine albumin concentration can arise. To compensate for variations in urine concentration in spot-check samples, it is helpful to compare the amount of albumin in the sample against its concentration of creatinine. This is termed the albumin/creatinine ratio (ACR) and microalbuminuria is defined as ACR $\geq$ 3.5 mg/mmol (female) or $\geq$ 2.5

mg/mmol (male), or, with both substances measured by mass, as an albumin to creatinine ratio between 30 and 300 µg albumin/mg creatinine (American Diabetes Assocation, 2009). However, it is important to note that these cut-offs may not be appropriate in all situations. Men with high muscle mass and members of various ethnic groups, including Mexican Americans, may demonstrate higher baseline levels of albumin (Mattix, Hsu, Shaykevich, & Curhan, 2002). Additionally, research suggests that levels as low as 0.32 mg/mmol of MAU (Zamora & Cubedda, 2009) or an ACR as low as 5 µg albumin/mg creatinine (Klausen, Scharling, & Jensen, 2006) may be associated with significant risk of CVD.

2.3.2 Microalbuminuria and Cardiovascular Disease

A notable link exists between MAU and both diabetes and CVD. T2D is the leading cause of end stage renal disease, which leads to increased mortality. The risk for developing MAU is greater among individuals with T2D with existing coronary heart disease, as research indicates such individuals secrete higher levels of albumin (Ismail, Becker, Strzelcyzk, & Ritz, 1999). Increased levels of albumin in turn predict the risk for diabetic nephropathy, including loss of kidney function and ESRD (Ibsen, et al., 2008; Keane, et al., 2003). MAU is also associated with increased risk of experiencing CVD events among individuals with T2D and mortality from CVD independent of other cardiovascular or diabetes risk factors (Soedamah-Muthu, Visseren, & Van Der Graaf, 2008; Valmadrid, Klein, Moss, & Klein, 2000). Based on such findings, MAU may represent a key diagnostic target for CVD risk among individuals with T2D. Furthermore, research involving specific ethnic populations, such as Turkish, Asian, and Latino,

suggests that MAU is an important clinical indicator among individuals with diabetes and HTN in predicting CVD risk.

2.3.3 Microalbuminuria in the Turkish Population

A growing body of research illustrates the importance of MAU as a predictor of CVD risk in the Turkish population. The study done by Col, Ocaktan, Ozdemir, Yalcin, and Tuncbilek (2004) of Turkish individuals with HTN, diabetes, or a combination of the two conditions reported that the prevalence of MAU among these three groups was 17.4%, 20.4%, and 22.1%, respectively. Furthermore, among those diagnosed with diabetes more than five years prior to the onset of the study were 2.15 times more likely to have MAU than those diagnosed more recently. The time of diagnosis was insignificant for individuals with HTN. Kozan, Ozcan, Sancaktar, and Kabakci (2011) reported an even greater prevalence of MAU in the Turkish hypertensive population. In this observational, cross-sectional study involving over 1,900 hypertensive patients, researchers found the prevalence to be 64.7%. Albumin levels tended to be greater among hypertensive individuals with documented coronary artery disease.

In spite of the known research, which suggests that individuals with HTN possess an increased prevalence of MAU, patients with this condition may not be adequately evaluated for CVD risk. In one observational study of approximately 1,000 Turkish hypertensive patients, researchers reported that, while many of the individuals possessed one or more known risk factors for CVD, disease risk management was inadequate. The prevalence of diabetes and MAU among these Turkish study participants was 31.1% and 11.1%, respectively. Yet, 79.3% of these patients possessed BP levels

indicative of a failure to control the HTN. Furthermore, about 12% of the patients failed to comply with antihypertensive medication regimens. Similarly concerning findings were observed among those subjects who also had diabetes. While the blood glucose levels were high in about one-third of subjects, blood glucose was not measured in 22.4% of these individuals (Kozan, 2011). Taken together with the two studies discussed previously, it appears that MAU may be prevalent among individuals with HTN alone or in conjunction with diabetes and that clinicians are not taking adequate steps to control the risk factors associated with CVD.

2.3.4 Microalbuminuria in Other Ethnic Populations

MAU impacts not only the Turkish population, but other ethnic populations as well. Among Asian populations, an association exists between MAU and diabetes. Park, et al. (1998) reported on the incidence and determinants of MAU in a study of Korean patients with T2D. Findings from this study indicated that the incidence of MAU in this group was 51.6 per 1000 person-years. Of those who did develop MAU, approximately 19% progressed to overt proteinuria by the end of the study follow-up period. Risk factors associated with development of MAU were diabetic retinopathy, poor glycemic control, and HTN. Tillan, Forouhi, McKeigue, and Chaturvedi (2005) reported on the significance of MAU among the Asian population. In this study, although albumin levels were generally lower than that for other ethnic groups, including African Caribbeans and Europeans, the presence of MAU was associated with a 2.5-fold increase in CVD and CVD-associated mortality.

Increased risk for CVD due to MAU also exists among the Latino population. In a cross-sectional study of Cuban-Americans with T2D, researchers investigated the influence of HTN and poor glycemic control on the risk for MAU (Zarini, Exebio, Gundupalli, Nath, & Huffman, 2011). Results indicated that MAU existed in 26% of the study group. When compared to individuals who did not meet the criteria for MAU, those with increased albumin levels also had a significantly higher incidence of HTN and elevated A1C levels. Additionally, among Cuban individuals with both poor glycemic control and HTN, the risk for developing MAU increased by 6.76-fold. Corona, et al. (2005) provided further support for the link between MAU and CVD in their study of over 1,500 Mexican Americans. Results indicated that 13.6% of the study population tested positive for MAU, and the probability of these individuals experiencing a myocardial infarction was 1.9-times greater than for individuals without MAU.

2.3.5 Summary

Whether or not the best method to diagnose MAU is to spot-check urine sample is a matter of discussion. It remains unclear whether microalbumin measurement alone is a sufficient screening method or a calculation of an albumin/creatinine ratio (ACR) is necessary to detect microalbumineric hypertensive patients. Published data have shown that the calculation of an ACR exhibited a higher sensitivity and specificity in the detection of microalbuminuria in various groups (American Diabetes Assocation, 2009). However, other studies concluded that the ACR did not provide any advantage compared with microalbumin measurement alone (Derhaschnig, et al., 2002). Additional

disagreement exists over the most appropriate cut-off levels of albumin to indicate MAU, as some studies suggest that lower levels of albumin may be predictive of CVD.

Maintaining adequate BP is an important therapeutic goal among individuals with T2D. The early detection of MAU among members of the Turkish population with HTN is fundamental in preventing and treating diabetes complications and improving individuals' renal and cardiovascular outcomes. Unfortunately, research suggests that clinicians are not addressing this issue adequately within this population. A greater awareness of the importance and utility of MAU detection in evaluating CVD risk is paramount.

3. ENVIRONMENTAL INFLUENCES ON CARDIOVASCULAR RISK FACTORS AMONG TURKISH IMMIGRANTS IN THE NETHERLANDS WITH TYPE 2 DIABETES

Abstract: This study examined the associations of serum cotinine and serum 25-hydroxyvitamin D with blood pressure and serum lipids in Turkish immigrants with type 2 diabetes (T2D) living in the Netherlands. A total of 110 participants, physician-diagnosed with T2D, aged 30 years and older were recruited from multiple sources from The Hague, Netherlands. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automated office blood pressure equipment. Serum cotinine was measured with a solid-phase competitive chemiluminescent immunoassay. Serum 25-hydroxyvitamin D was determined by electrochemiluminescence immunoassay (ECLIA). Serum lipids were determined by enzymatic assay and included total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein (LDL), and triglycerides (TG). Total cholesterol to HDL ratio (CHOL:HDL) and atherosclerosis-index (AI) were calculated. Statistical analysis included descriptives and multiple linear regressions. High cotinine levels (≥ 25 ng/mL), as a biomarker for smoke exposure, were associated with high CHOL:HDL ratio (B= 0.59, SE= 0.28, P<0.05) and high AI (B= 0.59, SE= 0.28, P<0.05). Low 25-hydroxyvitamin D levels (< 25 nmol/L) were associated with low DBP (B= -4.14, SE= 2.00, P<0.05). Environmental influences including smoking and vitamin D need to be further investigated in the Turkish immigrant population. Serum cotinine and vitamin D may be assessed as a standard of care for T2D management in the Turkish immigrant population.

Keywords: Type 2 diabetes; serum cotinine; 25-hydroxyvitamin D; Turkish immigrants; serum lipids; blood pressure

INTRODUCTION

As compared to the native Dutch population, the Turkish immigrants in the Netherlands show a higher prevalence of (i) type 2 diabetes (T2D), (ii) deficiency in vitamin D, (iii) tobacco smoking, and (iv) cardiovascular diseases (CVD) (Kriegsman, van Langen, Valk, Stalman, & Boeke, 2003; Uitewaal, Manna, Bruijnzeels, Hoes, & Thomas, 2004; van der Meer et al., 2008; Van Leest, van Dis, & Verschuren, 2002). It has been previously shown that low blood levels of vitamin D interfere with the proper functioning of insulin-producing beta-cells thereby suggesting a strong inverse association between T2D and vitamin D (Tuorkey & Abdul-Aziza, 2010). Additionally, disorders in vitamin D metabolism have been negatively associated with independent cardiovascular risk factors such as tobacco smoking (Brot, Jorgensen, & Sorensen, 1999). Facchini, Hollenbeck, Jeppesen, Chen, and Reaven (1992) have shown that chronic tobacco smokers are insulin resistant, hyperinsulinaemic, and dyslipidaemic when compared to nonsmokers hence substantiating the increased risk of coronary heart disease among smokers. Tobacco smoking also reduces the serum levels of vitamin D, as measured by circulating 25(OH)D (Brot et al., 1999). Smoking is associated with increases in plasma lipid levels including triglycerides (TG) and decreases in high-density lipoprotein cholesterol (HDL) (Facchini et al., 1992; Baynes, Boucher, Feskens, & Kromhout, 1997).

Smoking and vitamin D deficiency have been identified as risk factors for CVD (Ockene & Miller, 1997; Wang et al., 2008). Previous studies have consistently shown a relationship between smoking status, serum lipids and CVD risk (Nakamura et al., 2009). Tobacco smoking alone has been documented as an important risk factor for

atherosclerosis (Witteman, Grobbee, Valkenburg, van Hemert, Stijnen & Hofman, 1993). The atherosclerosis-index (AI) was found to be one of the most reliable indicators of lipid metabolism disorders (Mertz, 1980). Higher levels of total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL), and TG and lower levels of HDL have been found in individuals who were active smokers (Bruckert, Jacob, Lamaire, Truffert, Percheron, & de Gennes, 1992). Smoking and increased blood pressure (BP) levels also increase CVD risk accounting for more than 20% of the global premature deaths (Nakamura et al., 2008; WHO, 2002). Khalili et al. (1992) have found increased systolic blood pressure (SBP) in middle-aged men to be associated with increased CVD risk (Khalili, Nilsson, Nilsson and Berglund, 2002). However, several larger epidemiological studies have shown lower BP among smokers compared to nonsmokers (Omvik P, 1996). Biochemical markers of tobacco exposure can provide a reliable verification of tobacco smoke exposure, with serum cotinine accurately differentiating smokers from non-smokers (Twardella, Küpper-Nybelen, Rothenbacher, Hahmann, Wüsten, & Brenner, 2004; Jeemon et al., 2010).

Lower levels of vitamin D are associated with several CVD risk factors and the prevalence of CVD (Martins et al., 2007; Kendrick, Targher, Smits, & Chonchol, 2009). While some researchers found a significant increase in CHOL with increasing serum 25(OH)D, other researchers showed a significant decrease of CHOL levels (Jorde, Figenschau, Hutchinson, Emaus, & Grimnes, 2010; Melamed, Michos, Post, & Astor, 2008). A positive association between 25(OH)D levels and HDL has been reported (Jorde & Grimnes, 2011). A longitudinal study of 1762 subjects reported a negative association between 25(OH)D and TG (Jorde, Figenschau, Hutchinson, Emaus, & Grimnes, 2010).

There remains a paucity of data on the effects of 25(OH)D on serum lipids in addition to conflicting reports (Jorde & Grimnes, 2011). There is a strong association between 25(OH)D and BP (Jorde, Figenschau, Emaus, Hutchinson, & Grimnes, 2010; Scragg, Sowers, & Bell, 2007). Krause and co-workers have reported that SBP and diastolic blood pressure (DBP) levels were reduced by 6 mmHg after 6 weeks of UVB irradiation 3 times per week (Krause, Bühring, Hopfenmüller, Holick, & Sharma,1998). Skin exposure to ultraviolet B radiation is the main source of vitamin D and can be determined by measuring serum 25(OH)D (Forman et al., 2007). Several cross-sectional studies have shown that BP levels were significantly associated with 25(OH)D levels (Scragg, Sowers, & Bell, 2007; Judd, Nanes, Ziegler, Wilson, & Tangpricha, 2008; Martins et al., 2007).

Given the scarcity of studies available among the Turkish immigrant population and the high incidence of CVD, it is important to examine molecular biomarkers of modifiable health risks within this population. Therefore, the objective of this study was to examine the relationship between serum cotinine as an indicator of smoke exposure and 25(OH)D with BP and serum lipids.

METHODS AND PROCEDURES

Study participants

The study population consisted of 110 Turkish participants with T2D, aged 30 years and older. The investigation took place between March to May 2011 in a cross-sectional design. The participants were recruited from multiple sources in The Hague, Netherlands. During this 3-month period, approximately 300 letters written in Dutch and Turkish languages outlining the study were mailed to residents of The Hague with

Turkish surnames listed in the local telephone directory. The interested participants could respond to the invitation letter. Due to unknown addresses, 1% of the unopened letters were returned undeliverable. Posters and flyers were also displayed at a family physician's office, dietitian's office, health club center, mosque, hairdresser, grocery store, community center, and pharmacies where Turkish immigrants are known to visit. From this, 11 eligible participants were enrolled from the delivered mail, 60 participants were enrolled from the physician's office, and 20 participants from other sources. A local community representative recruited 19 eligible participants. Twenty-two potential participants did not qualify for the study because they were either not Turkish (n= 3), or did not have T2D (n= 8), or did not provide blood samples within the 3-month study period (n= 11). Interested participants were initially interviewed on the phone, at which time the study purpose was explained, and age and gender of the responders were recorded. To ascertain T2D status, each participant was asked for the age at diagnosis and initial treatment modalities. All participants were physician-diagnosed with T2D as having fasting serum glucose level of ≥ 6.1 mmol/L based on the classification recommended by the Dutch College of General Practitioners (NHG-standard 2006) (Bouma, Rutten, de Grauw, Wiersma, & Goudswaard, 2006). The study was performed according to Dutch legislation regarding Ethics and Human Research and approved by The Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek). Florida International University Institutional Review Board (FIU-IRB) also approved the study protocol. Informed consent was obtained from all participants prior to the commencement of the study.

Blood pressure

Blood pressure (BP) was measured according to standard procedure by using an automated office blood pressure equipment (Myers et al., 2011). Both SBP and DBP were measured on participants in a sitting position in a quiet room for several minutes while readings were being taken. Measurements were performed twice and the average reading taken as the individual's BP.

Laboratory examination

Venous blood (20 mL) was collected after an 8-12h overnight fast, by a certified phlebotomist who used standard laboratory techniques. Immediately after collection, blood samples were centrifuged; serum/plasma was separated, frozen and stored at -80°C for analysis. All measurements were performed in a hospital laboratory at Medisch Centrum Haaglanden. Serum cotinine was measured with a solid-phase competitive chemiluminescent immunoassay. Quantitative determination of total 25-hydroxyvitamin D in serum was determined by electrochemiluminescence immunoassay (ECLIA). Serum lipids were determined by enzymatic in vitro assay. The lipid panel included: CHOL, HDL, LDL, and triglycerides. Glycated hemoglobin (A1C) was determined by high-pressure liquid chromatography (HPLC).

Measurements of risk variables

A trained physician assistant (PA) fluent in both Dutch and Turkish languages, interviewed all participants. The PA assisted and completed questionnaires on age, smoking status, prescription medication(s) use including diabetes medications, lipid-

lowering drugs (LLD), and hypertension medications. The investigators reviewed the questionnaires and the participants were contacted to inquire about any missing information. Waist circumference (WC) was measured horizontally (to the nearest 0.1 cm) with a non-stretchable measuring tape placed midway between the lowest rib and the iliac crest with the participant standing at the end of a slight expiration. Tobacco smoke exposure was defined as having serum cotinine levels >25 ng/mL according to Medisch Centrum Haaglanden laboratory reference standards. Vitamin D deficiency was defined as having serum 25(OH)D levels <25 nmol/L (van der Meer et al., 2008). The atherosclerosis index (AI) was calculated from the equation: AI = (CHOL – HDL)/HDL (Hirata, Yamano, Suzuki, Miyagawa, & Nakadate, 2010).

Statistical analyses

Differences between two categories of smoke exposure and two categories of vitamin D status were assessed using the independent samples t-test for numerical values and the chi-square test for categorical variables. The impact of the independent variables, including serum cotinine and 25(OH)D, was assessed via separate multiple linear regression (MLR) models for each separate dependent variable including DBP, SBP, CHOL, HDL, LDL, TG, CHOL:HDL, and AI. All models were adjusted for age and diabetes medications. MLR for dependent BP variables were further adjusted for hypertension medications. MLR for dependent lipid variables were further adjusted for lipid-lowering drugs. Statistical analyses were performed using the SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA). Level of statistical significance was set at $P<0.05$.

RESULTS

Participants

The percentages or means and standard deviations of characteristics for Turkish immigrants with T2D classified by serum cotinine and related other characteristics are presented in Table 1. Participants with high cotinine levels (≥ 25 ng/mL) had a higher mean CHOL:HDL ratio (p= 0.026) and AI (p= 0.032) as compared to those with low cotinine levels (< 25 ng/mL).

The percentages or means and standard deviations of characteristics for Turkish immigrants with T2D classified by 25(OH)D are presented in Table 2. There was a higher percentage of females with low levels of 25(OH)D (< 25 nmol/L) as compared to males (p= 0.037). Participants with high levels of 25(OH)D (≥ 25 nmol/L) had a higher mean DBP (p= 0.033) as compared to those with low levels of 25(OH)D (< 25 nmol/L).

Multiple linear regression

Dependent variables in separate MLR models, adjusting for covariates are presented in Tables 3-5. Covariates included are age and diabetes medications. MLR for dependent BP variables were further adjusted for hypertension medications. MLR for dependent lipid variables were further adjusted for lipid-lowering drugs. For DBP, low 25(OH)D levels had a lower 4.14 mm/Hg than high 25(OH)D levels (p= 0.041) (Table 3). For CHOL:HDL, high cotinine levels had a higher 0.59 ratio than low cotinine levels (p= 0.035) (Table 5). For AI, high cotinine levels had a higher 0.59 ratio than low cotinine levels (p= 0.041) (Table 5).

DISCUSSION

The present study showed that CHOL:HDL ratio and AI are positively associated with serum cotinine levels indicating smoke exposure in Turkish participants with T2D. These findings corroborate other studies where there is a significant reduction in HDL levels in smokers than in non-smokers and an association between inhaled tobacco smoke and the atherosclerotic risk (Garrison, Kannel, Feinleib, Castelli, McNamara, & Padgett, 1978). According to the Framingham study, smokers who quit for more than one year have blood cholesterol levels similar to non-smokers (Garrison, Kannel, Feinleib, Castelli, McNamara, & Padgett, 1978). There is limited research available regarding the effect of smoking on lipid levels and its role in CVD (Garrison, Kannel, Feinleib, Castelli, McNamara, & Padgett, 1978; Brischetto, Connor, Connor, & Matarazzo, 1983; Craig, Palomaki, & Fladdow, 1989; Stubbe, Eskilsson, & Nilsson-Ehle, 1982). Several studies found tobacco smoking altered serum lipids and reported higher values of CHOL, TG, and LDL in smokers compared with non-smokers (Whig, Singh, Soni, & Bansal, 1992).

Previous cross-sectional studies have reported that smokers have lower BP than non-smokers (Berglund & Wilhelmsen, 1975; Erikssen & Enger, 1978; Stamler et al., 1975). Havlik, Garrison, Feinleib, Padgett, Castelli, & McNamara (1980) suggested that this could be due to the possible rebound of blood pressure due to nicotine deprivation of smokers who refrain from smoking before a medical examination. Another possible explanation could be due to the BP lowering effect of beta-blockers in hypertensive patients who smoke (Omvik, 1996). Given that our study population had T2D, who

commonly used hypertension medications, results did not show an association between smoking and BP levels.

Several studies did not find an association between 25(OH)D and TG or HDL which is in agreement with our findings (Chiu, Chu, Go, & Saad, 2004; John, Noonan, Mannan, & Boucher, 2005). Ford, Ajani, McGuire, & Liu (2005), however, found 25(OH)D to be inversely associated with hypertriglyceridemia but did not find an association with 25(OH)D and HDL. Further research is required to fully understand the link between 25(OH)D and lipid levels.

Looker, Dawson-Hughes, Calvo, Gunter, & Sahyoun (2002) using data from the National Health and Nutrition Examination Survey (NHANES III) found an inverse association between 25(OH)D levels and BP (Scragg, Sowers, & Bell, 2007). These findings are similar to this study where a negative association between 25(OH)D and DBP was found. Variations in skin color across ethnicities play an important role in low 25(OH)D levels and BP (Looker, Dawson-Hughes, Calvo, Gunter, & Sahyounm, 2002; Clemens, Adams, Henderson, & Holick, 1982). The inverse association between 25(OH)D levels and BP could be due to regional and ethnic differences in hypertension (Rostand, 1997). Forman et al. (2007) reported 25(OH)D levels to be inversely associated with risk of hypertension.

Our study has several strengths. The study was conducted during the Spring season to eliminate high or low sun exposure. Participants included were all of Turkish origin, a single origin, in which research concerning serum cotinine and 25(OH)D level and cardiovascular risk factors are limited. We used standardized protocols, including uniform anthropometric and biochemical measurements in a clinical setting. Serum

cotinine is more practical for use in clinical settings for the prediction of smoking status compared to urine cotinine (de Weerd, Thomas, Kuster, Cikot, Steegers, 2002). We adjusted the analysis for all major confounders of cotinine and 25(OH)D such as age, gender, WC, diabetes medications, lipid-lowering drugs, and hypertension medications.

There were a number of limitations for this study. First, because of the study's cross-sectional nature, our results do not establish causality and cannot be generalized to other populations. Additional statistical analyses were done to determine the potential confounding effect of medications use including hypertension medications and lipid-lowering drugs. Results of these tests verified that the direction did not change. Although BP and lipid levels are ethnically specific, our sample was not random and may not represent the Turkish immigrant population of the Netherlands. Second, given the small sample size (n= 110), these study findings need to be tested with a larger sample size. Further research should be done following cohorts to determine the effects of cotinine levels and 25(OH)D on morbidity and mortality.

CONCLUSION

The present study shows that environmental influences such as serum cotinine and 25(OH)D are significantly associated with BP and lipid levels. Among the CVD risk factors examined, CHOL:HDL ratio and AI were independent determinants of serum cotinine levels in smokers and DBP was an independent determinant of low 25(OH)D levels. Further studies are needed to confirm our results and to examine other environmental factors such as dietary intake that affect BP and lipid levels in Turkish immigrants with T2D. Research findings can contribute to the management of risk factors

for CVD in Turkish immigrants with T2D. Adding cotinine and 25(OH)D testing to standard diabetes care may be used to determine the potential risks for CVD and may direct management in the primary prevention of CVD. The results of this study could contribute to the evidence for the adverse effects of tobacco use and public health.

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Table 1 – Percentages or means and standard deviations for Turkish immigrants with type 2 diabetes) classified by serum cotinine (Netherlands, 2011

Variables	Serum cotinine (ng/mL)		
	High[a] (n= 31)	Low[b] (n= 79)	p
Age (years)	50.87±7.39	53.84±9.75	0.129
Female (%)	48.4	62.0	0.192
Waist circumference (cm)	103.13±9.08	105.77±10.97	0.237
Systolic blood pressure (mmHg)	139.32±24.34	142.61±20.11	0.470
Diastolic blood pressure (mmHg)	82.16±9.18	83.06±10.29	0.671
Total cholesterol (mmol/L)	4.96±1.25	4.76±1.12	0.405
HDL (mmol/L)	1.13±0.31	1.26±0.35	0.058
LDL (mmol/L)	3.06±1.09	2.75±0.94	0.130
Triglycerides	0.57±0.45	0.49±0.51	0.483
CHOL:HDL	4.68±1.57	3.99±1.37	**0.026**
Atherosclerosis-index[c]	3.68±1.64	3.00±1.39	**0.032**
Glycated hemoglobin (mmol/L)	3.92±0.25	3.91±0.24	0.882
Diabetes medications (%)	48.4	59.5	0.291
Lipid-lowering drugs (%)	54.8	49.4	0.606
Hypertension medications (%)	41.9	58.2	0.123

Abbreviations: HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; CHOL:HDL, total cholesterol to HDL cholesterol ratio.

[a]high cotinine is defined as ≥ 25 ng/mL

[b]low cotinine is defined as < 25 ng/mL

[c]Atherosclerosis-index = (Total cholesterol – HDL)/HDL

Table 2 – Percentages or means and standard deviations deviations for Turkish immigrants with type 2 diabetes classified by 25(OH)D (Netherlands, 2011)

	25(OH)D		
Variables	High[a] (n= 74)	Low[b] (n= 36)	p
Age (years)	53.03±9.41	52.94±8.91	0.965
Female (%)	51.4	72.2	**0.037**
Waist circumference (cm)	104.65±10.94	105.81±9.64	0.590
Systolic blood pressure (mmHg)	143.34±21.01	138.28±21.84	0.245
Diastolic blood pressure (mmHg)	84.22±10.08	79.92±9.18	**0.033**
Total cholesterol (mmol/L)	4.76±1.18	4.93±1.10	0.498
HDL (mmol/L)	1.23±0.36	1.21±0.29	0.699
LDL (mmol/L)	2.78±0.98	2.96±1.02	0.381
Triglycerides	0.52±0.49	0.51±0.50	0.888
CHOL:HDL	4.12±1.42	4.33±1.53	0.485
Atherosclerosis-index[c]	3.11±1.43	3.37±1.61	0.389
Glycated hemoglobin (mmol/L)	3.92±0.27	3.89±0.18	0.568
Diabetes medications (%)	55.4	58.3	0.771
Lipid-lowering drugs (%)	48.6	55.6	0.497
Hypertension medications (%)	51.4	58.3	0.491

Abbreviations: 25(OH)D, 25- hydroxyvitamin D; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; CHOL:HDL, total cholesterol to HDL cholesterol ratio.

[a]low 25-hydroxyvitamin D is defined as < 25 nmol/L

[b] high 25-hydroxyvitamin D is defined as ≥ 25 nmol/L

[c]Atherosclerosis-index = (Total cholesterol – HDL)/HDL

Table 3 – Multiple linear regression coefficients[a] and standard errors of serum cotinine and 25(OH)D for systolic blood pressure and diastolic blood pressure with

	Systolic blood pressure[a]			Diastolic blood pressure[a]		
Variables	B	SE	p	B	SE	p
Cotinine (≥ 25 ng/mL) [b]	0.91	4.18	0.828	-0.85	2.13	0.691
25(OH)D (< 25 nmol/L)[c]	-5.85	3.96	0.143	-4.14	2.00	**0.041**

Abbreviations: 25(OH)D, 25- hydroxyvitamin D; B, regression coefficient; SE, standard error.

[a]Dependent variables in separate multiple linear regression equations with covariates including age, gender, waist circumference, diabetes medications and hypertension medications.

[b]Difference (high cotinine – low cotinine) in dependent variables, adjusted for covariates in model

[c]Difference (low 25(OH)D – high 25(OH)D) in dependent variables, adjusted for covariates in model

Table 4 – Multiple linear regression coefficients[a] and standard errors of serum cotinine and 25(OH)D for total cholesterol, HDL, and LDL

	Total cholesterol[a]			HDL[a]			LDL[a]		
Variables	B	SE	p	B	SE	p	B	SE	p
Cotinine (≥ 25 ng/mL) [b]	0.21	0.23	0.359	-0.11	0.06	0.093	0.31	0.19	0.116
25(OH)D (< 25 nmol/L) [c]	0.22	0.22	0.331	-0.09	0.06	0.163	0.27	0.18	0.153

Abbreviations: 25(OH)D, 25- hydroxyvitamin D; CHOL, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; B, regression coefficient; SE, standard error.

[a]Dependent variables in separate multiple linear regression equations with covariates including age, gender, waist circumference, diabetes medications and lipid-lowering drugs.

[b]Difference (high cotinine – low cotinine) in dependent variables, adjusted for covariates in model

[c]Difference (low 25(OH)D – high 25(OH)D) in dependent variables, adjusted for covariates in model

Table 5 – Multiple linear regression coefficients[a] and standard errors of serum cotinine and 25(OH)D for triglycerides, CHOL:HDL, and atherosclerosis-index

	Triglycerides[a]			CHOL:HDL[a]			Atherosclerosis-index[a]		
Variables	B	SE	p	B	SE	p	B	SE	p
Cotinine (≥ 25 ng/mL) [b]	0.07	0.11	0.535	0.59	0.28	**0.035**	0.59	0.28	**0.041**
25(OH)D (< 25 nmol/L) [c]	-0.002	0.10	0.985	0.46	0.27	0.088	0.53	0.27	**0.054**

Abbreviations: 25(OH)D, 25- hydroxyvitamin D; TG, triglycerides; CHOL:HDL, total cholesterol to HDL cholesterol ratio; B, regression coefficient; SE, standard error.

[a]Dependent variables in separate multiple linear regression equations with covariates including age, gender, waist circumference, diabetes medications and lipid-lowering drugs.

[b]Difference (high cotinine – low cotinine) in dependent variables, adjusted for covariates in model

[c]Difference (low 25(OH)D – high 25(OH)D) in dependent variables, adjusted for covariates in model

Note: Atherosclerosis-index = (Total cholesterol – HDL)/HDL

4. OBESITY AND SERUM HIGH SENSITIVITY C-REACTIVE PROTEIN LEVELS AMONG TURKISH IMMIGRANTS IN THE NETHERLANDS WITH TYPE 2 DIABETES

Abstract: The study examined the associations of anthropometric measures of obesity with high sensitivity C-reactive protein (hs-CRP) levels in Turkish immigrants with type 2 diabetes (T2D) living in the Netherlands. A total of 110 participants, physician-diagnosed with T2D, aged 30 years and older were recruited from multiple sources from The Hague, Netherlands. Serum hs-CRP levels were measured with immunoturbidimetric assay. Glycated hemoglobin (A1C) was determined by high-pressure liquid chromatography. Measures of obesity including: body weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR) were determined. Statistical analysis included descriptive statistics and multiple linear regressions (MLR) stratified by gender. Participants with hs-CRP levels >10 mg/L (n= 17) were excluded from the analysis. Females had higher BMI (P= 0.007), HC (P< 0.001), and WHtR (P= 0.011) as compared to males. Conversely, males had higher weight (P= 0.007), and WHR (P<0.001) than females. MLR showed that after controlling for covariates, hs-CRP was positively associated with WC (B= 0.10, SE= 0.05, P<0.05), WHR (B= 17.37, SE= 8.24, β= 0.332, P<0.05) in males and hs-CRP was positively associated with WHtR (B= 8.90, SE= 4.41, β= 0.376, P<0.05) in females. Gender-specific associations between obesity measures and hs-CRP level need to be further investigated in the Turkish immigrant population. Hs-CRP assessment may be added as a standard of care for T2D management within this population.

Keywords: Type 2 diabetes; low-grade inflammation; high sensitivity C-reactive protein; Turkish; immigrant; obesity

INTRODUCTION

Cardiovascular disease (CVD) is a significant cause of morbidity and mortality among individuals with type 2 diabetes (T2D) (Uitewaal, Goudswaard, Ubink-Veltmaat, Bruijnzeels, Hoes, & Thomas, 2004(a); Soinio, Marniemi, Laakso, Lehto, & Rönnemaa, 2006). Recent literature suggests T2D to be a state of low-grade inflammation (Pickup, 2004). Low-grade systemic inflammation is associated with an increased risk of CVD, where elevated serum C-reactive protein (CRP) levels are a reliable predictor of CVD and CVD risk factors (Soinio et al., 2006; Ujcic-Voortman, Baan, Verhoeff, Krol, & Seidell, 2011; Onat, Sansoy, Yildirim, Keleş, Uysal, & Hergenç, 2001; Onat, Can, & Hergenç, 2008). There are significant associations between obesity and increased circulating serum CRP levels in persons with obesity-related diseases (Onat et al., 2001; Huffman, Gomez, & Zarini, 2009; Huffman, Whisner, Zarini, & Nath, 2010). In the Netherlands, Turkish immigrants form the largest ethnic minority group with 388,967 members according to 2011 census (CBS, 2011). Several studies showed a higher prevalence of T2D, obesity, and elevated serum CRP levels among Turkish immigrants when compared to the indigenous Dutch (Uitewaal et al., 2004(a); Ujcic-Voortman et al., 2011; Uitewaal, Manna, Bruijnzeels, Hoes, & Thomas, 2004(b); Kriegsman, van Langen, Valk, Stalman, & Boeke, 2003; van Leest, van Dis, & Verschuren, 2002). Poor glycemic control combined with high prevalence of obesity in Turkish immigrants with T2D increases CVD risk. There is a higher prevalence of obesity among Turkish females as compared to their male counterparts (Uitewaal et al., 2004(b); van Leest et al., 2002). A prospective cohort of the Turkish Adult Risk Factor (TARF) study, which included 1046

Turkish adults, found CRP levels to be greater for females than for males (Onat et al., 2001).

Obesity is a determinant of CRP levels (Onat et al., 2001). There is lack of agreement as to which indicator of obesity best predicts CVD risk (Connelly, Hanley, Harris, Hegele, & Zinman, 2003; Mojiminiyi, Al Mulla, & Abdella, 2009; Can et al., 2009; de Koning, Merchant, Pogue, & Anand, 2007; Schneider et al., 2010). Body mass index (BMI) is an independent determinant of CRP level in females, whereas waist circumference (WC) is an independent determinant of CRP level in males (Connelly et al., 2003). Other studies found waist-to-hip ratio (WHR) to have a stronger correlation with CRP levels as opposed to BMI or WC (Snijder et al., 2003). However, Mojiminiyi et al. (2009) found WHR to show the weakest correlations with CRP levels and reported BMI to be the most strongly associated anthropometric measure with CRP levels in patients with T2D. Caan et al. (1994) suggest that non-obese versus obese individuals could exhibit the same WHR, which could remain constant during weight change. Other findings suggest that waist-to-height ratio (WHtR) is a better predictor of CVD than the more widely used BMI (Can et al., 2010; Schneider et al., 2010). Including either hip circumference (HC) or height into WC may offer additional information on CVD risk than WC alone (Can et al., 2009). Along with inconsistencies of obesity measurements and CRP levels, there is scarcity of studies regarding the association of CVD risk factors with CRP levels in a Turkish population with T2D living in the Netherlands (Ujcic-Voortman et al., 2011). Therefore, the objective of this study was to examine the relationship between measures of obesity and CRP levels within this population.

Examining these factors could provide important insights into the possible role of successful diabetes management.

METHODS AND PROCEDURES

Study participants

The study population consisted of 110 Turkish participants with T2D, aged 30 years and older. The investigation took place between March and May 2011 in a cross-sectional design. The participants were recruited from multiple sources in The Hague, Netherlands. During this 3-month period, approximately 300 letters written in Dutch and Turkish languages outlining the study were mailed to residents of The Hague with Turkish surnames listed in the local telephone directory. The interested participants could respond to the invitation letter. Due to unknown addresses, 1% of the unopened letters were returned back. Posters and flyers were also displayed at a family physician's office, dietitian's office, health club center, mosque, hairdresser, grocery store, community center, and pharmacies where Turkish immigrants are known to visit. From this, 11 eligible participants were enrolled from the delivered mail, 60 participants were enrolled from the physician's office, and 20 participants from other sources. A local community representative recruited 19 eligible participants. Twenty-two potential participants did not qualify for the study because they were either not Turkish (n= 3), or did not have T2D (n= 8), or did not provide blood samples within the 3-month study period (n= 11). Interested participants were initially interviewed on the phone, at which time the study purpose was explained, and age and gender of the responders were recorded. To ascertain T2D status, each participant was asked for the age at diagnosis and initial treatment

modalities. All participants were physician-diagnosed with T2D as having fasting serum glucose level of ≥ 6.1 mmol/L based on the classification recommended by the Dutch College of General Practitioners (NHG-standard 2006) (Bouma, Rutten, de Grauw, Wiersma, & Goudswaard, 2006). The study was performed according to Dutch legislation regarding Ethics and Human Research and approved by The Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek). Florida International University Institutional Review Board (FIU-IRB) also approved the study protocol. Informed consent was obtained from all participants prior to the commencement of the study.

Anthropometric measurements

All anthropometric measurements were obtained using standard techniques (Schneider, 2007) with the participant wearing light clothing without shoes. Height (rounded to the nearest 0.1 cm) was determined using a wall-mounted stadiometer, and weight (to the nearest 0.1 kg) was determined using a digital electronic clinical scale. BMI was calculated as weight (kg) divided by height (m2). WC was measured horizontally (to the nearest 0.1 cm) with a non-stretchable measuring tape placed midway between the lowest rib and the iliac crest with the participant standing at the end of a slight expiration. Hip circumference (HC), the level of the widest diameter around the gluteal protuberance, was measured to the nearest 0.1 cm. WHR (WC in cm divided by HC in cm) and WHtR (WC in cm divided by height in cm) were calculated.

Laboratory examination

Venous blood (20 mL) was collected after an 8-12h overnight fast, by a certified phlebotomist who used standard laboratory techniques. Immediately after collection, blood samples were centrifuged; serum/plasma was separated, frozen and stored at -80°C for analysis. All measurements were performed in a hospital laboratory at Medisch Centrum Haaglanden. The Roche Tina-quant® Cardiac C-reactive Protein (Latex) High Sensitive (CRPHS) immunoturbidimetric assay was used for the in vitro quantitative determination of hs-CRP levels in the range from 0.1-20 mg/L. This assay is proven to be a suitable method for screening large populations (Lolekha, Chittamma, Roberts, Sritara, Cheepudomwit, & Suriyawongpaisal, 2005). A1C was determined by high-pressure liquid chromatography (HPLC) using the Bio-Rad VARIANT™ II TURBO Hemoglobin A1C Program. This method is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Consensus Committee, 2007).

Measurements of risk variables

A trained physician assistant (PA) fluent in both Dutch and Turkish languages, interviewed all participants. The PA assisted and completed questionnaires on age, smoking status, prescription medication(s) use including nonsteroidal anti-inflammatory drugs (NSAIDs), lipid-lowering drugs (LLD), hypertension medications, and diabetes medications. The investigators reviewed the questionnaires and the participants were contacted to inquire about any missing information. Current smokers were defined as

having smoked at least 100 cigarettes per lifetime and reported smoking every day or some days (CDC, 2002).

Statistical analyses

Participants who had hs-CRP levels >10 mg/L (n= 17) were assumed to have acute phase infection, thus were excluded from the analysis, leaving a study sample of n= 93 participants (42 males and 51 females). Differences between males and females were assessed using the independent samples t-test for numerical values and the chi-square test for categorical variables. The impact of hs-CRP for males and females were assessed via separate multiple linear regression models for each separate dependent variable including weight, BMI, WC, HC, WHR, and WHtR. All models were adjusted for age, A1C, smoking, taking NSAIDs, taking LLD, taking hypertension medications, and taking diabetes medications. Statistical analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA). Level of statistical significance was set at $P<0.05$.

RESULTS

Participants

Percentages or means and standard deviations of characteristics for Turkish immigrants with type 2 diabetes by gender are presented in Table 1. Females had a higher BMI ($p= 0.007$), HC ($P<0.001$) and WHtR ($p= 0.011$) than males. Conversely, males had a higher weight and WHR than females ($p= 0.007$, $P<0.001$ respectively).

Multiple linear regression

Multiple linear regression coefficients and standard errors for hs-CRP and selected anthropometry measurements for males and females are presented in Table 2. After adjusting for covariates, including age, A1C, smoking, NSAIDs, LLD, hypertension medications, and diabetes medications, hs-CRP increases 0.10 mg/dL for every 1 cm increase in WC in males (p= 0.037). Additionally, hs-CRP increases 17.37 mg/dL for every 1 unit of WHR in males (p= 0.043). Hs-CRP increases 8.90 mg/dL for every 1 unit of WHtR in females (p= 0.050).

DISCUSSION

The main findings of our study were that hs-CRP associations were stronger with anthropometric indices in males than in females. These findings may be related to body fat differences between males and females. Females tend to have a higher percentage of body fat than do males, regardless of equivalent amounts of liver and intra-abdominal fat (Westerbacka et al., 2004). Onat, Can, Hergenç, Uğur, and Yüksel (2011) reported CRP levels were much higher in females as compared to males. Our results suggest that WHtR was a better measure than BMI, WC, WHR, weight, or HC in reflecting hs-CRP levels in Turkish females with T2D. This finding confirms the findings of other cross-sectional studies (Can et al., 2009; Schneider et al., 2010). Results from the Turkish Heart Study suggest that WHtR is a better predictor of CVD risk as compared to BMI, WC, and WHR (Can et al., 2009). Schneider et al. (2010) observed similar findings, where WHtR was the strongest predictor of CVD risk and mortality and discouraged the use of BMI. Our study shows a significant difference in WHR in males with respect to hs-CRP levels.

These findings are in accordance with other studies where WHR indicated a correlation with hs-CRP level in patients with T2D (Mojiminiyi et al., 2009). Connelly et al. (2003) found measures of total body fat were more strongly correlated with hs-CRP levels than measures of fat distribution including the WHR. Mojiminiyi et al. (2009) found gender-dependent differences in the pattern of fat distribution. Although other studies found BMI correlated with hs-CRP levels in patients with T2D more strongly (Mojiminiyi et al., 2009), our results revealed that BMI was not significant with hs-CRP levels as compared to WC. WC and HC measure different patterns of body fat distribution, with independent effects on CVD, which could be poorly reflected in the WHR (Mojiminiyi et al., 2009). Research suggests that anthropometric measures of abdominal obesity are a better indicator of CVD than BMI (de Koning et al., 2007; Schneider et al., 2010). The use of WHtR rather than WHR is recommended to assess CVD risk in Turkish adults (Can et al., 2009).

Various mechanisms have been suggested for the association between obesity and hs-CRP levels. Hs-CRP is synthesized by both the liver and the adipose tissue in response to proinflammatory cytokines such as interleukin-6 (IL-6) (Anty et al., 2006). Consequently, both tissues may contribute to the elevated serum CRP levels found in obesity (Ujcic-Voortman et al., 2011; Anty et al., 2006). Elevated hs-CRP levels in females with T2D could be due to a higher BMI and higher percentage of body fat (Flores-Alfaro, Parra-Rojas, Salgado-Bernabé, Chávez-Maldonado, & Salazar-Martinez, 2008; Al-Daghri et al., 2010). Higher inflammatory stress in females than in males with T2D could describe their higher CVD risk (Saltevo, Kautiainen, & Vanhala, 2009). In the KORA (Cooperative Research in the Region of Augsburg) study, a large proportion of

the variation in hs-CRP levels was explained by body fatness and the association was particularly strong for females (Thorand et al., 2006). Even though, a significant proportion of the variation in hs-CRP level could not be explained by measures of obesity (Connell et al., 2003).

There are a number of other factors that have been found to affect hs-CRP levels including age, smoking, A1C, medications, alcohol and ethnicity (Ujcic-Voortman et al., 2011; Onat et al., 2008; Pai, Hankinson, Thadhani, Rifai, Pischon, & Rimm, 2006). Turkish males have a higher prevalence of smoking as compared to Dutch males (Uitewaal et al., 2004(b)). The TARF Study found smoking status to be a major determinant of elevated hs-CRP level in Turkish males independent of WC (Onat et al., 2008). Higher insulin resistance in relation to obesity may play a role in poor A1C status and can largely explain elevated hs-CRP level in patients with T2D (Onat, Uyarel, Hergenç, Karabulut, Albayrak, & Can, 2007). Previous studies have shown that certain medications including NSAIDs, LLD, angiotensin-converting enzyme (ACE) inhibitors, and diabetes medications, affect serum hs-CRP levels (Prasad, 2006). Additionally, moderate alcohol consumption has shown an inverse association with hs-CRP levels (Pai et al., 2006). Due to the low reporting of alcohol consumption in our study population, we did not include this as a control variable in our analysis. The TARF Study suggested that the association between hs-CRP levels and CVD might be even stronger for the Turkish population as compared to most Western populations (Onat et al., 2001).

Our study has several strengths. Participants included were all of Turkish origin, a single origin, in which research concerning hs-CRP level and measures of obesity is scarce. We used a standardized protocol, including uniform anthropometric and

biochemical measurements in a clinical setting. Serum hs-CRP is a more sensitive assay for the prediction of CVD compared to traditional assays for circulating C-reactive protein levels (Pearson et al., 2003). Hs-CRP levels >10 mg/L reflect acute inflammation and were excluded from the analysis (Chou, Hsu, Liu, Teng, Wu, & Ko, 2010). NSAIDs, LLD, hypertension medications, diabetes medications, smoking and A1C, which might have influenced the association of hs-CRP levels, were controlled for in the analysis (Onat et al., 2008; Prasad, 2006).

There were a number of limitations for this study. First, because of the study's cross-sectional nature, our results do not establish causality and cannot be generalized for other populations since measures of obesity are known to be ethnic-specific. Second, given the small sample size (n= 93), these study findings need to be tested in a larger sample size and in a prospective manner. Additionally, the results obtained for hs-CRP from one laboratory measurement may not be reproducible.

CONCLUSION

The present study shows that gender differences exist in the association of anthropometric indices of obesity and hs-CRP levels. Among the measures examined, WC and WHR were independent determinants of serum hs-CRP levels in males. The WHtR was an independent determinant of serum hs-CRP levels in females. Further studies are needed to confirm our results and to examine the factors of obesity that affect serum hs-CRP levels in Turkish immigrants with T2D. Research findings can contribute to the management of risk factors for CVD in Turkish patients with T2D. Adding hs-CRP

testing to standard diabetes care may be used for determining the potential risk for CVD and may direct management in the primary prevention of CVD.

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Table 1 – Percentages or means and standard deviations of characteristics for Turkish immigrants with type 2 diabetes by gender (Netherlands 2011)

Variables	Males (n= 42)	Females (n= 51)	p
Age (y)	51.71±9.35	54.47±8.73	0.146
Weight (kg)	89.10±11.33	81.85±13.77	**0.007**
Body mass index (kg/m^2)	30.72±3.85	33.71±6.07	**0.007**
Waist circumference (cm)	106.17±9.23	103.16±10.97	0.161
Hip circumference (cm)	106.93±6.20	114.65±10.50	**<0.001**
Waist-to-hip ratio	0.99±0.06	0.90±0.06	**<0.001**
Waist-to-height ratio	0.62±0.06	0.66±0.08	**0.011**
hs-CRP (mg/dL)	2.95±2.65	3.50±2.58	0.313
Glycated hemoglobin (mmol/L)	52.17±14.63	51.57±15.84	0.853
Smoking (%)	35.7	25.5	0.285
NSAIDs (%)	61.9	62.7	0.934
Lipid-lowering drugs (%)	45.2	64.7	0.060
Hypertension medications (%)	59.5	56.9	0.796
Diabetes medications meds (%)	57.1	62.7	0.583

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; NSAIDs, Non-steroidal anti-inflammatory drugs.

Table 2 – Multiple linear regression coefficients and standards errors of selected anthropometry measurements for hs-CRP

	hs-CRP [a]					
	Males (n= 42)			Females (n= 51)		
Variables	B	SE	p	B	SE	p
Weight (kg)	0.05	0.04	0.137	0.02	0.03	0.359
Body mass index (kg/m^2)	0.07	0.11	0.540	0.09	0.06	0.127
Waist circumference (cm)	0.10	0.05	**0.037**	0.05	0.03	0.111
Hip circumference (cm)	0.08	0.07	0.254	0.05	0.03	0.129
Waist-to-hip ratio	17.37	8.24	**0.043**	2.83	5.73	0.625
Waist-to-height ratio	10.16	7.77	0.200	8.90	4.41	**0.050**

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; B, regression coefficient; SE, standard error, β, beta coefficient.

[a]Dependent variable in separate multiple linear regression equations with covariates including age, glycated hemoglobin, smoking, non-steroidal anti-inflammatory drugs, lipid-lowering drugs, hypertension medications, and diabetes medications.

5. MICROALBUMINURIA AND HYPERTENSION AMONG TURKISH IMMIGRANTS IN THE NETHERLANDS WITH TYPE 2 DIABETES

Abstract: This study examined the associations of microalbuminuria (MAU), as determined by albumin-to-creatinine ratio (ACR), with hypertension (HTN) among Turkish immigrants with type 2 diabetes (T2D) living in the Netherlands. A total of 110 participants, physician-diagnosed with T2D, aged 30 years and older were recruited from multiple sources from The Hague, Netherlands in a cross-sectional design. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using automated office blood pressure equipment. Urine albumin was measured by immunoturbidimetric assay. Urine creatinine was determined using the Jaffe method. MAU was defined as ACR ≥ 3.5 mg/mmol for females and/or ACR ≥ 2.5 mg/mmol for males. Positive MAU was present in 21% of Turkish immigrants with T2D. Adjusted logistic regression analysis indicated that the odds of having MAU were 6.6 times higher in hypertensive than those that were normotensive, 95% CI (1.19, 36.4). These findings suggest that HTN and MAU may be assessed as a standard of care for T2D management for this population. Prospective studies of diabetes outcomes are recommended to further verify these findings.

Keywords: Kidney function; microalbuminuria; hypertension; blood pressure; cardiovascular disease; type 2 diabetes; Turkish immigrants

INTRODUCTION

Chronic kidney disease is a worldwide problem that carries a significant risk for cardiovascular morbidity and mortality (Eknoyan, 2004). A small but abnormal albumin excretion in urine is known as microalbuminuria (MAU). MAU is a widely known predictor of diabetic nephropathy, essential hypertension (HTN), and cardiovascular disease (CVD) (Keane et al., 2003; Ibsen, Olsen, Wachtell, Borch-Johnsen, Lindholm, & Mogensen, 2008). It is imperative that MAU be measured in all subjects with type 2 diabetes (T2D) and HTN, so that renal and cardiovascular adverse events can be properly managed and prevented (ADA, 2009; Basi, Fesler, Mimran, & Lewis, 2008).

Microalbuminuria can be diagnosed, ranging between 30–300 mg, from a 24-hour urine collection (ADA, 2005). Measuring MAU by albumin-specific urine dipstick alone, without simultaneously measuring creatinine, can give false-negative and/or false-positive results due to variations in urine concentration (ADA, 2005). To compensate for these variations in spot-check urine samples, it is useful to evaluate the amount of MAU against its concentration of creatinine (Basi, Fesler, Mimran, & Lewis, 2008). This is defined as the albumin/creatinine ratio (ACR) and MAU is defined as ACR $\geq$ 3.5 mg/mmol for females or ACR $\geq$ 2.5 mg/mmol for males (Jerums & MacIsaac, 2002). The Kidney Disease Outcomes Quality Initiative guidelines report that ACR measurement in a first-morning spot urine collection is a reliable method for kidney failure (NKF, 2002).

Hypertensive T2D individuals with MAU are at increased risk of developing end-stage renal disease (ESRD) (Ismail, Becker, Strzelczyk, & Ritz, 1999). Maintaining adequate blood pressure and glycemic control plays a fundamental role in preventing renal and CVD events in individuals with T2D (Zarini, Exebio, Gundupalli, Nath, &

Huffman, 2011). The high prevalence of T2D and CVD in the Turkish immigrant population in the Netherlands merits further assessment of diabetes complications (Kriegsman, van Langen, Valk, Stalman, & Boeke, 2003; Van Leest, van Dis, & Verschuren, 2002). The Trabzon Hypertension Study showed that HTN is common among the Turkish adult population and uncontrolled hypertensives are at high risk of cardiovascular morbidity and mortality (Erem, Hacihasanoglu, Kocak, Deger, & Topbas, 2009). A cross-sectional observational study done in Turkey demonstrated that Turkish hypertensive patients were not adequately evaluated for CVD risk (Kozan, 2011).

Given that T2D is the main cause of chronic kidney disease, it is essential to initiate screening and management programs for diabetic nephropathy, HTN, and CVD globally (Atkins, 2005; Parving, Lewis, Ravid, Remuzzi, & Hunsicker, 2006). Taking into account the scarcity of studies available among the Turkish immigrant population, it is important to examine biomarkers of modifiable health risks within this population. Therefore, the objective of this study was to examine the relationship between MAU as an indicator of kidney failure and HTN in patients with T2D. It was hypothesized that individuals with T2D who have HTN will have an increased likelihood of having elevated MAU.

METHODS AND PROCEDURES

Study participants

This study was a cross-sectional design and the sample consisted of 110 Turkish immigrants with T2D, aged 30 years and older. The participants were recruited from multiple sources in The Hague, Netherlands. During a 3-month period, approximately

300 letters written in Dutch and Turkish languages outlining the study were mailed to residents of The Hague with Turkish surnames listed in the local telephone directory. The interested participants could respond to the invitation letter. Due to unknown addresses, 1% of the unopened letters were returned undeliverable. Posters and flyers were also displayed at a family physician's office, dietitian's office, health club center, mosque, hairdresser, grocery store, community center, and pharmacies where Turkish immigrants are known to visit. From this, 11 participants were enrolled from the delivered mail, 60 participants were enrolled from the physician's office, and 20 participants from other sources. A local community representative recruited 19 eligible participants. Twenty-two potential participants did not qualify for the study because they were either not Turkish (n= 3), or did not have T2D (n= 8), or did not provide blood samples within the 3-month study period (n= 11). Interested participants were initially interviewed on the phone, at which time the study purpose was explained, and age and gender of the responders were recorded. To ascertain T2D status, each participant was asked for the age at diagnosis and initial treatment modalities. All participants were physician-diagnosed with T2D as having fasting serum glucose level of $\geq$ 6.1 mmol/L based on the classification recommended by the Dutch College of General Practitioners (NHG-standard) 2006 (Bouma, Rutten, de Grauw, Wiersma, & Goudswaard, 2006). The study was performed according to Dutch legislation regarding Ethics and Human Research and approved by The Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek). Florida International University Institutional Review Board (FIU-IRB) also approved the study protocol. Informed consent was obtained from all participants prior to the commencement of the study.

Blood pressure

Blood pressure (BP) was measured according to standard procedure by using an automated office blood pressure equipment (Myers et al., 2011). Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with participants in a sitting position in a quiet room for several minutes while readings were being taken. Measurements were performed twice and the average reading taken as the individual's BP.

Laboratory examination

Venous blood (20 mL) was collected after an 8-12h overnight fast, by a certified phlebotomist using standard laboratory techniques. Immediately after collection, blood samples were centrifuged, serum/plasma was separated, frozen and stored at -80°C for analysis. All measurements were performed in a hospital laboratory at Medisch Centrum Haaglanden. Urinary albumin was measured by immunoturbidimetric assay using Tina-quant Albumin, which is hardly influenced by endogenous and exogenous interfering factors (Hubbuch, 1991). Urinary creatinine was measured by a modified Jaffe method first described in 1886 (Jaffe, 1886). Glycated hemoglobin (A1C) was determined by high-pressure liquid chromatography (HPLC) using the Bio-Rad Variant II Turbo Hemoglobin A1C assay. This method is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Consensus Committee, 2007). Serum cotinine analysis was performed with an Immulite 1000 system (Siemens Medical

Solutions Diagnostics), a solid-phase competitive chemiluminescent immunoassay, using the manufacturer's Nicotine Metabolite Assay kit.

Measurements of risk variables

A trained physician assistant (PA) fluent in both Dutch and Turkish languages, interviewed all participants. The PA assisted and completed questionnaires on age, smoking status, prescription medication(s) use including diabetes medications, lipid-lowering drugs (LLD), and hypertension medications. The pilot investigator reviewed the questionnaires and the participants were contacted to inquire about any missing information. All anthropometric measurements were obtained using standard techniques (Schneider, 2007) with the participant wearing light clothing without shoes. Height (to the nearest 0.1 cm) was determined by using a wall-mounted stadiometer, and weight (to the nearest 0.1 kg) was determined by using a digital electronic scale. BMI was calculated as weight (kg) divided by height (m^2). Hypertension was defined as: SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or use of hypertension medications. Smokers were defined as having serum cotinine levels ≥ 25 ng/mL according to Medisch Centrum Haaglanden laboratory reference standards. The ACR was calculated to determine the presence of MAU and defined as ACR ≥ 3.5 mg/mmol for females or ACR ≥ 2.5 mg/mmol for males (Jerums & MacIsaac, 2002).

Statistical analyses

Chi-square tests were performed to compare percentage differences between participants with postive and negative MAU. Unadjusted and adjusted logistic regression

models were conducted to investigate the extent to which HTN is associated with an increased likelihood of having positive MAU. The logistic regression model was adjusted for age, gender, BMI, glycated hemoglobin (A1C), diabetes medications, lipid-lowering drugs (LLD), hypertension medications, and serum cotinine. Statistical analyses were performed using the SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA). Level of statistical significance was set at $P<0.05$.

RESULTS

Participants

Percentages or means and standard deviations of characteristics for Turkish immigrants with type 2 diabetes by microalbuminuria are presented in Table 1. Positive MAU was present in 21% (n= 23) of Turkish immigrants with T2D. There was a significantly higher percentage in males (32.6%) with positive MAU (87.5%) as compared to females (12.5%) (p= 0.011). There was a significantly higher percentage of participants with A1C levels ≥ 53 mmol/m (31.7%) with positive MAU as compared to participants with A1C levels < 53 mmol/m (14.5%) (p= 0.032). There was a significantly higher percentage of participants that were hypertensive (28.0%) with positive MAU as compared to participants that were normotensive (5.7%) (p= 0.007). There was a marginally significant higher percentage of participants with BMI ≥ 30 kg/m^2 (26.4%) as compared to participants with BMI < 30 kg/m^2 (10.5%) (p= 0.052).

Logistic regression

Unadjusted and adjusted odds ratios of positive MAU by hypertension status are presented in Table 2. Unadjusted odds ratio indicated that the odds of of having positive MAU were 6.4 times higher in hypertensive than those that were normotensive, 95% CI (1.41, 29.15). Adjusted odds ratio showed that after controlling for covariates, including age, gender, BMI, A1C, LLD, diabetes medications, hypertension medications, and serum cotinine, the odds of having positive MAU were 6.6 times higher in hypertensive than those that were normotensive, 95% CI (1.19, 36.39).

DISCUSSION

As compared to the native Dutch population, the Turkish immigrants in the Netherlands show (i) higher prevalence of T2D, (ii) higher prevalence of CVD, and (iii) lower prevalence of HTN (Kriegsman, van Langen, Valk, Stalman, & Boeke, 2003; Van Leest, van Dis, & Verschuren, 2002; Agyemang, Ujcic-Voortman, Uitenbroek, Foets, & Droomers, 2006). The lower prevalence of HTN among the Turkish could be due to awareness and increased treatment levels with hypertension medications. Nevertheless, our study revealed that hypertensive Turkish individuals with T2D were more likely to have elevated ACR compared to those that were normotensive. Additionally, positive MAU was present in 21% of our study population increasing their risk for CVD.

In the Systolic Hypertension in the Elderly Program (SHEP), SBP was a strong predictor of decline in renal function (Young, Klag, Muntner, Whyte, Pahor, & Coresh, 2002). In the Framingham study, pulse pressure predicted CVD in individuals aged more than 60 years, whereas DBP was the strongest predictor in those aged more than 50 years

(Franklin et al., 2001). We determined hypertension, identified as SBP ≥140 mmHg or DBP ≥ 90 mmHg or use of hypertension medications, to be a better measure of MAU given our aging study population. Boulatov, Stenehjem, and Os (2001) reported a higher prevalence of MAU in individuals with HTN and observed a significant relationship between ambulatory BP and ACR.

The results of this study showed that having HTN increased the likelihood of having positive MAU among Turkish immigrants with T2D living in the Netherlands. Our results suggest that hypertensives have a significantly higher percentage having positive MAU compared to normotensives. Ravid, Brosh, Ravid-Safran, Levy, and Rachmani (1998) indicated that risk factors including BP, BMI, A1C levels, male gender, and plasma cholesterol categorize individuals for poor renal and adverse CVD events. Maintaining adequate BP and glycemic control in individuals with T2D is an essential therapeutic goal for management of CVD (Zarini et al., 2011). Several studies showed that in hypertensive individuals with T2D, tight BP control can reduce the risk for diabetic nephropathy (Thomaseth, Pacini, Morelli, Tonolo, & Nosadini, 2008; UKPDS, 1998).

Various studies have reported remission and/or regression of MAU in individuals with T2D (Araki et al., 2005; Gaede, Tarnow, Vedel, Parving, & Pedersen, 2004). Araki et al. (2005) reported remission/regression of MAU in approximately 50% of individuals with T2D in a 6-year prospective study. Antihypertensive therapy and improved glycemic control were independent predictors for remission to normoalbuminuria in individuals with T2D (Gaede, Tarnow, Vedel, Parving, & Pedersen, 2004). A prospective study conducted by Mogensen et al. (2000) revealed that ACR decreased by 50% with a

combination treatment of antihypertensives. Furthermore, the authors suggested the beneficial therapeutic control of blood pressure for the prevention of diabetic nephropathy and CVD.

Various mechanisms have been suggested for MAU measurements and the best method to identify MAU has yet to be determined. Several studies showed a good correlation between MAU measurement alone and ACR in spot urine collection and 24-hour urine collection (Rodby, Rohde, Sharon, Pohl, Bain, & Lewis, 1995; Derhaschnig, Kittler, Woisetschläger, Bur, Herkner, & Hirschl, 2002). Gender specific cut-off values are recommended for ACR (Connell, Hollis, Tieszen, McMurray, & Dornan, 1994) and it is recommended that a positive result needs confirmation by 24-hour urine collection (Derhaschnig et al., 2002). However, research findings demonstrated a higher sensitivity of ACR than MAU measurement alone (James, Fotherby, & Potter, 1995).

Our study has several strengths. Participants included were all of Turkish origin, a single origin, in which research concerning MAU, HTN, T2D and CVD are limited. We used a standardized protocol, including uniform anthropometric and biochemical measurements in a clinical setting. We adjusted the analysis for all major confounders of MAU such as age, gender, BMI, A1C, LLD, diabetes medications, hypertension medications, and smoking.

There were a number of limitations for this study. First, because of the study's cross-sectional nature, our results do not establish causality and cannot be generalized for other populations. Our sample was not randomly selected and may not represent the Turkish immigrant population of the Netherlands. Second, given the small sample size (n= 110), this study's findings need to be tested with a larger sample size.

CONCLUSION

The present study shows that MAU is significantly associated with HTN in individuals with T2D. Further studies are needed to confirm our results and to examine other risk factors such as dietary intake that affect BP levels in Turkish immigrants with T2D. Research findings can contribute to the management of risk factors for CVD in Turkish patients with T2D. Adding MAU testing to standard diabetes care may be used for determining the potential risk for CVD and may direct treatment in the primary prevention of CVD. Further research should be done following cohorts to determine the effects of MAU and HTN for person with T2D on CVD morbidity and mortality.

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Table 1 – Percentages or means and standard deviations of characteristics for Turkish immigrants with type 2 diabetes by microalbuminuria (Netherlands, 2011)

	MAU		
Variables	Positive* (n= 23)	Negative (n= 87)	p
Age (years)			0.194
<55	26.5	73.5	
≥55	16.4	83.6	
Gender			**0.011**
Male	32.6	67.4	
Female	12.5	87.5	
Body mass index (kg/m^2)			**0.052**
<30	10.5	89.5	
≥30	26.4	73.6	
Glycated hemoglobin (mmol/m)			**0.032**
<53	14.5	85.5	
≥53	31.7	68.3	
Diabetes medications			0.336
No	16.7	83.3	
Yes	24.2	75.8	
Lipid lowering drugs			0.545
No	18.5	81.5	
Yes	23.2	76.8	
Serum cotinine (ng/mL)			0.189
<25	17.7	82.3	
≥25	29.0	71.0	
Hypertension			**0.007**
Normotensive	5.7	94.3	
Hypertensive	28.0	72.0	

Abbreviations: MAU; microalbuminuria.

*Positive MAU is defined as albumin-to-creatinine ratio ≥ 3.5 mg/mmol for females and/or albumin-to-creatinine ratio ≥ 2.5 mg/mmol for males.

Note: Hypertensive was defined as: systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or use of hypertension medications.

Table 2 – Unadjusted and adjusted* odds ratios of having positive microalbuminuria by hypertension status

	Unadjusted			Adjusted*		
Parameter	OR	95% CI	p	OR	95% CI	p
Normotensive[a]	1.00			1.00		
Hypertensive	6.42	1.41, 29.15	**0.007**	6.58	1.19, 36.39	**0.031**

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Reference category for p-value.

*Covariates included in the logistic regression analysis were age, gender, body mass index, glycated hemoglobin, diabetes medications, lipid-lowering drugs, and serum cotinine.

Note: Positive microalbumiuria is defined as albumin-to-creatinine ratio ≥ 3.5 mg/mmol for females and/or albumin-to-creatinine ratio ≥ 2.5 mg/mmol for males.

Note: Hypertensive was defined as: systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or use of hypertension medications.

6. SUMMARY AND DISCUSSION

The association of tobacco smoke, vitamin D status, anthropometric parameters, and kidney function in Turkish immigrants living in the Netherlands with type 2 diabetes (T2D) were studied. Cotinine levels, as a biomarker for smoke exposure, were positively associated with total cholesterol to HDL ratio (CHOL:HDL) and atherosclerosis-index (AI). Serum 25-hydroxyvitamin D [25(OH)D] levels were negatively associated with diastolic blood pressure (DBP). Gender-specific associations between anthropometric measures and high sensitivity C-reactive protein (hs-CRP) levels showed that hs-CRP was positively associated with waist circumference (WC) and waist-to-hip ratio (WHR) in males. There was a marginally significant association between hs-CRP and waist-to-height ratio (WHtR) in females only. Microalbuminuria (MAU) was present in 21% of Turkish immigrants with T2D. The odds of having positive MAU, as determined by albumin-to-creatinine ratio (ACR), were 6.58 times higher in participants with hypertension than those without hypertension adjusting for age, gender, body mass index, glycated hemoglobin, diabetes medications, lipid-lowering drugs, and serum cotinine.

The finding that smoke exposure is positively associated with symptoms of atherosclerosis is consistent with Venkatesan, Hemalatha, Bobby, Selvaraj, and Sathiyapriya (2006). In addition we found CHOL:HDL to be positively associated with smoke exposure. Our findings agree with other studies where there is a significant reduction in HDL levels in smokers than in non-smokers and an association between inhaled tobacco smoke and the atherosclerotic risk (Venkatesan, Hemalatha, Bobby, Selvaraj, & Sathiyapriya, 2006; Garrison, Kannel, Feinleib, Castelli, McNamara, & Padgett, 1978). Whig, Singh, Soni, and Bansal (1992) reported that tobacco smoking

predicted higher levels of total cholesterol (CHOL), low-density lipoprotein (LDL), and triglycerides (TG). However, we found no significant association with CHOL, LDL, and TG. There is limited research available regarding the effect of smoking on lipid levels and its role in CVD (Garrison, Kannel, Feinleib, Castelli, McNamara, & Padgett, 1978; Brischetto, Connor, Connor, & Matarazzo, 1983; Craig, Palomaki, & Fladdow, 1989; Stubbe, Eskilsson, & Nilsson-Ehle, 1982). This study additionally hypothesized that smoke exposure would be positively associated with blood pressure (BP). However, the results did not show an association between smoking and BP levels. Previous cross-sectional studies have reported that smokers have lower BP levels than non-smokers and suggested that this could be due to the possible rebound of blood pressure due to nicotine deprivation of smokers who refrain from smoking before a medical examination (Havlik, Garrison, Feinleib, Padgett, Castelli, & McNamara, 1980).

Several studies did not find an association between 25(OH)D and TG or HDL which is in agreement with our findings (Chiu, Chu, Go, & Saad, 2004; John, Noonan, Mannan, & Boucher, 2005). This study did not find a significant association of 25(OH)D with TG, HDL, and LDL. Ford, Ajani, McGuire, & Liu (2005), however, found 25(OH)D to be inversely associated with hypertriglyceridemia but did not find an association with 25(OH)D and HDL. Further research is required to fully understand the link between 25(OH)D and lipid levels. Findings of this study support the hypothesis that 25(OH)D will be negatively associated with BP. These findings are similar to other studies using data from the National Health and Nutrition Examination Survey (NHANES III) where researchers reported an inverse association between 25(OH)D levels and BP (Scragg, Sowers, & Bell, 2007; Looker, Dawson-Hughes, Calvo, Gunter, & Sahyoun, 2002). The

inverse association between 25(OH)D levels and BP could be due to regional and ethnic differences in hypertension (Rostand, 1997). Forman et al. (2007) reported 25(OH)D levels to be inversely associated with risk of hypertension. It is further hypothesized that 25(OH)D would be negatively associated with smoke exposure. Given the small sample size of active smokers in this study, finding a significant association with smoke exposure and 25(OH)D levels may be attributed to lack of power.

An association between abdominal obesity and elevated hs-CRP levels was found, which is in agreement with other studies (Onat, 2001; Shen, Farrell, Penedo, Schneiderman, & Orth-Gomer, 2010). These findings may be related to body fat distribution differences between males and females. These results suggest that WC and WHR were better measures than weight, body mass index (BMI), hip circumference, and WHtR in reflecting hs-CRP levels in Turkish males with T2D. Results from the Turkish Heart Study suggested that WHtR is a better predictor of CVD risk as compared to BMI, WC, and WHR (Can et al., 2009). These findings are similar to ours in which we found that WHtR was significant in reflecting hs-CRP levels in Turkish females with T2D. Research suggests that anthropometric measure of abdominal obesity is a better indicator of CVD (de Koning et al., 2007; Schneider et al., 2010).

Finally, the association between kidney function and HTN was assessed. The study supports the hypothesis that MAU will be positively associated with HTN. Boulatov, Stenehjem, and Os (2001) reported a higher prevalence of MAU in individuals with HTN and observed a significant relationship between ambulatory BP and ACR. Our results suggest that the odds of having a positive MAU are higher in hypertensives compared to normotensives. Ravid, Brosh, Ravid-Safran, Levy, and Rachmani (1998)

observed a similar finding where risk factors including BP, BMI, A1C levels, male gender, and plasma cholesterol categorized individuals for poor renal and adverse CVD events. Maintaining adequate BP and glycemic control in individuals with T2D is an essential therapeutic goal for management of CVD (Zarini et al., 2011).

7. STRENGTHS AND LIMITATIONS

This study had several strengths. Participants included were all of Turkish origin, a single origin providing homogeneity. Research concerning serum cotinine, 25(OH)D, hs-CRP, ACR, and CVD in individuals with T2D are limited. We used a standardized protocol, including uniform anthropometric and biochemical measurements in a clinical setting. All measurements were performed in a hospital laboratory at Medisch Centrum Haaglanden.

Serum cotinine is more practical for use in clinical settings for the prediction of smoking status compared to urine cotinine (de Weerd, Thomas, Kuster, Cikot, Steegers, 2002). The study was conducted during the Spring season to eliminate high or low sun exposure. We adjusted the analysis for all major confounders of cotinine and 25(OH)D such as age, gender, WC, diabetes medications, lipid-lowering drugs, and hypertension medications.

Serum hs-CRP is a more sensitive assay for the prediction of CVD compared to traditional assays for circulating C-reactive protein levels (Pearson et al., 2003). Hs-CRP levels >10 mg/L reflect acute inflammation and were excluded from the analysis (Chou, Hsu, Liu, Teng, Wu, & Ko, 2010). NSAIDs, LLD, hypertension medications, diabetes medications, smoking and A1C, which might have influenced the association of hs-CRP levels, were controlled for in the analysis (Onat et al., 2008; Prasad, 2006).

Research finding demonstrated a higher sensitivity of ACR compared with MAU measurement alone (James, Fotherby, & Potter, 1995). We adjusted the analysis for all major confounders of MAU such as age, gender, BMI, A1C, diabetes medications, LLD, hypertension medications, and smoking.

There were a number of limitations for this study. First, because of the study's cross-sectional nature, the results do not establish causality and cannot be generalized to all Turkish and other populations. Although CVD risk factors are ethnically specific, the sample was not random and may not represent the Turkish immigrant population of the Netherlands. Second, given the small sample size (n= 110) sufficient power was not always reached. Study findings need to be tested with a larger sample size.

8. FUTURE RESEARCH

Our findings indicate that serum cotinine, 25(OH)D, hs-CRP, and MAU may be assessed as a standard of care for T2D management in the Turkish immigrant population. Further research should be conducted following cohorts to determine the effects of these biomarkers on CVD morbidity and mortality. Prospective studies of diabetes outcomes are recommended and need to be further investigated in the Turkish immigrant population living in the Netherlands.

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2002	Bachelor of Science, Premedical/Preprofessional Track Certification: Biotechnology Florida Atlantic University Boca Raton, Florida
2006	Master of Public Health Graduate Certificate: Environmental and Occupational Health Graduate Certificate: Epidemiology Florida International University Miami, Florida
2007	Doctoral Candidate, Public Health Specialization: Environmental and Occupational Health Florida International University Miami, Florida

PROFESSIONAL EXPERIENCE

2006	Research and Evaluation Assistant U.S. Department of Health & Human Services Substance Abuse and Mental Health Services Administration Rockville, Maryland
2007-2008	Research Assistant South Florida Asthma Consortium Miami, Florida
2009-2010	Graduate Research Assistant Department of Environmental and Occupational Health Florida International University Miami, Florida
2011-2012	Graduate Research Assistant Department of Dietetics and Nutrition Florida International University Miami, Florida

ACADEMIC HONORS

2004-2005	The National Chancellor's List
2005-2006	The National Chancellor's List
2005-2006	Delta Epsilon Iota Academic Honor Society
2007-2008	Cambridge Who's Who Among Executives and Professionals
2008-2012	Golden Key International Honour Society
2012	Sigma Xi – The Scientific Research Society of North America

CONFERENCE PRESENTATIONS

Fisher, S.K., Blau, G.M., & Sukhram, S.D. Addressing suicide issues in systems of care. Presentation at the 19th Annual Research Conference: A system of care for children's mental health: Expanding the research base, Tampa, Florida, February 22-24, 2006.

Sukhram, S., Nair, R., Villalba, K., Melchior, M., Kunkle, B. MPH, and Gasana, J. Association between exposure to cats and dogs and risk of asthma. Poster presentation at the Florida Environmental Health Association 60th Annual Conference, Palm Beach, Florida, August 11-15, 2008.

Smith, B., Sukhram, S., Melchior, M., Sukhram, A., and Gasana, J. Occupational asthma and domestic Cleaners. Poster presentation at the Sunshine Education and Research Center for Occupational Health and Safety at the University of South Florida, Tampa, Florida, November 21, 2008.

Sukhram, S.D., Zarini, G., and Huffman, F.G. Obesity and serum high sensitivity C-reactive protein in Turkish immigrants with type 2 diabetes. Presentation at Experimental Biology, San Diego, California, April 21-25, 2012.

PUBLICATIONS IN PREPARATION

Sukhram, S.D., et al. Environmental influences on cardiovascular risk factors among Turkish immigrants in the Netherlands with type 2 diabetes.

Sukhram, S.D., et al. Obesity and serum high sensitivity C-reactive protein levels among Turkish immigrants in the Netherlands with type 2 diabetes.

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