Analyzing Anthropometry And Metabolic Variables Associated With Microalbumin And C-Reactive Protein As Markers Of Early Glomerular Dysfunction Among Mauritian Patients Suffering From Type II Diabetes.

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Abstract

Microalbuminuria and C-reactive are of particular importance in the early diagnosis of diabetic nephropathy. The present study was undertaken to determine microalbumin and CRP levels in patients suffering from type 2 diabetes and to assess possible correlation with metabolic and anthropometric variables. Ninety-two subjects; aged between 21-64 years of which 49 were healthy individuals and 43 patients suffering from type II diabetes constituted the study groups. Anthropometry measurements included blood pressure, body mass index and waist-hip ratio. Fasting venous blood samples and urine samples were collected from all subjects. Determination of biochemical parameters included fasting blood glucose, total cholesterol, HDL-cholesterol, triglycerides; HbA1c and C-reactive protein were measured. Urinary microalbumin and creatinine were also determined. Main findings showed that urine microalbumin, expressed as albumin-to-creatinine ratio was higher among diabetic patients (2.5±3.8) compared to non diabetic subjects (0.8±1.1) and were found to be significantly correlated with risk factors linked to diabetic related vascular diseases. In conclusion, based on present findings one could argue that urinary microalbumin may possibly be used as a marker for early indication of diabetic nephropathy.

INTRODUCTION

Type 2 diabetes is increasingly being recognized as an inflammatory condition associated with insulin resistance and vascular endothelial dysfunction (Nayak and Roberts, 2006, Lin et al., 2006). Alternatively, a chronic low-grade inflammatory condition has been proposed to underline increased risk for atherosclerotic disease, including renal dysfunction and cardiovascular disease (Brunner 2008) suggesting a possible link between the high incidence of macrovascular complications and diabetes. There is also some evidence to suggest that inflammatory condition may induce insulin resistance, thus increases risk of cardiovascular disease (Festa 2000, Pitsavos et al., 2007). Hyperglycaemia contributes largely to the development of endothelial dysfunction in diabetes (Hadi et al., 2007) and ultimately leads to albumin loss. Deckert et al., (1989) have reported that albumin leakage into the urine reflects widespread vascular damage and predispose to greater penetration of atherogenic lipoproteins into the arterial wall. Urinary albumin is therefore considered as an important marker for glomerular dysfunction (Satchel et al., 2008). Slightly elevated albumin excretion in urine, called microalbuminuria, is of particular importance in the early diagnosis of diabetic nephropathy (Tobe et al.,2002, Stehouwer and Smulders, 2006) Microalbuminuria arises from an increase passage of albumin through the glomerular filtration barrier (Satchel et al., 2008) as a result of increased permeability of the glomerular capillary wall, an increased intraglomerular pressure, or both (Tobe et al.,2002, Stehouwer and Smulders, 2006).

In addition, hyperglycaemia can increase intraglomerular pressure as well as alter the charge selectivity of the glomerular capillary wall, thereby increasing its permeability (Stehouwer and Gall, 2002). Some data suggest that microalbuminuria in type 2 diabetes, is associated not only with increased glomerular passage of albumin but also with an absence of a compensatory process in tubular reabsorption of albumin (Stehouwer and Gall, 2002).

Chronic low-grade inflammation and endothelial
dysfunction play a fundamental role from initiation and progression of atherothrombosis and the development of cardiovascular disease and microalbuminuria in diabetic patients, as well as in non diabetic individuals, is associated with both of them (Stehouwer and Gall, 2002). Therefore, it may be possible that endothelial dysfunction and chronic low-grade inflammation underlie the association between microalbuminuria and cardiovascular disease in type 2 diabetes (Stehouwer and Gall, 2002).

Certain studies have revealed increased plasma concentration of inflammatory markers and mediators such as C-reactive protein, fibrinogen, sialic acid, and IL-6 in patients with cardiovascular disease (Ridker et al., 2002, Ridker, 2003). Among them, C-reactive protein (CRP) has been demonstrated as an independent risk factor and a strong predictor of cardiovascular events.

CRP is an acute-phase protein produced mainly by liver cells under the control of interleukin-6 in response to tissue damage, infection, inflammation and malignant neoplasia (Stenhouwer and Gall, 2002). Previous findings have revealed that CRP is also produced by vascular smooth muscle cells and endothelial cells (Ridker et al., 2002). Increased levels of serum CRP, measures by highly sensitive assay (CRP-hs) are generally regarded as a sign of inflammation, and may identify individuals at moderate or high risk of cardiovascular disease (Savoya and Schiffrin, 2007).

Ridker et al. (2002) have shown that plasma CRP level is a powerful indicator of ischaemic cardiovascular events in patients with stable or unstable angina, and may even predict cardiovascular events among apparently healthy individuals. Observational studies have also suggested an independent role for CRP in the development of insulin resistance and diabetes, but it is unclear whether the association is a causal one or the consequence of inefficacy of the adipose tissue and other confounding factors (Nayak et al. 2006, Ridker et al. 2002). However, knowledge about a possible relationship of inflammation with urinary albumin in the microalbuminuria range is limited and controversial. Nayak et al., (2006) have reported a positive correlation between inflammatory markers and type 2 diabetes whilst Duncan et al, 2005) have shown the correlation is only within a certain groups. No such correlation data are presently available for Mauritian patients suffering from diabetes.

Over the past 20 years, the Mauritian population has been faced with a significant increased in the number of people suffering from type II diabetes. For instance, between 1986 and 1997 there has been a 40% increase in persons with diabetes. Mauritius is one of the countries with highest rate of prevalence of diabetes in the world with nearly one in five of our adults above the age of 30 years is suffering from diabetes (MOHQL, 2004).

The present study was undertaken to determine microalbumin and CRP levels among type 2 diabetic and non diabetic subjects and to establish possible correlation with metabolic and anthropometric variables.

MATERIALS AND METHOD

The study population consisted of ninety two subjects of whom 49 were healthy individuals and 43 patients with type 2 diabetes. Volunteers were informed of the details of the study and eligibility was determined after they completed a participant questionnaire and a consent form. The population sample included male and female subjects aged between 18-65 years, grouped in two categories: (a) individuals in good health, considered as control and (b) patients with type 2 diabetes.

Subjects below the age of 18 and above 65 years including smokers, pregnant women, patients with type I diabetes and patients suffering from inflammatory diseases such as rheumatoid arthritis were excluded from the study.

The study design incorporated a baseline survey which consisted of a self administered questionnaire on lifestyle and disease status. Subjects were assessed on information pertaining to diabetes-related diseases, smoking habits, alcohol intake and nutrient intake.

ANTHROPOMETRICAL MEASUREMENTS

Body height and weight were taken according to a standardized protocol. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²), and was used as an index of obesity. The WHO classification of BMI was used to estimate the degree of obesity, and is as follows: BMI>25 = overweight, BMI 25-29.9 = pre-obesity and BMI>30=obesity. Height was measured without shoes and waist and hip circumferences were measured using a standard clinician’s tape. Waist measurement was taken around the narrowest part of the waist at midpoint between umbilicus and xipoid, and hips measurement was taken at the widest point around the hips. The waist hip ratio (WHR) was determined by dividing the
waist measurement by the hip measurement. WHR is a specific indicator of central adiposity as opposed to general obesity indicated by BMI (Nayak et al., 2006). A WHR ≥ 0.96 for men and ≥ 0.83 for women defined by WHO (WHO, 1980) is significant for visceral obesity. Weight was taken using a standard balance.

**BLOOD PRESSURE MEASUREMENT**

Blood pressure (BP) was measured according to standard procedure using a mercury sphygmomanometer (Accoson, England) prior to taking of blood samples. Both systolic and diastolic blood pressures were measured after a 10 minutes rest with subjects in a sitting position. Measurements were performed twice and the second reading was taken as the individual’s blood pressure. The WHO (2002) definition of hypertension was used, i.e. systolic blood pressure ≥ 140 mm Hg and diastolic pressure ≥ 90 mmHg.

**BLOOD SAMPLES AND URINE COLLECTION**

Fasting blood samples were collected from subjects in a seated position after an overnight fast of at least 12 hours. The specimen was taken from the cubital fossa of the chosen arm by standard technique. A total of 10.0 ml of blood was collected and 2.5 ml were dispensed in appropriate coated and uncoated blood collecting tubes for determination of respective biochemical analytes.

**BIOCHEMISTRY**

**PLASMA**

For purpose of this study, the following biochemical investigations were carried out to determine: fasting blood glucose, total cholesterol, HDL-cholesterol, triglycerides, HbA1c and CRP-hs concentration on plasma or blood specimens, and microalbumin and creatinine concentration on urine specimens. All tests were carried out using commercially available kits. Dry chemistry reagents strips were used for urine analysis. Tests were carried out as per procedures laid out in the information sheets accompanying the kits.

Biochemical assays were performed using (i) Targa BT2000 plus automated chemistry analyzer (Biomedica Instruments SPA., Italy), for plasma glucose, total cholesterol and triglycerides. Urine creatinine was determined using HUMAN Biomedica and Diagnostica, (Germany). A Cobas Mira Plus automated chemistry analyzer (Roche Diagnostics, France) was used for determination of CRP-hs and HDL-cholesterol determination. An automated glycohaemoglobin analyzer (HLC-723G7,TOSOH Bioscience, Japan) was used for HbA1c determination and a PC-PIA-MAS gamma counter (STRATEC Biomedical Systems), for urine microalbumin determination.

Glucose, total cholesterol, triglycerides and HDL were determined using commercially available kits (Biosystem S.A., Barcelona (Spain). Low density lipoprotein (LDL-cholesterol) was calculated using the Friedewald formula. Plasma Glucose and HbA1c analysis were performed on the same day of blood collection. Plasma and serum were separated after centrifugation from blood collected in heparin and plain tube and stored at -20C until analyzed for lipids profile and CRP-hs. HDL-cholesterol was determined after centrifugation following precipitation of low density lipoprotein and chylomicron fractions by phosphotungstic acid in the presence of magnesium ions. The HDL-cholesterol concentration was determined in the supernatant using the CHOD-PAP method under the same principle as for total cholesterol. Triglycerides estimation was carried out by enzymatic colorimetric test (GPO-PAP method) using reagents from HUMAN Biomedica and Diagnostica, (Germany). Glycated haemoglobin (HbA1c) was assayed in EDTA whole blood based on the principle of high-performance liquid chromatography assay using an automated glycohaemoglobin analyser (HLC-723G7,TOSOH Bioscience, Japan).

**URINE ANALYSIS**

First morning urine samples from participants were collected in plain sterile urine containers on two different days and tested for proteinuria by urine protein dipsticks. The urine samples for both days were after that kept at 4-8 C for microalbumin and creatinine assays.

**URINE ALBUMIN AND MICROALBUMIN**

Urine albumin excretion was estimated by using urine protein reagent strips from Uritek Diagnostics. Urinary microalbumin concentration was determined by radioimmunoassay technique according to protocol on a PC-PIA-MAS Gamma Counter (STRATEC Biomedical Systems). Albumin was assayed based on the principle of a radioimmunological competition assay using commercially available RIA kits (Immunotech,Beckman coulter company, Czech Republic). For validation, control samples from the reagents kit and microalbumin control from APTEC Diagnostics were used. Tests for urine creatinine were performed on Targa BT2000 plus based on the principle of
Jaffe reaction. Commercially available reagents were from Biolabo Reagents (France).

**MICROALBUMIN-TO-CREATININE RATIO (UACR)**

UACR, used as a measure of albumin excretion in the microalbuminuric range, was calculated by dividing microalbumin concentration (mg/L) by urine creatinine concentration (mmol/L) and the results were expressed as mg/mmol. The UACR is more convenient to perform than a 24-hr urine collection for microalbumin estimation, and the results of these two tests have been shown to correlate highly (Tobe et al. 2002). The urine albumin-to-creatinine ratio (UACR) cut-off point of less than 30mg/mmol as recommended by the American Diabetes Association was used to define microalbuminuria. (ADA 2000). Reference ranges used for microalbuminuria in this study was 2.5-29.9 mg/mmol for male and 3.5-29.9 mg/mmol for female.

**QUALITY CONTROL**

Appropriate external quality controls were used for each test. Quality control reagents were treated similar as samples for determination of respective analytes. QC's from Serodos (human), Randox, TOSOH, Biosystems, Immunoteck were used for glucose & microalbumin, lipids, HbA1c, CRP and creatinine. Results were calculated against CRP-hs standard (human IgG) provided with the kit reagent system. Protein controls (level 1 and 2) from Biosystem S.A. (Barcelona, Spain) were used to validate results.

**STATISTICS**

Statistical analyses were performed on biochemical and anthropometrical measurements using SPSS software (version 10.0)... Results are expressed as mean ± SD. The comparisons among groups were done using statistical hypothesis procedure. The p value <0.05 was set as the level of significance. Pearson’s correlation test was used for correlation studies

**RESULTS**

Table 1.0 shows a summary statistics of biochemical and anthropometric variables in the study population.

**DISCUSSION**

Available literature suggests that inflammatory condition may induce insulin resistance leading to diabetes and ultimately may be a significant factor contributing towards the development of diabetic vascular complications (Brunner
et al., 2008). Furthermore the association of microalbuminuria with an increase risk of cardiovascular disease in diabetic population has been reported elsewhere (Nakamura et al., 2004) Although, CRP is a particularly useful marker of chronic inflammation and is being regarded as a marker of choice to assess cardiovascular risk (Shishedor et al., 2003) there is still some controversy regarding the relationship between these two markers (Nayak et al., 2006, Nayak and Bhakha, 2005).

Findings from the study showed a significant increase in microalbumin concentration in diabetic patients as compared to non diabetic subjects. This increase can be interpreted as an early sign of nephropathy changes in the diabetic patients, which could possibly be attributed to the degradation of the glomerular basement membrane and in particular, damage to the surface layer on the endothelium, the glycocalyx. As a result, there is an increased filtration of albumin through the damaged glomerular filtration barrier and hence an increased albumin loss in the urine.

In relation to lipids profile, it was observed that total cholesterol and LDL-cholesterol were unexpectedly lower among the diabetic patients. This may possibly be attributed to the intake of cholesterol lowering drugs or to the intake of low fat and carbohydrate diets. However, there was an increase in triglyceride levels in diabetic patients as compared to non-diabetic subjects. This increase in triglycerides is a common feature of diabetes mellitus. It is suggested that in diabetes, there is a reduced action of insulin on adipose tissue resulting in suppression of lipolysis. This result in reduced hydrolysis of stored triglycerides and hence non-esterified fatty acids are greatly increased (Nayak and Bhakha, 2005).

Salient findings from this study revealed a positive correlation between microalbuminuria and most of the risk factors of the metabolic syndrome; fasting glucose, HbA1c, triglycerides, blood pressure and body mass index, all characteristic features of metabolic syndrome whose underlying pathophysiology is thought to be related to insulin resistance (Kahn et al., 2005). Therefore, it can be argued that microalbuminuria may be related to insulin resistance in the prediction of cardiovascular events. Our findings also appear to be consistent with previous reports indicating the association of microalbuminuria with an increase risk of cardiovascular disease in diabetics (Stehouwer and Gall, 2002).

This study has shown a non significant increase in CRP-hs levels among diabetic patients. This finding could possibly be attributed to inherent inflammatory state associated with diabetes mellitus. However, no correlation was observed between CRP-hs and anthropometric variables as well as lipids profile with exception of triglycerides. This finding may therefore, suggest that CRP-hs act as an independent marker for cardiovascular risk factor and this is consistent with findings reported by (Nayak et al.,2006, Nayak and Bhakha, 2006), Furthermore, no correlation was also found between microalbuminuria and CRP-hs, thus suggesting that these inflammatory markers occur independently of each other. Festa et al. (2000) and Nakamura et al. (2004) have shown possible relationship between CRP-hs and microalbuminuria.

Positive correlations were found between CRP-hs and fasting glucose, HbA1c and triglycerides, all common features in diabetes mellitus. These finding are suggestive that CRP-hs may predict the development of diabetes. This is consistent with the findings of Pradhan et al. (2003) where they concluded that elevated levels of CRP-hs predict the development of type 2 diabetes. Contrarily, Brunner et al., (2008) suggested that it is unclear whether this association is causal for diabetes or is the consequence of the inefficacy of the adipose tissue.

Results from this study would tend to indicate that microalbuminuria could be used as an inflammatory marker in diabetes mellitus for diagnosis of early onset early diabetic nephropathy. The objective of this study was to correlate microalbumin with metabolic variables and CRP among in patients suffering from type 2 diabetes. Statistical analyses undertaken in this work has clearly demonstrated that there are definite correlations between the variables parameters studied in subjects with and without diabetes.

Our findings clearly showed that increased microalbumin and CRP levels were correlated cardiovascular risk factors such as hypertension and waist to hip ratios (p < 0.05) and based these results lend us to conclude that that the increased microalbumin and CRP correlated with metabolic variables in Mauritian type-2 diabetic patients. Although it is known that in diabetic cases, the underlying pathology may be occulted by other diseases that can cause deviation in laboratory measurements It should be pointed out that the present findings are not to be confounded with factors leading to the aberrant levels of CRP and microalbumin, especially being given that metabolic syndrome is known to
be associated with a cluster of risk factors that are directly or indirectly linked with onset of cardiovascular diseases (Subratty et al., 2007).

In conclusion, measurement of CRP, microalbumin, and waist to hip ratio along with the blood pressure should form part of a protocol to be recommended for all type 2 diabetic patients to reduce the cardiovascular risk.

References


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