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Original Article

Red blood cell distribution width and diabetes-associated complications

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ABSTRACT

Aim: Red blood cell distribution width (RDW) is a marker of cardiovascular morbidity and mortality. However, there is little data on the relationship between RDW and diabetes-associated complications. The aim was to investigate whether there is any association between RDW, nephropathy, neuropathy and peripheral arterial disease (PAD) in a type 2 diabetic population.

Methods: This study included 196 diabetic patients with proliferative diabetic retinopathy. All subjects were investigated for diabetic nephropathy, diabetic neuropathy and PAD. Participants underwent 24-h blood pressure monitoring and were analysed for markers of the metabolic syndrome, inflammation, and insulin resistance.

Results: 57% of the participants had diabetic nephropathy, 46% had diabetic neuropathy while 26% had PAD. No significant association was found between RDW, diabetic neuropathy and PAD (p = NS). However, RDW was strongly associated with diabetic nephropathy ($p = 0.006$), even following adjustment for potential confounding variables. Multivariate logistic regression analysis showed RDW (odds ratio [OR] 1.64, 95% confidence interval [CI] 1.15–2.35, $p = 0.006$), estimated glomerular filtration rate (OR 0.98, 95% CI 0.96–0.99, $p < 0.001$), night-time diastolic blood pressure (OR 1.07, 95% CI 1.03–1.11, $p = 0.001$) and erythrocyte sedimentation rate (OR 1.03, 95% CI 1.004–1.05, $p = 0.019$) to be independently associated with diabetic nephropathy.

Conclusions: This is the first study to report lack of association between RDW, neuropathy and PAD in subjects with type 2 diabetes mellitus. More importantly, RDW was shown to be significantly associated with diabetic nephropathy in a type 2 diabetic population with advanced proliferative retinopathy independent of traditional risk factors, including diabetes duration and glycaemic control.

1. Introduction

Red blood cell distribution width (RDW) is a measure of variation of the volume of red blood cells, thus "width" referring to the width of the distribution curve (distribution width) and not the width of the cells. It is routinely assessed as part of a standard complete blood count [1]. Increased RDW levels are seen in iron deficiency anaemia, folate and vitamin B12 deficiency anaemia, and in recent haemorrhage; it is related to impaired erythropoiesis or erythrocyte degradation [1]. Higher RDW levels have also been shown to be associated with increased cardiovascular mortality in the general population [2,3] and with increased cardiovascular morbidity and mortality in patients with known heart failure [4] and coronary artery disease (CAD) [5]. Increased RDW levels have also recently been shown to be strongly and independently associated with adverse cardiovascular outcomes in CAD patients undergoing percutaneous coronary interventions both in the acute [6,7] and elective settings [8].

In keeping with these studies, RDW is currently being recognised as a novel prognostic marker that reflects oxidative stress and chronic inflammation in patients with cardiovascular disease [9]. Diabetes mellitus is a chronic condition characterised by increased oxidative stress and vascular inflammation with consequent accelerated atherosclerosis and increased cardiovascular morbidity and mortality. The association between RDW and diabetes mellitus in stable CAD patients undergoing elective percutaneous coronary intervention has recently been investigated by Tsuboi et al. [9] whereby increased RDW was significantly associated with increased long-term all-cause mortality in diabetic patients after a mean follow-up duration of 3.9 years. In addition, data from the National Health and Nutrition Examination Survey

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(NHANES) have shown that higher RDW levels are associated with increased adjusted odds of myocardial infarction, heart failure, stroke and nephropathy in a nationally representative sample of USA adults with diabetes mellitus; the odds of developing diabetic retinopathy were not significantly increased [10]. However, there are no data on the relationship between RDW, peripheral arterial disease and peripheral neuropathy in a selected type 2 diabetic population.

We therefore sought to evaluate whether there is any significant and independent association of RDW with diabetic nephropathy, diabetic neuropathy and peripheral arterial disease in a type 2 diabetic population of long duration. This was performed by univariate analyses followed by multivariate analysis to adjust for multiple possible confounding variables, including anthropometric, and haematological ones as well as blood pressure, smoking, hyperinsulinaemia, insulin resistance and inflammatory markers.

Our study population comprised type 2 diabetic patients with proliferative diabetic retinopathy requiring laser treatment. Patients with proliferative retinopathy must have had sufficient exposure to chronic hyperglycaemia for a sufficiently long duration to have developed this complication. Studying such patients therefore allowed us to better investigate risk factors in addition to hyperglycaemia in the development of diabetes-associated macro- and microvascular complications. The statistical power of our study was also increased by studying such a high-risk population. Furthermore, studying a large number of factors allowed us to be in a better position to control for possible confounding factors and therefore to identify true associations of RDW with diabetic complications including diabetic nephropathy in type 2 diabetes mellitus.

2. Subjects, materials and methods

2.1. Subjects

A total of 209 diabetic patients were randomly selected and enrolled in this cross-sectional study, as approved by the University of Malta Research Ethics Committee. The inclusion criteria were the presence of type 2 diabetes mellitus (DM) and of proliferative retinopathy. Subjects were randomly selected (every fifth patient was chosen) from the list of subjects (available from the Ophthalmic outpatients, Mater Dei Hospital, Malta) who had undergone laser treatment for proliferative diabetic retinopathy between April 2006 and April 2008. Exclusion criteria included the presence of autoimmune disease, persistent urinary tract infection (making it impossible to assess for microalbuminuria) and normoalbuminuric renal impairment (eGFR < 60 mL/min/1.73 m^2). All participants gave written informed consent.

2.2. Experimental protocol

Patients were examined at the Diabetes Centre, Mater Dei Hospital, after an overnight fast. No medication was taken on the morning of the examination. Patients who were treated with insulin discontinued injections after 22:00 on the day preceding the examination. All patients were assessed for a past medical history of hypertension, hypercholesterolaemia, ischaemic heart disease (IHD), peripheral arterial disease (PAD), cerebrovascular accident (CVA)/transient ischaemic attack (TIA), as well as neuropathy. Medications taken and smoking history were also noted. In addition, all subjects underwent routine physical examination. Height and weight were measured using a calibrated balance and a stadiometer with the subject wearing light indoor clothing without shoes. Body mass index (BMI) was calculated as weight (kg) divided by height in metres squared. Waist index was calculated as waist circumference (cm) divided by 94 for men and 80 for women [11]. Office blood pressure was measured in the supine position after 5 min of rest. Metabolic syndrome was defined according to the International Diabetes Federation criteria [12].

The presence of distal peripheral neuropathy (DPN) was assessed using vibration perception threshold (VPT) as a marker of DPN. VPT is a well-established and validated marker of large fibre nerve function and has been shown to be reproducible [13], to correlate well with nerve conduction studies [14], and to predict foot ulceration [15], amputation, and mortality [16]. VPT was measured at the first metatarsophalangeal joints using a Horwell neurothesiometer in a two-step manner starting from 50 V with decreasing stimulation and then starting from 0 V with increasing stimulation. The subjects were asked to report when they began to feel or stopped feeling vibration [11]. The mean of the two measurements for the least sensitive foot was used in further analysis. DPN was defined as VPT ≥ 25 V [11].

Ankle-brachial pressure index (ABI) was determined in all subjects using a conventional Doppler machine (Bidop ES-100V3, Hadeco Inc, Kawasaki, Japan) as an indicator of PAD. It has been shown that ABI may be reliably used in diabetic patients, with the exception of those with ABI higher than the critical value, suggestive of medial arterial calcification (MAC) [17,18]. The lower value of ABI in either limb was used for analysis. Subjects were categorised into three groups according to the ABI measurements obtained: subjects with values between 0.9 and 1.29 were the reference group, those with an ABI < 0.9 were diagnosed with PAD [19], while ABI ≥ 1.3 was indicative of MAC [20]; the latter were thus excluded from the analysis.

Urinary albumin excretion was determined for each patient to identify subjects with diabetic nephropathy, defined by the presence of microalbuminuria (i.e. an albumin/creatinine ratio [ACR] greater than or equal to 2.5 mg/mmol in men or 3.5 mg/mmol in women) or macroalbuminuria (i.e. an ACR greater than or equal to 30 mg/mmol) in at least two out of three early-morning urine samples, in the absence of haematuria, pyuria, or a positive urine culture. Urine albumin was measured by an immunoturbidometric technique (Roche Diagnostics, Mannheim, Germany). Creatinine was measured by a kinetic colorimetric test using the Jaffe reaction (Roche Diagnostics).

Blood samples were taken in the overnight fasting state and the following investigations performed: full blood count (including RDW), renal and liver functions, total protein and albumin values, uric acid values, fasting plasma glucose (FPG), and fasting lipid profiles. Estimated glomerular filtration rate (eGFR) was calculated using the six-variable Modified Diet Renal Disease (MDRD) formula [21]. Haemoglobin A1c (measured using high performance liquid chromatography) was taken as a marker of glycaemic control. Erythrocyte sedimentation rate (ESR) and high sensitivity C reactive protein (hsCRP) were measured as markers of inflammation. hsCRP was measured by the Roche (Latex) assay. Serum insulin was assayed using an immunoenzymometric assay (IMMULITE 2000 insulin) and insulin resistance was estimated using the homeostatic model assessment (HOMA-IR) [22]. Glucose, total cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride concentrations were measured using Roche enzymatic in vitro tests (gluco-quant glucose/HK, cholesterol CHOD-PAP, HDL-C plus, and triglyceride GPO-PAR, respectively).

All patients had 24-h ambulatory blood pressure monitoring performed using TONOPORT V machines; means were calculated for systolic (SBP), diastolic (DBP) and arterial blood pressure (MAP), for both daytime and night-time. Pulse pressure (PP) was also calculated. A non-dipping blood pressure profile was defined as a nocturnal decline of mean arterial pressure of less than 10%.