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Marker of lipid peroxidation related to diabetic nephropathy in Indonesian type 2 diabetes mellitus patients

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ABSTRACT

Objective: Even though diabetes patients exhibit an increased oxidative stress, its correlation with diabetic nephropathy is not fully understood. The purpose of this study was to determine whether lipid peroxidation marker correlates well with eGFR and UACR in type 2 diabetes mellitus patients.

Methods: We collected urine and serum samples of Indonesian type 2 diabetes mellitus outpatients with normo- and microalbuminuria at a Local Government Clinic (from ages: 39–74 years). Urinary 8-iso-$PGF_{2\alpha}$ was measured by ELISA, the serum malondialdehyde by TBARS assay, and urinary albumin by BCG albumin assay. eGFR was calculated using the corrected-Cockcroft–Gault (CG), MDRD, and CKD-EPI equation. Other necessary data were obtained through questionnaires.

Results: The results showed that the increasing level of malondialdehyde was mildly correlated with the decline in eGFR (MDRD). In contrary, there was a significant positive correlation between 8-iso-$PGF_{2\alpha}$ concentration and eGFR based on the corrected-CG, MDRD study, and CKD-EPI equation ($r = 0.457$, $p < 0.001$; $r = 0.424$, $p < 0.001$; $r = 0.443$, $p < 0.001$). This relationship still persisted in the normoalbuminuric subjects ($n = 43$) ($r = 0.491$, $p = 0.001$; $r = 0.461$, $p = 0.002$; $r = 0.455$, $p = 0.002$). The multivariate analysis showed that 8-iso-$PGF_{2\alpha}$ together with fasting plasma glucose was the most predictive factor for the high 2-quantile eGFR (adjusted OR 1.001, (95% CI, 1.000–1.001)). However, there was no significant correlation between UACR with malondialdehyde ($r = 0.268$, $p = 0.050$) and 8-iso-$PGF_{2\alpha}$($r = -0.030$, $p = 0.808$). UACR itself was inversely correlated with eGFR based on the corrected-CG, the MDRD, and CKD-EPI ($r = -0.232$, $p < 0.05$; $r = -0.228$, $p < 0.05$; $r = -0.232$, $p < 0.05$).

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Conclusions: Increased 8-iso-$PGF_{2\alpha}$ and malondialdehyde in type 2 diabetes mellitus patients may play a role in the pathophysiologic significance of diabetic nephropathy, even while considering the effect of potential confounders.

1. Introduction

Diabetes and its associated complications, such as diabetic nephropathy have become a health problem of considerable magnitude. Diabetes cases in Indonesia (20–79 years) is predicted to reach a number of 14 million in 2035 [1]. Microvascular complications cause diabetic retinopathy and nephropathy, in which hyperglycemia plays an important role in their pathogenesis [2]. A cohort study which involved 1283 type 2 diabetes mellitus patients at the National Center General Hospital Dr. Cipto Mangunkusumo, Indonesia, in 2003–2006 showed that eventhough the prevalence of patients with creatinine serum $\geq$2 mg/dL was only 5.8%, but the prevalence of patients with estimated Glomerular Filtration Rate (eGFR) <60 ml/min/1.73 m^2 reached 13.2% based on the Modification of Diet in Renal Disease (MDRD) to 43.7% based on the BSA-corrected Cockcroft–Gault (CG) [3]. This phenomenon raised the need to investigate and validate a non-invasive early biomarker for diabetic nephropathy.

Hyperglycemia is one of the factors that create a state of constant and progressive damage to the vascular wall, manifested by endothelial dysfunction [2]. It is not clear to what extent the damage of vascular wall is caused by hyperglycemia. However, some studies found that in diabetes (type 1 and type 2), increased flux of free fatty acids and glucose is associated with increased mitochondrial reactive oxygen species (ROS) production and, as a consequence, increase the oxidative stress [4]. Hyperglycemic condition stimulates the generation of ROS through a number of enzymatic and non-enzymatic pathways. These include glucose oxidative phosphorylation, the polyol pathway, advanced-glycation end products, leakage during mitochondrial respiratory processes, NADPH oxidase activation, and uncoupling nitric oxide synthase (NOS) [4]. Malondialdehyde and 8-iso-Prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) are known as reliable oxidative stress biomarkers based on BOSS (Biomarker Oxidative Stress Study) [5]. Malondialdehyde is a highly reactive nucleophylic agent which could attack macromolecules, including amino acid or sulfhydril moiety of protein. It is generated by both lipid peroxidation and as a by-product of prostaglandin and thromboxane synthesis [6].

Apart from malondialdehyde, 8-iso-$PGF_{2\alpha}$ is also a lipid peroxidation product with prostaglandin-like structure produced primarily from esterified arachidonic acid in tissues by non-enzymatic reactions catalyzed by free radicals such as superoxide anion ($O_2^{\bullet -}$), in vivo. Urinary 8-iso-$PGF_{2\alpha}$ is one of the major urinary metabolite of F_2-isoprostane series which is not influenced by the lipid content of the diet [7] and is believed to be a useful marker for oxidative stress which can be assayed by non-invasive means.

Li et al. (2012) reported that high levels of malondialdehyde may link to the decline of kidney function with age [8]. It is also reported that malondialdehyde increases in diabetes and contributes to the atherosclerotic plaque deposits [9]. Increased 8-iso-$PGF_{2\alpha}$ synthesis is also reported to be responsible in part for the increase in renal TGF-β, a well-known mediator of diabetic nephropathy, in type 1 diabetes experimental rats [10].

However, it is still unclear whether oxidative stress is an initiating or progressing cause or it is just the consequence of diabetic nephropathy in type 2 diabetes mellitus patients.

Therefore, the aim of this study was to test the hypothesis that oxidative stress measured as serum malondialdehyde and urinary 8-iso-$PGF_{2\alpha}$ are associated with diabetic nephropathy in Indonesian type 2 diabetes mellitus patients, while considering the effect of potential confounders.

2. Patients and methods

2.1. Study design and study population

A prospective study of 16 men and 87 women (58.20 ± 7.84 years old) participants from the Local Government Clinic at Pasar Minggu District, South Jakarta, Indonesia was conducted and this is the first report of the results which were analyzed cross-sectionally. This study was approved prior to conduct by the Local Ethical Committee, Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo Hospital (No. 633/H2.F1/ETIK/VI/2013), and all subjects gave their written informed consent. Venous blood samples from patients were collected, and sera from individual subjects were transferred in several aliquots to prevent frequently freeze-thawing. Subsequently, the samples were stored at −20 °C until further analysis. The analysis was conducted up to 6 months after sample collection, except for the serum malondialdehyde, which was less than 1 month.

Apparatus: Spectrophotometer double beam (Shimadzu 1610), ELISA microplate reader (Biotek Elx808 and Thermo Scientific), and Microcentrifuge (Beckman and Labsco).

Chemicals: 1,1,3,3-tetraethoxypropane (Sigma–Aldrich, USA), creatinine (Merck, German), 8-iso-$PGF_{2\alpha}$ ELISA Kit (Abnova, USA), thiobarbituric acid (Merck, German), trichloroacetic acid (Merck, German), sodium hydroxide (Merck, German), picric acid (Merck, German), and distilled water.

2.2. Urine and serum sample collections and storage

Blood was collected (5 ml) from each participant by a venipuncture into vacutainers tube without anticoagulant. The blood was centrifuged at 3000 rpm for 10 min, separated