Early detection of diabetic nephropathy induced by vascular endothelial dysfunction: emerging new biomarkers

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ABSTRACT

Diabetic nephropathy is the leading cause of kidney failure all over the world. Vascular endothelial dysfunction (VED) is thought to play a key role in progression of diabetic nephropathy. This study aimed to evaluate the potential new biomarker; asymmetric dimethylarginine (ADMA) for early detection of diabetic nephropathy. Furthermore, the role of oxidative stress in pathogenesis of vascular endothelial dysfunction was investigated via determination of 8-isoprostane, nitric oxide and glutathione peroxidase.

The diabetic subjects under study were divided into non-dialyzed nephritic patients, hemodialyzed patients (pre- and post- hemodialysis) and kidney transplanted patients together with normal healthy control. The results showed drastic elevation in the levels of ADMA and 8-isoprostane in the diabetic patients before and after hemodialysis sessions when compared with normal control group. In contrast, ADMA and 8-isoprostane levels were improved following kidney transplantation. In the meantime, the levels of nitric oxide and GSHPx were statistically decreased in all groups except in those patients undergo kidney transplantation. In conclusion, there are associations between oxidative stress markers with the progression of VED, while increased ADMA level predict the induction of nephropathy in poor glycemic control patients and hence it could be used as a useful promising biomarker for early detection of diabetic nephropathy. Targeting on reducing oxidative stress and lowering ADMA levels may be specific therapeutic interventions to prevent diabetic nephropathy progression.

Keywords: *Diabetic nephropathy, vascular endothelial dysfunction, ADMA, 8-isoprostane & oxidative stress.*

INTRODUCTION

Despite intensive glycemic control, individuals with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are predisposed to developing vascular complications, which include cardiomyopathy, atherosclerosis, nephropathy, retinopathy, and neuropathy. Indeed, there is a striking correlation between the incidence of cardiovascular diseases and mortality rates in diabetic patients ***(Sharma et al., 2012)***.

The effects of chronic hyperglycemia, hyperlipidemia and hypertension in patients with diabetes mellitus (DM) take places these individuals at high risk for microvascular and macrovascular complications. Approximately 80% of patients with T2DM will succumb to cardiovascular complications such as stroke, peripheral arterial disease, and heart disease**.** Although DM carries a 1.5- to 4.5-fold risk of cardiovascular mortality, the microvascular complications of DM (e.g., retinopathy, nephropathy, and neuropathy) can have devastating effects on patient's quality of life ***(Centers for Disease Control and Prevention, 2007)***.

The microvascular and macrovascular complications of DM are believed to be caused by a process known as oxidative stress. Intracellular oxidative stress occurs when the production of reactive oxygen species (by-products of normal metabolism) exceeds the capacity of the cell's antioxidants to neutralize them. Endothelial cells chronically exposed to oxidative stress favor the induction of specific long-term complication pathways ***(Unger, 2008)***.

Diabetic nephropathy is the leading cause of kidney failure all over the world. The overall risk of developing diabetic nephropathy varies between about 10% of T2DM to about 30% of T1DM ***(Assal et al., 2009)***.

Compelling evidence has been provided that both insulin-dependent and non-insulin-dependent diabetic patients are under conditions of oxidative stress and that the complications of diabetes mellitus (there after indicated as diabetes) could be partially mediated by oxidative stress ***(Davı` et al 2005; Ceriello 2006)***.

High glucose generates reactive oxygen species (ROS) as a result of glucose auto-oxidation, metabolism and

formation of advanced glycation end-products. Reactive oxygen species are important mediators of vascular complications in diabetes ***(Miyata et al., 2008)***.

Asymmetric dimethylarginine (ADMA) is a naturally occurring endogenous L-arginine metabolite with the capacity to inhibit all three isoforms of NO synthases. ADMA and, to a greater extent, its stereoisomer symmetrical dimethylarginine (SDMA), which has no effect on NO synthases, have been reported to accumulate in patients with renal failure. Although ADMA is excreted by the kidneys to some extent, the major metabolic pathway is degradation by the dimethylarginine dimethylaminohydrolases (DDAHs) $DDAH_1$ and $DDAH_2$ into dimethylamine and L-citrulline. *In vitro*, hyperglycemia impairs DDAH activity in vascular smooth muscle cells and the endothelium, thereby contributing to elevated ADMA levels among diabetic patients ***(Assal et al., 2009)***.

The role of increased plasma ADMA concentrations in endothelial dysfunction and vascular injury has been studied in various conditions such as preeclampsia, diabetes, stroke, and peripheral vascular and coronary heart disease. It has also been proposed that increased ADMA blood levels

contribute to progression of chronic kidney disease, but so far only experimental data exist in support for this hypothesis. This notion is of considerable interest, because plasma ADMA concentrations were found to be already increased in early stages of renal disease, and the kidney itself seems to be an important organ of ADMA metabolism ***(Assal et al., 2009)***.

Isoprostanes are prostaglandin (PG)-like substances that are produced *in vivo* independently of cyclooxygenase (COX) enzymes, primarily by free radical induced peroxidation of arachidonic acid. The formation of PG-like compounds during auto-oxidation of polyunsaturated fatty acids was first reported in the mid-1970s, but isoprostanes were not discovered to be formed *in vivo* in humans until 1990. F_2-isoprostanes are a group of 64 compounds isomeric in structure to cyclooxygenase-derived $PGF_{2\alpha}$. Other products of the isoprostane pathway are also formed *in vivo* by rearrangement of labile PGH_2-like isoprostane intermediates. These include E_2- and D_2-isoprostanes, cyclopentenone-A_2- and J_2-isoprostanes, and highly reactive acyclic-ketoaldehydes (isoketals) ***(Grosso et al., 2008)***.

In addition to being markers of oxidative stress, F_2-isoprostanes seemed to be mediators of important biological effects. The first to be revealed was the vasoconstriction of renal glomerular arterioles, as demonstrated by the direct infusion of 15-F_{2t}-IsoP, previously indicated as 8-epi-$PGF_{2\alpha}$ or 8-iso-$PGF_{2\alpha}$ (the most tested and generally evaluated isomer of the F_2-IsoP series), into the renal artery. It seems to act through the activation of receptors analogous or identical to those for the thromboxane A_2 (TxA_2) receptors (TPr's). This effect is believed to be very important in the explanation of the hepato-renal syndrome, in which the initial production of F_2-IsoPs would occur in the liver; the latter would induce vasoconstrictory effects in the kidney, resulting in the full feature of renal failure ***(Comporti et al., 2008)***.

AIM OF THE WORK

The aim of this study was to evaluate the best early prognostic biomarkers in Egyptian patients with type 2 DM complicated with diabetic nephropathy induced by vascular endothelial dysfunction (VED); receiving either conservative treatment or regular hemodialysis or even undergo kidney transplantation. This was achieved through the evaluation of:

- Asymmetric dimethylarginine (ADMA) and nitric oxide levels, to evaluate the role of VED in progression of diabetic nephropathy.
- 8-isoprostane & plasma glutathione peroxidase to evaluate the potential link between oxidative stress and pathogenesis of diabetic nephropathy.

In addition, lipids profile was assessed to reflect the presence of microvascular complications.

Moreover, to assess any correlation between these parameters and diabetic nephropathy stages.

Review of literature

Diabetes Mellitus (DM):

The rapidly increasing prevalence of diabetes mellitus worldwide is one of the most serious and challenging health problems in the 21st century ***(Van den Oever et al., 2010)***.

According to the World Health Organization, diabetes mellitus now affects about 220 million people worldwide, and the growth in its prevalence represents a global health crisis already accounting for more than 10% of the total healthcare expenditure in many countries. In the USA, over 24 million children and adults (almost 8% of the entire population) have diabetes, whereas another 57 million have prediabetes and are thus likely to develop the disease unless they make lifestyle changes. Diabetes without proper treatment can cause many complications, with cardiovascular diseases accounting for up to 80% of premature mortality ***(World Health Organization, 2009).***

The number of people with diabetes grows faster than expected. In 2007, 246 million people (roughly 6%) were affected worldwide and it is estimated that this will increase to 380 million, or 7.3% by 2025. Furthermore, it is estimated

that there are even more people (308 million or 8.1%) with impaired glucose tolerance (IGT). These people have a significant risk of developing type 2 diabetes mellitus (T2DM) ***(Van den Oever et al., 2010)***.

Type 2 diabetes mellitus (T2DM) accounts for 90%–95% of all diabetes and is more common in people older than 45 who are overweight. There is strong evidence that genetics plays an important role as well. However, the prevalence of T2DM is becoming higher in children and young adults because of the higher rate of obesity in this population ***(Van den Oever et al., 2010)***.

Central obesity and insulin resistance next to diabetes, high cholesterol and high blood pressure form the most important risk factors for cardiovascular disease (CVD). CVD is the major cause of death in people with T2DM.

Diabetes is also the leading cause of blindness, renal failure, and lower limb amputations ***(International Diabetes Federation, 2006)***.