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## Diabetic nephropathy and antioxidants

Majid Tavafi <sup>1,\*</sup>

<sup>1</sup>Department of Anatomy, Lorestan University of Medical Sciences, Faculty of Medicine, Khoram Abad, Iran.

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### ABSTRACT

**Context:** Oxidative stress has crucial role in pathogenesis of diabetic nephropathy (DN). Despite satisfactory results from antioxidant therapy in rodent, antioxidant therapy showed conflicting results in combat with DN in diabetic patients.

**Evidence Acquisitions:** Directory of Open Access Journals (DOAJ), Google Scholar, Pubmed (NLM), LISTA (EBSCO) and Web of Science have been searched.

**Results:** Treatment of DN in human are insufficient with rennin angiotensin system (RAS) blockers, so additional agent ought to combine with this management. Meanwhile based on DN pathogenesis and evidences in experimental and human researches, the antioxidants are the best candidate. New multi-property antioxidants may be improved human DN that show high power antioxidant capacity, long half-life time, high permeability to mitochondrion, improve body antioxidants enzymes activity and anti-inflammatory effects.

**Conclusions:** Based on this review and our studies on diabetic rats, rosmarinic acid a multi-property antioxidant may be useful in DN patients, but of course, needs to be proven in clinical trials studies.

*Implication for health policy/practice/research/medical education:*

Treatment of diabetic nephropathy (DN) in human by rennin angiotensin system blockers is inadequate and antioxidants ought to couple with this treatment. Classical antioxidants have not shown beneficial effect in diabetic patients. New multi -property antioxidants may be ameliorate human DN that show high power antioxidant capacity, anti inflammatory effect, long half-life time, high permeability to mitochondrion and improve body antioxidants enzyme activity. Rosmarinic acid, multi properties antioxidant may be useful in DN patients, but of course, needs to be proven in clinical trials studies.

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### 1. Context

**D**iabetic nephropathy is the common cause of leading to end-stage of renal disease (ESRD) (1). Diabetic nephropathy is a progressive and irreversible renal disease characterized by the accumulation of extra cellular matrix in glomerular mesangium and kidney intersti-

tial tissue that eventually leads to renal failure (2).

### 2. Evidence Acquisition

Directory of Open Access Journals (DOAJ) Google Scholar, Pubmed (NLM), LISTA (EBSCO) and Web of Science were searched with key words relevant to diabetic nephropathy, antioxidants and

\*Corresponding author: Dr. Majid Tavafi, Department of Anatomy, Lorestan University of Medical Sciences, Faculty of Medicine, Khoram Abad, Iran. Tel: +989188527146, Fax: +989188527146, E-mail: tavafi.m@lums.ac.ir

oxidative stress.

### 3. Results

Several mechanisms are thought to be involved in the pathogenesis of diabetic nephropathy and its complications, all of them originating from hyperglycemia. Some of these pathways are: increasing and activation of intra-renal rennin angiotensin system (RAS), formation of advanced glycation end products (AGEs), polyol pathway activation, aldol reductase activation, activation of protein kinase C (PKC), increase of some cytokines – such as insulin like growth factor-1 (IGF-1), transforming growth factor beta (TGF- $\beta$ )- and the oxidative stress pathway (1-5).

There are many evidences that oxidative stress plays a key role in the most pathogenic pathways of diabetic complications (6). Free radicals such as superoxide can induce cell and tissue injuries throughout lipid peroxidation, activation of nuclear factor of Kappa-Beta (7), production of peroxynitrite, PKC activation and induction of apoptosis. Furthermore, reactive oxygen species (ROS) and other free radicals can directly induce injury. Oxidative stress activate pathogenic pathways such as RAS, polyol pathway, PKC-B and AGEs (1-8). AgII activate NADPH oxidase that leads to the superoxide ions formation (1-7-9). AGEs can induce ROS production and activate PKC by induction of oxidative stress in mesangial cell (10).

Experimental researches established the role of oxidative stress as a central factor in onset and progression of diabetic nephropathy (11-14). Human studies also showed that oxidative stress markers such as 8-oxodG (oxo-2'-deoxyguanosine) (15-19), 8-iso PGF2 (20) and MDA (21) increased in diabetic patients.

Interestingly, oxidative stress has been suggested as a common product of much of mecha-

nisms that are involved in pathogenesis of diabetic nephropathy (2-13). In fact, in the tangle web of diabetic nephropathy pathogenesis, oxidative stress activates other pathogenic pathways, other pathways make injury via oxidative stress, and oxidative stress directly leads to injury. Thus, inhibition of oxidative stress may constitute a focal point for multiple therapeutic synergies.

#### *3.1. Results from antioxidant therapy in experimental diabetic nephropathy*

Experimental researches showed that antioxidant therapy ameliorated or inhibited diabetic nephropathy in rodents. There are reports that glomerular hypertrophy inhibited through consumption of herbal antioxidants extract such as garlic, ginger (22) and ginkgo biloba (23) in diabetic animals. In addition, glomerular hypertrophy inhibited by antioxidants such as Vit E and alpha tochopherol in diabetic rats (24-25).

Our studies showed that glomerular number conserved by administration of satureja khazestanica essential oil (SKEO) and rosmarinic acid (RA) in diabetic rats (26-27).

Some researchers reported the amelioration of glomerulosclerosis with use of root extract of *lindera strychnifolia* (28), extract of aerial parts of *Aster koraiensis* (29), SKEO (26) and rosmarinic acid (27). There are reports that glomerulosclerosis inhibited or decreased by Vit C, alpha lipoic acid, Vit E (30-32) and ferolic acid, a polyphenolic antioxidant in diabetic rats (33). Coenzyme Q10 also ameliorated glomerulosclerosis and improved tissue antioxidant enzymes in diabetic rat (34).

Inhibition of increased serum MDA in treated diabetic rats reported with use of rosemary extract (35), Vit E (13), ginger (36), alpha lipoic acid (32), SKEO (26) and rosmarinic acid (27).

Some researchers reported the amelioration

of creatinine in the diabetic animals treated by extract of the root of *lindera strychnifolia* (29), ginkgo biloba extract (37), Vit E and tocotrienol (13-38), SKEO (39), RA (40) and fenugreek (14).

Therefore based on the findings antioxidant therapy must be one of the most important strategies in combat with diabetic nephropathy progression.

### 3.2. Results from antioxidant therapy in diabetic patients

Studies in this field are limited and there are no enough data from human studies. Ceriello reported that Vit E have failed to show beneficial effect on diabetic complication (41) and high dose of Vit E fail to prevent albuminuria while lower doses exacerbate renal injury (42,43).

Two intervention studies that used Vit E or  $\beta$ -carotene supplementation in patients with type 2 diabetes did not show a positive effect on the development of type 2 diabetes (44,45).

Antioxidant supplementation studies have produced conflicting results in endothelial function, retinal blood flow and renal function outcomes (46,47).

Oral Vit E treatment normalized elevated baseline creatinine clearance in diabetic patients with type 1 diabetic without inducing a significant change in glycemic control in an 8-month randomized double-masked placebo-controlled crossover trial (48).

Gaede et al reported that Vit E (680 mg/day) and Vit C (1250 mg/day) combination significantly improved renal function in Type 2 diabetes (47).

Treatment with high-dose benfothiamine reduced albuminuria in patients with Type 2 diabetes (49).

HbA1C levels were significantly reduced by high dose Vit E antioxidant supplementation, suggesting that antioxidants may have some ad-

vantage in protecting against the complications of type 2 diabetes (50).

Bardoxolone methyl, a novel synthetic triterpenoid with antioxidant and anti-inflammatory properties, improved kidney function in patients with advanced DN already receiving RAS blockers, with few adverse events (51). Silymarin a herbal drug with antioxidant and anti-inflammatory effects reduces urinary excretion of albumin, TNF- $\alpha$ , and MDA in patients with diabetic nephropathy and may be considered as a novel addition to the anti-diabetic nephropathy treatment (52).

## 4. Discussion

At the present DN manages by means of RAS blockers. Drugs such as angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) currently are the main strategy of DN management. However, despite RAS inhibition DN progress to ESRD in a large proportion of diabetic patients (53). In other word in addition to activation of RAS system, other pathways are involved in the DN pathogenesis and combined therapy must be introduced to block pathways.

Based on molecular mechanisms of diabetic nephropathy pathogenesis that mentioned in introduction, and increase of oxidative stress markers in experimental and diabetics patient, there is no doubt that oxidative stress plays a pivotal or central role in the initiation and progression of diabetic complications (5-41-54). Besides epidemiological studies have demonstrated association between inflammatory and oxidative stress markers with cardiovascular and renal outcomes in chronic kidney disease (CKD) and ESRD (55-57). Thus combined therapy with antioxidants and anti-inflammatory agent may be leads to satisfactory results.

The most known free radicals involving in the diabetic nephropathy pathogenesis are reactive oxygen species (ROS) such as superoxide ( $-O_2^-$ ), hydroxyl ( $-OH$ ), and peroxy ( $-RO_2$ ) and non radical species such as hydrogen peroxide ( $H_2O_2$ ) and hydrochlorous acid (HOCl) and reactive nitrogen species produced from Similar pathways, which include the radicals nitric oxide ( $-NO$ ) and nitrogen dioxide ( $-NO_2$ ), as well as the nonradical peroxy nitrite ( $ONOO^-$ ), nitrous oxide ( $HNO_2$ ), and alkyl peroxy nitrates (RONOO). Of these,  $-O_2^-$ ,  $-NO$ ,  $H_2O_2$ , and  $ONOO^-$  have been the most widely investigated in the diabetic kidney (58). There are a number of enzymatic and no enzymatic sources of ROS in the diabetic kidney, including auto oxidation of glucose, transition metal-catalyzed Fenton reactions, advanced glycation, polyol pathway flux, mitochondrial respiratory chain deficiencies, xanthine oxidase activity, peroxidase, nitric oxide synthase (NOS) and NADPH oxidase (58).

Human body combat against free radicals by natural defense with antioxidant enzymes and exogenous antioxidants. Reactive oxygen species can be eliminated by a number of enzymatic and no enzymatic antioxidant mechanisms. Super oxide dismutase (SOD) immediately converts  $\bullet O_2^-$  to  $H_2O_2$ , which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase (GPX) in the mitochondria, catalase that invert  $H_2O_2$  to  $O_2$  and  $H_2O$ . Another enzyme is glutathione reductase, which regenerates glutathione that is used as a hydrogen donor by GPX during the elimination of  $H_2O_2$ . No enzymatic antioxidants include vitamins A, C and E; glutathione;  $\alpha$ -lipoic acid; carotenoids; trace elements like copper, zinc and selenium; coenzyme Q10 (CoQ10); and cofactors like folic acid, uric acid, albumin, and vitamins B1, B2, B6 and B12 (59).

In diabetic nephropathy, structural injury de-

velops over years before clinical and laboratory abnormalities such as albuminuria, hypertension, or declining glomerular filtration rate appear (60). Thus, waiting for clinical or laboratory manifestation of DN without initiating treatment may hinder the efforts that prevent progression to ESRD.

Since oxidative stress appears to play an important role as an early etiologic factor in diabetic nephropathy and later progression (33), we suggest antioxidant therapy as one of the most important treatment strategies for diabetic patients without nephropathy for the prevention and slowing of diabetic nephropathy before reaching to ESRD.

Antioxidant supplementation studies have shown conflicting results in endothelial function and renal function outcomes in diabetic patients (46). Antioxidants per se have demonstrated minimal renoprotection in humans despite positive preclinical research findings. However, the classical antioxidants, such as vitamins E and C, do not appear to be helpful (41). Some clinical evidences for the effectiveness of antioxidants on the treatment of diabetic nephropathy have not been established and there are several reports that indicated the absence of improvement and even worsening of diabetic nephropathy with antioxidant treatment (61). According to these studies antioxidant supplementation such as vitamin use, may not be the ideal antioxidant strategy in human diabetic nephropathy. However, some studies that used combined antioxidants therapy or antioxidant with anti-inflammatory agent showed that improvement of albuminuria, HbA1C and MDA in diabetic patients (49-52).

Why the antioxidant therapy in diabetic patients has not shown suitable results despite of pivotal role of oxidative stress in diabetic injury induction? Some researchers believe in, may be classical antioxidants have low penetration in to specific cellular organelles such as mitochondrion (58-59).

May be said that conventional antioxidants are neutralize reactive oxygen molecules on a one-for-one basis, whereas hyperglycemia-induced overproduction of superoxide is a continuous process. Based on observations of the beneficial effects of over expression of antioxidant enzymes in mouse models, what is needed is a new type of antioxidant, a catalytic antioxidant, such as an SOD/catalase mimetic (62). The most our information's about DN and antioxidant have been yield from diabetic animal model studies, but rodent models of diabetic atherosclerosis, cardiomyopathy, and, indeed, any diabetic microvascular complications do not recapitulate major aspects of the phenotypes of human diabetic complications. Large animal models such as pigs or nonhuman primates, in which diabetic cardiovascular diseases(CVD) more closely resembles that in humans, have been generated (5,63,64).

However, the limitation of clinical study results on the beneficial effects of antioxidant vitamins in DN management should not discourage us from more basic and clinical research on this issue because relation between oxidative stress and diabetic nephropathy were established. The experimental data demonstrate that the metabolic abnormalities of diabetes cause mitochondrial superoxide over production. This increased superoxide production is the central and major mediator of diabetes tissue damage (5). In future studies the new antioxidants will be preferred that show multiple effect against DN pathogenesis pathways.

Therefore, new strategies with combination of classic antioxidants as well as new antioxidants – synthetic or plant origin should be implemented in the treatment of diabetes. Because of in diabetic nephropathy, structural injury develops over years before appearing of clinical and laboratory findings, ARBs and antioxidant therapy must be

started after diabetes identification to prevent and slowing of reaching to diabetic nephropathy or ESRD. RA a phenolic compound showed properties including: antioxidant (65-66), anti inflammatory (67,68), reducing NF $\kappa$ B, increasing glutathione transferase, anti Bcl-2 activity (59) and scavenger of peroxynitrite (69). Moreover we found that RA ameliorates DN in rats (27) and improve activity of kidney SOD, GPX and catalase in gentamicin induced nephrotoxicity(70) .

However, based on DN pathogenesis that reviewed and evidences from animal and human studies, for achieving successful antioxidant therapy in future, the new suggestions introduce to investigate in animals and especially clinical trial studies.

- Continuous use of antioxidants with long half-life time.
- Increase of antioxidant concentration in specific cellular organelle such as mitochondrion.
- Explore and use of antioxidants that show anti-inflammatory, blood lowering pressure effect and increase body antioxidant enzymes activity.
- Test of new antioxidants in diabetic pigs or primates.
- Investigation of antioxidants that improve activity of SOD, GPX and catalase in kidney such as rosmarinic acid (70) and olive leave extract (71) in clinical trial researches.

## 5. Conclusions

Treatment of DN in human by RAS blockers is inadequate and antioxidants ought to couple with this treatment. Classical antioxidants have not shown beneficial effect in diabetic patients. New multi -property antioxidants may be ameliorate human DN that show high power antioxidant capacity, anti inflammatory effect, long half-life time, high permeability to mitochondrion and improve body antioxidants enzyme activity. Ros-

marinic acid, multi properties antioxidant may be useful in DN patients, but of course, needs to be proven in clinical trials studies.

### Conflict of interest

The authors declared no competing interests.

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