

## Therapeutic Potential effects of pyridoxine and /or ascorbic acid on Microalbuminuria in diabetes mellitus patient's: a randomized controlled clinical study

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### Abstract

**Objectives:** This study aimed to evaluate the therapeutic potential effects of ascorbic acid or and pyridoxine on diabetic renal microalbuminuria.

**Methods:** This was a cross-sectional study on patients with diabetes mellitus at Al-Yarmouk teaching hospital from January to December 2012, Iraq-Baghdad. Twenty one patients with diabetes mellitus (D.M), 8 IDDM and 13 NIDDM were selected from, the duration of disease were ranged from 2-12 years for both type (10 females and 11males) and all enrolled patients ages were ranged from 28-65years. The concentration of total protein in urine was calculated by a biuret colorimetric assay and the urine creatinine level was measured by a modified Jaffe test. Statistical analysis: results are expressed as mean  $\pm$  SD, for comparisons of two groups, Student's t-test was used and statistical significance was accepted at p values < 0.05.

**Results:** pyridoxine produced significant reduction in urinary albumin:creatinine ratio in patients with Type II D.M with the current therapy p<0.05 except with glimepiride p >0.05 while the Ascorbic acid showed significant effect on albumin:creatinine in patients with Type II D.M after six week of treatment p <0.05except on patient that treated with glibenclamide or glimepiride p<0.05. Combined effects of ascorbic acid 500 mg/day and pyridoxine 40mg/day on urinary albumin:creatinine produced significant reduction in albumin: creatinine ratio in both Type I D.M and Type II D.M p <0.05.

**Conclusions:** Dual synergistic effects of ascorbic acid and pyridoxine produced more beneficial effects than either ascorbic acid or pyridoxine in amelioration of diabetic microalbuminuric nephropathy.

### Key words:

Pyridoxine, Ascorbic Acid, Microalbuminuria

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### INTRODUCTION

Microalbuminuria define as when the kidney excrete small amounts of albumin into the urine, or when there is an rising permeability for albumin in the renal glomerulus apparatus also microalbuminuria can be diagnosed from a 24-hour

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urine collection (between 30–300 mg/24 hours) or, more frequently, from eminent concentrations in a spot sample (30 to 300 mg/L), mutually must be measured on at least two of three measurements over a 2-3 month period <sup>1</sup>.

Normally up to 150 mg a day of protein may be excreted by a normal person, primarily called Tamm-Horsfall protein. Proteinuria is distinct as a protein/creatinine ratio greater than 45 mg/mmol (which is equivalent to albumin/creatinine ratio of greater than 30 mg/mmol or approximately 300 mg/g), normally albumin /creatinine ratio < 2.5 in male and < 3.5 in female <sup>2</sup>.

Proteinuria principally requests appropriate diagnosis and the most common cause is diabetic nephropathy; so good glycemic control may slow the progression of diabetic nephropathy and medical management via angiotensin converting enzyme (ACE) inhibitors, which are classically first-line remedy for proteinuria but; in patients whose proteinuria is not restricted with ACE inhibitors, the addition of an aldosterone antagonist or angiotensin receptor blocker (ARB) may promote the reduction in the protein loss diabetic nephropathy . <sup>3</sup>

Pyridoxal phosphate the metabolically dynamic form of pyridoxine is concerned in many aspects of macronutrient metabolism , gene expression and commonly serves as a coenzyme for a lot of reactions and can assist facilitation of decarboxylation, transamination, racemization, and interconversion reactions and many studies concern the link of pyridoxine with diabetic nephropathy management . <sup>4</sup>

Ascorbic acid is called L-hexuronic acid, since it is derivative from glucose, many animals are capable to create it, but humans necessitate it as part of their nutrition. <sup>5</sup>

Ascorbate frequently acts as an antioxidant; it typically reacts with oxidants of the reactive oxygen species, such as the hydroxyl radical formed from hydrogen peroxide. Such radicals are damaging to cell at the molecular level due to their probable interaction with nucleic acids, proteins, and lipids.

Occasionally these radicals start chain reactions and ascorbate can cease these chain reactions by electron transfer and because many antioxidants have a role in diabetic nephropathy like vitamin E so ascorbic acid introduced in this study. <sup>6</sup>

While; albuminuria is an important indicator for the beginning and development of renal diseases, the mechanism by which albuminuria is caused still remains a subject of dispute and many studies have investigated the tubular role in the postglomerular processing of albumin on the onset of albuminuria<sup>7</sup>. Beneath physiological circumstances the slit diaphragm may preserve this steadiness in response to changes in filtration pressure. This would need steady reorganization of the podocyte foot process and the slit diaphragm apparatus, but the mechanisms underlying the turnover of slit diaphragm proteins are mainly unidentified and recent studies have provided confirmation that podocytes have a means function in the progress of albuminuria <sup>8</sup>.

The confirmation implicates that the metabolic consequences of hyperglycemia as the majority important contributory factor in the expansion of diabetic nephropathy a study of randomized clinical trials first established that insistent control of blood sugar decreases the progress of nephropathy, as well as other microvascular complications, in type 1 diabetes and repeat renal biopsies have recognized that the renal lesions of diabetic nephropathy may reverse after long-term pancreas transplantation moreover; hyperglycemia leads to augment generation of reactive oxygen species; depletion of the reduced form of nicotinamide dinucleotide , activation of the polyol pathway, which can direct to de novo-synthesis of diacylglycerol and amplified protein kinase C activity; alterations in the hexosamine pathway; and non-enzymatic protein glycation (advanced glycosylation end products), all of which have been concerned in development of diabetic nephropathy as well as other diabetic microvasculopathies. <sup>9-11</sup>

So this study aimed to evaluate the therapeutic potential effects of ascorbic acid or and pyridoxine on diabetic renal microalbuminuria.

**Patients and methods**

This study was carried out in Departments of Pharmacology and internal medicine, College of Medicine, Al-mustansiriya University, Baghdad – Iraq, from October to December 2012. It is approved by scientific jury of Department of Pharmacology and internal medicine and licensed by board of medical college.

Twenty one patients(10 females and 11males) with diabetes mellitus (D.M), 8 IDDM (with or without metformine ) and 13 IDDM (on glibenclamide or glimepiride with or without metformin) were selected from Al-Yarmouk teaching hospital, the duration of disease ranged from 2-12 years for both type and all enrolled patients ages were ranged from 28-65years. Instantaneously earlier to the collection phase, the patients provided a spot mid-stream urine sample. The urine A/C ratio was determined on spot urine specimens. The concentration of total protein in urine was calculated by a biuret colorimetric assay (Cobas Integra Analyzer, F Hoffman-La Roche, Basel, Switzerland), and the urine creatinine level was measured by a modified Jaffe test (Hitachi 7170 autoanalyzer, Hitachi, Tokyo, Japan). The urine A/Cratio was obtained by dividing the urinary protein concentration by the urine creatinine concentration. <sup>12, 13</sup>

Random urine sample sample can estimate urinary albumin:creatinine ratio the normal range 2.5mg/mmol and 3.5mg/mmol in male and female respectively.

$$\frac{\text{Urine albumin } (\mu\text{g/dL})}{\text{Urine creatinine (mg/dL)}} = \text{UACR in mg/g} \sim \sim \text{Albumin excretion in mg/day}$$

UACR (urinary albumin creatinine ratio) is a ratio between two measured substances, unlike a dipstick test for albumin, it is unaffected by variation in urine concentration.

The urine A/C ratio was measured before and after intake pyridoxine tablet 40mg or ascorbic acid tablet 500mg or both through 6 weeks duration. The drugs were purchased from private pharmaceutical company, pyridoxine 40 mg (kontam pharmaceutical co.ltd, china) and ascorbic acid 500mg (Remedicaltd.Limassol-cyprus,Europe) The characteristic of this study summarized in table (10).

**Table 1:** The characteristic of study

Number	21 patients 13NIDDM 8IDDM
Gender	10 females and 11males
Age	28-65 years
Duration of disease	2-15 years
Current therapy	Insulin, metformin, glibenclamide and glimepiride
Additional therapy	Ascorbic acid or pyridoxine or both

Statistical analysis: results are expressed as mean ± SD, for comparisons of two groups, Student’s t-test was used and statistical significance was accepted at p values < 0.05.

**RESULTS**

Pyridoxine produced significant effects on urinary albumin:creatinine ratio in patients with Type II D.M with the current therapy except with Glimepride ( table 2).

**Table 2:** effects of pyridoxine 40mg/day on urinary albumin:creatinine mg/mmol after six weeks regarding the current treatment in Type II D.M (13 patients).

Type II D.M	Before (mean ±SD)	after (mean ±SD)	P value
Glibenclamide	5.45±0.336	2.56±0.674	<0.05*
Glimepiride	4.66±0.55	4.11±0.321	>0.05
metformin	4.77±0.372	3.22±0.612	<0.05*
Glibenclamide +insulin	5.23±0.732	3.11±0.462	<0.05*

<0.05\*: significant effects

Ascorbic acid showed significant effect on albumin:creatinine in patients with Type II D.M after

six week of treatment except on patient that treated with Glibenclamid or Glimepride (table 3).

**Table 3:** effects of ascorbic acid 500 mg/day on urinary albumin: creatinine mg/mmol after six weeks regarding the current treatment in Type II D.M (13 patients).

Type II D.M	Before (mean ±SD)	after (mean ±SD)	P value
Glibenclamid	5.45±0.336	4.33±0.424	>0.05
Glimepride	4.66±0.55	4.11±0.221	>0.05
Metformin	4.77±0.372	3.99±0.682	<0.05*
Glibenclamid+insulin	5.23±0.732	3.27±0.342	<0.05*

<0.05\*: significant effects

In type I D.M pyridoxine and ascorbic acid effects showed significant effects on urinary albumin:creatinine ratio regarding P value <0.05 table( 4).

**Table 4:** effects of ascorbic acid 500 mg/day and pyridoxine 40mg/day on urinary albumin:creatinine mg/mmol after six weeks regarding the current treatment in Type I D.M(8 patients).

Type I D.M	ascorbic acid 500 mg/day			pyridoxine 40mg/day		
	Before (mean ±SD)	after (mean ±SD)	P value	Before (mean ±SD)	after (mean ±SD)	P value
insulin	5.66±0.578	3.11±0.442	<0.05*	5.66±0.578	2.99±0.261	<0.05*
Insulin + Metformin	5.77±0.322	2.99±0.765	<0.05*	5.77±0.322	3.11±0.437	<0.05*

<0.05\*: significant effects

Combined effects of ascorbic acid 500 mg/day and pyridoxine 40mg/day on urinary microalbuminuria showed significant reduction in albumin: creatinine ratio in both Type I D.M and Type II D.M table (5).

**Table 5:** Combined effects of ascorbic acid 500 mg/day and pyridoxine 40mg/day on albumin: creatinine ratio in both Type I D.M and Type II D.M

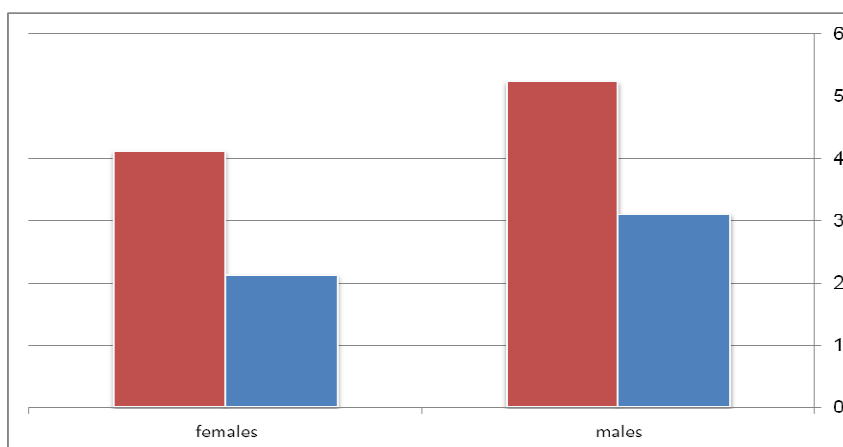
A/C ratio mg/mmol	Type I D.M			Type II D.M		
	Before (mean ±SD)	after (mean ±SD)	P value	Before (mean ±SD)	after (mean ±SD)	P value
	5.66±0.578	2.01±0.212	<0.05*	5.66±0.578	2.33±0.221	<0.05*
	5.77±0.322	1.19±0.765	<0.05*	5.77±0.322	1.99±0.127	<0.05*

<0.05\*: significant effects A/C: albumin: creatinine Regarding gender differences in the reduction of albumin:creatinine ratio after combined therapy of ascorbic acid 500 mg/day and pyridoxine 40mg/day table FEMALE respond more than male ( 6) and figure (1).

**Table 6:** gender differences in the reduction of albumin:creatinine ratio after combined therapy of ascorbic acid and pyridoxine.

Gender	Number	A/C ratio mg/mmol		P value
		before(mean ±SD)	after(mean ±SD)	
male	11	5.23±0.732	3.11±0.732	<0.05*
female	10	4.12±0.336	2.12±0.336	<0.05*

<0.05\*: significant effects A/C: albumin: creatinine



**Figure 1:** gender differences in the reduction of albumin:creatinine ratio after combined therapy of pyridoxine and ascorbic acid .

## DISCUSSION

Diabetic nephropathy is a progressive turn down in glomerular filtration rate, associated by proteinuria and other end-organ complications such as retinopathy, diabetic nephropathy progresses to renal disease via a number of stages starting as normoalbuminuria, microalbuminuria, macroalbuminuria and then end-stage renal disease these increased by hyperglycemia and hypertension. Renal disease in diabetic patients is characterized by hyperfiltration and hyperperfusion in addition to structural abnormalities and metabolic changes and any renal cell types are pretentious by hyperglycemic damage like podocytes, mesangial endothelial cells, tubular epithelial cells, interstitial fibroblasts, and vascular endothelia. These lead to thickening of basement membranes, mesangial extension and hypertrophy and glomerular epithelial cell failure.<sup>14-17</sup>The present study showed that pyridoxine alone exhibited significant reduction in microalbuminuria and albumin/creatinine ratio both in type I D.M and type II D.M regardless of gender differences except in patients that treated with Glimpeptide.

The beneficial effect of pyridoxine has been confirmed in multiple animal models of diabetes and in phase II clinical trials. Though, the mechanism of pyridoxine effects is inadequately understood but the possible mechanism is scavenging of pathogenic reactive carbonyl species establish to be important in diabetes, and the pathogenicity of reactive carbonyl species is a methylglyoxal which may be due to modification of serious arginine residues in matrix proteins and interfering with renal cell-matrix interactions, this methylglyoxal effect can be inhibited by pyridoxine. The physiological reactive carbonyl species 3-deoxyglucosone can damage glomerular mesangial cells. So; the therapeutic effect of pyridoxine is achieved, via protection of renal cell-matrix from damage by a variety of reactive carbonyl species.<sup>18</sup>

Moreover; hyperglycemia changes lipids and proteins by nonenzymatic binding of glucose residues via a

sequence of biochemical reactions, leading to the creation of advanced glycation end products that induce direct injury to the mesangial cells and podocytes in diabetic glomerular region, also glycation end products mediate their actions by receptor-dependent or -independent mechanisms. The receptors for glycation end products are presented on podocytes, and the inhibition of their activity minimize the mesangial expansion. Consequently, inhibition of glycation end products formation seems to be an striking therapeutic choice that may alter the pathogenesis and retard the progression of diabetic albuminuria. Inhibitors of advanced glycation end products are aspirin, thiamine, thiazolidinediones, carnosines, angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptors blocker (ARBs), and pyridoxine<sup>19, 20</sup>.

Williams et al studied were done in patients with mild to moderate type 1 and type 2 diabetic nephropathy, pyridoxine appeared to have a significant consequence in dropping the slope of creatinine change from baseline compared with placebo, and the effect was even more remarkable among the subgroup with type 2 diabetes. Although pyridoxine did not affect urine albumin excretion, it significantly reduced plasma advanced glycation products level, decreasing toxic effects of reactive oxygen species and scavenging reactive carbonyl compounds.<sup>21</sup>

All these studies supports results of present study and explained the beneficial effects of pyridoxine in improving microalbuminuria in diabetic patients in spite of short duration (6weeks) and small daily dose 40mg/day. Additionally; first advance to reduce the development of advanced glycation end products by interference at one of the many steps concerned in the formation of advanced glycation end products, such as by aminoguanidine. Aminoguanidine was the first compound planned to inhibit advanced glycation end products formation. Metformin, which is characteristically used in the treatment of type 2 diabetic patients, has some structural similarities to



aminoguanidine it reduces methylglyoxal, an important precursor of advanced glycation end products formation in type 2 diabetes. So; the beneficial effects of metformin in type 2 diabetic patients, related to these specific effects on advanced glycation end products accumulation and pyridoxine is a natural potent inhibitor of the formation of advanced glycation end products and striking effects of pyridoxine, in delayed advance of nephropathy and retinopathy, have been confirmed in diabetic rats when combined with metformin.<sup>22-24</sup>

Co-administration of metformin plus Pyridoxine appears to decrease the frequency of insulin resistance, reduce insulin requisite and as a result lessen the incidence of adverse effects linked to high-dose insulin therapy.<sup>25</sup>

In the present study the using metformin in both type of diabetes in conjugation with either pyridoxine or ascorbic acid showed significant reduction in albumin/creatinine ratio, these finding correspond with the previous studies.

Oxidative stress have a responsibility in the pathogenesis of diabetic complications and it is thought that agents that regularize reactive oxygen species might be helpful in the regression of diabetic nephropathy, even though it has not been directly tested in this study since hyperglycemia-induced generation of free radicals, associated to the development and advance of diabetic nephropathy so ameliorating oxidative stress via antioxidants may be an proficient advance for reducing diabetic complications.<sup>26</sup>

Abundant clinical trials investigated the consequence of the antioxidant vitamin E on the prevention of diabetic complications. Free radicals can trigger quite a lot of damaging pathways in diabetes including accelerated arrangement of advanced glycation end products and polyol pathway which have been established to be concerned in microvascular complications<sup>27</sup>.

sources of oxidative stress in diabetes may be nonenzymatic or enzymatic pathway, the non-

enzymatic sources of oxidative stress initiate from the oxidation of glucose since hyperglycemia can directly cause increased reactive oxygen species generation also, glucose autoxidation and generate hydroxyl radicals also, glucose reacts with proteins in a non-enzymatic way causing formation of Amadori products followed by development of advanced glycation end products. Moreover; in hyperglycemia, there is enhanced metabolism of glucose through the sorbitol pathway, which also augment production of oxidative species while Enzymatic sources of reactive species in diabetes include NOS, NAD(P)H oxidase and xanthine oxidase enzymes<sup>28</sup>. Non-enzymatic antioxidants include vitamins A, C and E; glutathione;  $\alpha$ -lipoic acid, carotenoids and pyridoxine.<sup>29</sup> Vitamin C is a water-soluble vitamin that prevents lipid peroxidation and both Enzymatic and Non-enzymatic glucose oxidation and generations of Hydroxyl radicals.<sup>30</sup>

Newly; it has been postulated that antioxidant efficiency of vitamins such as C and E is fractional since these antioxidants act as scavengers of reachable surplus reactive species in a mode correspond to come near to oxidative stress connected clinical problems.<sup>31, 32</sup>

Furthermore; sharp direction of prominent doses of ascorbic acid has been exposed to diminish negative sound effects of oxidative stress, such as smoking, on endothelial function and microvascular flow<sup>33</sup>.

Ascorbic acid antioxidant may refill endogenous antioxidant pools in patients with diabetes and consequently attach to the resistance alongside oxidative stress.<sup>34</sup> Nonetheless, unremitting oral treatment abortive to display an increased accessibility assessed at the level of individual capillaries in diabetic patients following a 2-4 week treatment period with ascorbic acid though, some studies do summit to a encouraging contact of oral ascorbic acid in diabetic patients<sup>35</sup>.

Numerous mechanisms of ascorbic acid may be rapid, within hours after intravascular and oral administration, as indicated via a numeral of

preceding studies <sup>36</sup>, and the treatment era in the present study was elected on the foundation of this fact.

Ascorbic acid is a water-soluble agent, allow for fast interactions between circulating blood constituents and the endothelium, as is raxofelast, another water-soluble antioxidant which was exposed to proceed endothelial function after only 1 week of oral treatment in Type II diabetes <sup>37</sup>. Extra feasible property of more long-term treatment cannot be prohibited and may even be caused by miscellaneous mechanisms than those of acute effects. The patients in the present study served as their own matched controls due to the cross-over design.

In adding to defending lipids basement membrane of glomerular mesangial cells from peroxidation, vitamin C is consideration to scavenge reactive oxygen species and therefore guard endothelium derived NO, via inhibition formation of peroxynitrite <sup>38</sup>. The acute consequence of vitamin C in improving endothelial function in patients with Type II diabetes has been accredited to this mechanism. <sup>39</sup>

The be deficient in of an result of oral vitamin C in patients with Type II diabetes may narrate to the foundation of the oxidant stress. Vitamin C is effectual in scavenging extracellular radicals, but comparatively ineffective at scavenging intracellular radicals, and it is probable that the final is a more significant source of radical's generation in diabetes than in other circumstances linked with increased oxidative stress, chiefly smoking. It is improbable that treatment for a longer period would extra considerably augment intracellular concentrations of vitamin C since, at lower daily doses of 100-500 mg, plasma concentrations plateau after 3 weeks, saturating intracellular ascorbic acid levels <sup>40</sup>. We cannot, though, eliminate the opportunity that a longer duration of treatment with a diverse dose of vitamin C may be efficient in lowering oxidative stress and or humanizing endothelial function. It is also potential that high doses may have an unfavorable effect: there is proof that high

concentrations may cause oxidative damage to DNA and may certainly lower NO bioactivity <sup>41</sup>. So in the present study the dose of ascorbic acid was within the intermediate range to avoid DNA damage ,and most of previous studies support the beneficial effects of ascorbic acid alone lowering diabetic renal deterioration via amelioration of A/C ratio ,thus ;extracellular antioxidant( ascorbic acid) with intracellular antioxidant(pyridoxine) produced more significant effects in reduction of oxidative stress and then amelioration of A/C ratio in diabetic patients .Unfortunately ; oxidative stress marker not measured directly in this study .

Moreover; gender responding to the effects of antioxidant was different in this study and the effects of gender has been explained by choosing sex-specific factors for the designation of abnormal ranges of A/C . Though, this sex-specific method has not been established separately, and race-specific factors have not been strong-minded. <sup>42</sup>

It has earlier been documented that ACR values are biased in relation to albumin excretion rate (AER) by determinants of urine creatinine excretion, particularly gender. Creatinine is generated in muscle, and urinary excretion is the main character for generated creatinine, so muscle mass is a major factor of urine creatinine excretion. Since men have greater muscle mass than women, men have greater urine creatinine excretion than women, and, on typical, men have lower ACR values than women for a given AER. As a result, gender-specific thresholds have been developed, recommended, and used for the classification of urine ACR, with lower threshold levels used for men than women. Also; women had only a somewhat minor occurrence than did men if we had used the unadjusted measure for microalbuminuria, A/C of 25 mg/g or more . Population studies of albumin excretion rate have also shown that men excrete albumin at a superior rate than women. In compare, other population based studies account privileged occurrence of microalbuminuria among women than among men.

Nevertheless, modification of creatinine excretion in a conduct analogous to that used here would lead to high predominance of microalbuminuria in men than in women<sup>43-48</sup>

### CONCLUSION

Dual synergistic effects of ascorbic acid and pyridoxine produced more beneficial effects than either ascorbic acid or pyridoxine in amelioration of diabetic microalbuminuric nephropathy.

### Conflict of interest

All other authors have no conflict of interest to declare.

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