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Evaluation and study the effect of asymmetric dimethylarginine in chronic renal failure patients treated by hemodialysis

تقدير ودراسة تأثير غير المتناظرة ثنائي مثيل الارجنين لدى مرضى العجز الكلوي الذين تتم معالجتهم بالغسل الدموي

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Abstract:

In term of morbidity and mortality, chronic renal failure (CRF) is among the most serious chronic disease which result from the permanent loss of kidney function and without a regular course of dialysis or kidney transplantation , the disease is fatal.

Recent studies indicated that in end stage renal disease(ESRD) , there is a new cardiovascular risk factors include: Homocysteine (Hcy) and Asymmetric dimethylarginine(ADMA).

Hyperhomosyct(e)inaemia (HHC) a consistent finding in uraemic patients is now widely recognized as an independent risk factor for vascular disease . HHC is associated with an increased risk of myocardial infarction, stroke, and venous thromboembolism.

The other risk factor asymmetric dimethyl arginine (ADMA), endogenous inhibitor of nitric oxide synthase (NOS) is also elevated in chronic renal failure (CRF).

The aim of this study was to determine the effect of ADMA concentration on renal function in hemodialysis patients (HD).also urea and creatinine and electrolyte was examined.

Blood samples were obtained from 20 apparently healthy subjects (10 male and 10 female), patients with renal failure, treated by hemodialysis, 25(12 male and 13 female). Plasma concentration of ADMA was significantly higher than those of control (P-value $\geq$ 0.05).

الخلاصة:

لازالت حالات قصور الكليه المزمن تمثل مشكله صحية كبيره . خصوصا" وان حالات الوفيات من جراء هذا المرض ازدادت بشكل ملحوظ في الاونه الاخيره وتنتج عادة" من فقدان الكلية لوظيفتها بشكل جزئي أو كلي .

وبدون إجراء العلاج المتمثل إما بالغسل الدموي المنتظم أو زرع الكليه فان المرض سيعد خطرو مميت.

تشير الدراسات ألحديثه إلى وجود مخاطر أخرى متمثله بمعايير جديدة لها تأثير على إمراض القلب المتسببة بالوفاة لدى هؤلاء المرضى وهي:

الهوموسيستين , والعامل المتسبب في تثبيط الانزيم المسؤول عن تخليق اوكسيد النتريك NO (غير المتناظره ثنائي مثيل ارجنين Asymmetric dimethylarginine ADMA) .

إن ارتفاع الهوموسيستين له علاقة بإمراض القلب المتمثلة بالسكتة القلبية والجلطة الدماغية وتصلب الأوعية الدموية(الشرايين).كما إن زيادة تركيز الهوموسيستين أصبح ألان منتشرا" لدى مرضى الكله ويعتبر عامل خطورة ومؤشر لإمراض القلب .عامل الخطورة الأخر ADMA وجد إن تركيزه يرتفع لدى مرضى العجز الكلوي

إن الهدف من الدراسة هو تعيين تاثير زيادة تركيز المركب غير المتناظرة ثنائي مثيل الارجنين لدى مرضى العجز الكلوي الذين يتم علاجهم عن طريق الديلزه الدموية .

لقد تم تجميع نماذج الدم من 20شخص يمثلون المجموعة الضابطة ظاهريا (الاصحاء10 ذكور و10 إناث) إما مرضى العجز الكلوي عددهم 25 مريض(12 ذكور و 13 إناث) ووجد إن تركيز المركب (ADMA) يزداد بشكل معنوي لدى مرضى العجز الكلوي مقارنة بالمجموعة الضابطة(P-value $\geq$ 0.05).

Introduction:

Asymmetric Dimethylarginine (ADMA) is an endogenous molecule which can be detected in human blood and urine. It shows structural homology to the amino acid L-arginine, and it acts as an inhibitor of nitric oxide (NO) synthesis. Dimethylarginine is formed during proteolysis of methylated proteins. Protein methylation is ubiquitously present mechanism of post-translation modification of proteins. It results in a modification of the tertiary structure and the function of proteins. This process is catalyzed by a group of enzymes named S-adenosylmethionine protein N-methyltransferase (protein methylases I and II) (1). The name of these enzymes suggest their molecular function: They transfer one or more methyl groups from the methyl group donor S-adenosylmethionine to L-arginine residues within proteins or polypeptides. Accordingly, depending on the number of transferred methyl groups, NG-monomethyl-L-arginine (MMA) and NG, NG-dimethyl-L-arginine. ADMA are formed by the activity of protein methylases I, and NG-monomethyl-L-arginine and NG, NG-L-dimethyl-arginine symmetric dimethyl arginine (SDMA) are formed by the activity of protein methylases II. ADMA pathway was cited (3).

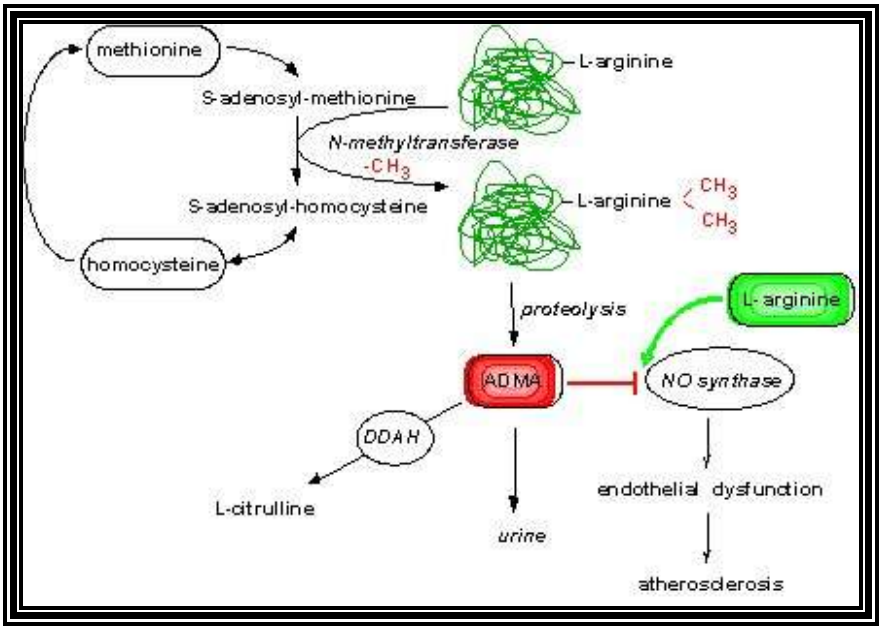

Figure (1) Pathway of ADMA (3).

Free circulation ADMA and SDMA are then released after degradation of such methylated protein residues. Methyl groups contained, group donor S-adenosylmethionine, an intermediate in the metabolism of Hcy. There are numerous experimental data showing that the vascular endothelium plays a central role in the maintenance of physiological vascular tone and vascular structure. One of the major mediators that are released by healthy endothelial cells is NO (4). NO is formed by the enzyme NOS from the amino acid precursor L-arginine. NO is involved in a vast number of regulatory processes within the cardiovascular system. Beside its potent vasodilatory effect, NO also acts as an endogenous inhibitor of platelet aggregation. Furthermore , NO inhibits the adhesion of monocytes and leukocytes at the healthy vascular endothelium an effect that , once disturbed , precedes the immigration of inflammatory cells into the vascular wall at sites that later become plaques . It inhibits the proliferation of vascular smooth muscle cells; this might be of great importance during the development of reestenosis after angioplasty (5).

The serum creatinine level dose not rise until at least half of the kidney nephrons are destroyed or damaged because creatinine level rise and fall more slowly than BUN levels. Creatinine levels are often preferred to monitor renal function on long term basis

Chronic renal failure is the only pathological condition that causes a significant increase in the serum creatinine level and it is not affected by hepatic protein metabolism (6 , 7) . More than 90% of urea is excreted through the kidneys, losses through the gastrointestinal tract and skin accounting for most of the remaining minor fraction. Urea is neither actively reabsorbed nor secreted by the tubules but is filtered freely by the glomeruli. In a normal kidney (40-70) % of the highly diffusible urea moves passively out of the renal tubule and into the interstitium, ultimately to reenter plasma and this depend on the renal output (8).

Impairment of renal function may be responsible for various electrolyte abnormalities including negative balances of sodium, potassium, calcium, phosphorus, and magnesium (9).

Methods:

The instruments used in this study:

1- High performance Liquid Chromatography . Shimadzu LC-6A
2-UV-Vis. Spectrophotometer (Cecil 7200)
3-Cooling centrifuge (Hitachi ,Japan).
4- MSE minor 35 centrifuge (England).
5- Water bath (Thamastst T-22S) .

Subjects:

Blood samples were obtained from healthy volunteers 20, their age range between (30-50) years, 25 patients with CRF on regular hemodialysis, their age range (22-63). Patients attending in dialysis department in Al-Kadumia teaching hospital.

Plasma analysis of ADMA:

An Hyper Performance Liquid Chromatography (HPLC) method based on that described by Rambausek *et al* (10) was developed for the measurement of ADMA, this measurement required an extraction step using bond elut solid phase cation exchange (SCX) column to remove the interfering substances and to concentrate the sample .0.1 ml of plasma was mixed with 0.1 ml of 40µmol/L solution of the standard ADMA and 0.8 ml of phosphate –buffered saline (PBS). This mixture was applied to Oasis MCX solid phase extraction column for extraction of ADMA. The column was consecutively washed with 1.0 ml of 100 mmol/L HCl and 1.0 ml methanol. Analyte was eluted with 1.0 ml of concentrated ammonia /water /methanol (10 /40 /50). After evaporation of the solvent under nitrogen, ADMA was derivatized with OPA reagent containing 3 mercaptopropanoic acid. The derivative was separated by isocratic reversed phase chromatography on a C-18 column (3.9 * 150 mm; 5µm particle size waters). Potassium phosphate buffer 50 mmol/L; pH= 6.5 containing 8.7 % acetonitrile was used as mobile phase at a flow rate of 1.1 ml/min and a column temperature of 30˚C . UV- Vis detection was performed at excitation and emission wavelengths of 340 mm. After elution the Analyte, strongly retained compounds were quickly eluted by a strong solvent flush with 50% acetonitrile, resulting in a total analysis time of 30 min (11).

Urea and creatinine were done by colorimetric method (Randox kit). Serum potassium and sodium were determined by using flame photometer.

Results and discussion:

The calibration curve of ADMA was shown in figure (2).The concentration of ADMA in µmol/L was shown in table (1). There was significant difference in ADMA concentration between patients and control P-value=0.001, the plasma levels of ADMA in HD patients were in the range of (1.9±0.5) µmol/L which was significantly higher than their level in control subjects (0.6±0.3) µmol/L. These findings are consistent with (Mac Allister *et al* (12).

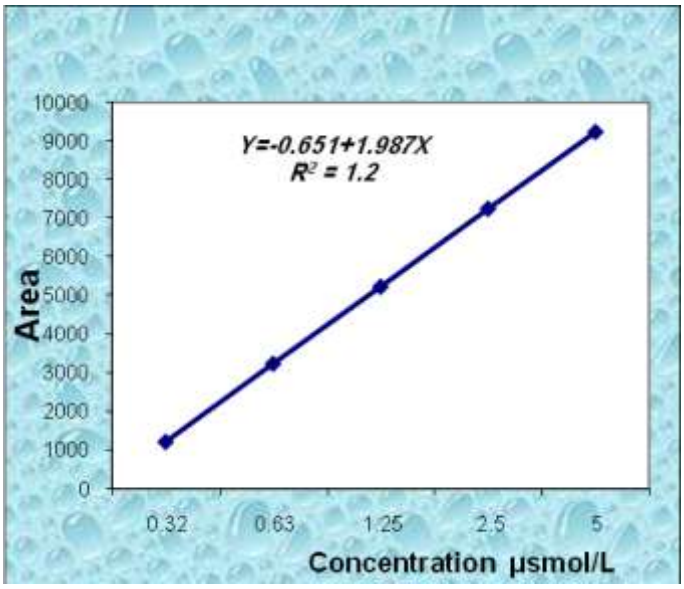

Figure (2) Calibration curve of ADMA.

Table (1) Concentration of ADMA in patient group and control

	HD	Control	p-value
	no. 10	no. 10	
ADMA, µmol/l M±SD	1.9±0.5	0.6±0.3	0.001

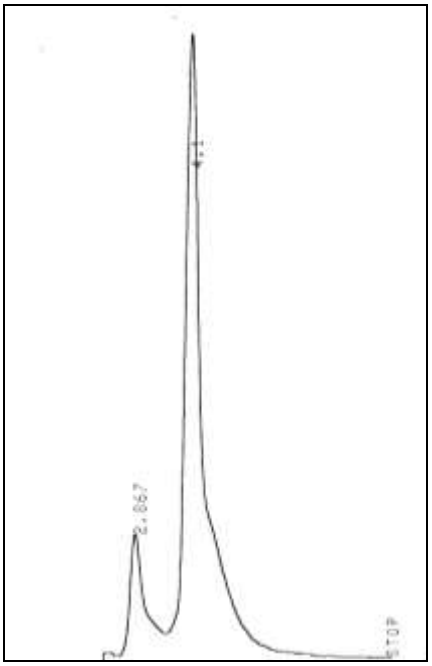

Figure (3): HPLC chromatogram of ADMA as standard.

It has been suggested that some of the features of CRF might result from the inhibition of NO production (13), and in uremic patients it has been shown to inhibit NO synthase activity in murine endothelial cells. Also Lorenzo (14) showed that guanidine and uremic compounds have a dose dependent inhibitory effect on NO synthesis.Concentration of 10 μmol/L of ADMA inhibited NO synthesis by 31.8 % .Guanidine compounds that accumulate in patients that suffering from renal failure are potent inhibitors of NO synthesis.

Hyperhomocyst (e) inemia increases plasma ADMA, an effect that is temporally related to a decline in endothelial vasodilator function. There is a relationship between ADMA accumulation and oxidative stress. ADMA is derived from the breakdown of proteins that contain methylated arginine residues, a process that occurs in all cell types, the methylated arginine residues are excreted in the urine or, in the case of ADMA and L-NMMA, are metabolized by the enzyme DDAH. The activity of DDAH appears to be critical in regulating ADMA levels. In isolated vascular rings, inhibition of DDAH activity results in gradual vasoconstriction. This vasoconstriction is reversed by the addition of L-arginine to the medium. . These results suggest that ADMA is constantly being produced during normal protein turnover, and DDAH is essential in preventing accumulation of ADMA. Accumulation of ADMA in a number of metabolic disorders (including hyperhomocyst (e) inemia is related to oxidative stress. ADMA accumulates under these conditions because the activity of DDAH is impaired by oxidation. The sensitivity of DDAH to oxidative stress is conferred by a sulfhydryl moiety in the catalytic site that is required for enzyme activity. Modification of this sulfhydryl moiety alters enzyme activity. Hcy mounts an oxidative attack on DDAH, forming a disulfide bond with the sulfhydryl group in the catalytic site. Endothelial DDAH activity is reduced by Hcy; furthermore, Hcy binds directly to recombinant DDAH to inhibit its activity. In addition to its vulnerability to oxidative stress, this sulfhydryl moiety can be nitrosylated, which provides a mechanism for a reversible form of DDAH inhibition (15).

The purpose of measurement of urea and creatinine was to prove that the patients involved in this study have clinical features of CRF after recurrent measurement of urea and creatinine which are a highly significant as shown in table (2).Elevated serum levels of creatinine and urea are pathogenetic of renal insufficiency.

There was an increase in the levels of serum urea and creatinine in patients groups and control.

Table (2) Urea and creatinine levels in patient groups and control.

	Patients HD	Control	p-value
S.Urea mg/dl	104.2±82.2	42.8±8.5	0.001
S.Cre. mg/dl	3.5±1.5	1.2±0.4	0.001

Urea excretion is decreased in CRF, when the kidney ability to excretion is severely impaired. This may cause greatly increased concentration of urea in the blood (uremia) and other body fluid (16). Urea is an ideal marker molecule and a performance parameter to compare treatment dose in different technique in spite of its moderate toxicity and it's currently usage as a marker of renal replacement therapy (RRT) adequacy because it's easily measurable and, present the end product of

protein metabolism. Its accumulation during kidney failure defines the requirement for dialysis while its deamination defines the efficiency for treatment (17).

The creatinine levels is a more reliable parameter than urea level for identification of renal dysfunction, since the serum level of creatinine rises earlier than that of urea and the formation of creatinine is largely independent of protein metabolism, in contrast to the formation of urea (18) and because creatinine has lower back diffusion from tubules lumen to poritubular blood (19).

The mean value of electrolyte (Na^{+} , K^{+} , Ca^{++} , Mg^{++} , PO_4^{-3}) in patient group and control were presented in table (3)

Table (3) Electrolytes levels in patients and control.

	Patients	Control	P-value
S.Na^{+} meq/L	125±9.0	142.1±5.8	0.001
S.K^{+} meq/L	4.4±0.8	4.8±0.8	0.001
S.Ca^{++} mg/dl	6.4±1.1	10.0±1.1	0.001
S.PO_4^{-3} mg/dl	4.3±0.8	3.7±0.8	0.001
S.Mg^{++} mg/dl	2.2±0.6	2.2±0.6	0.24

Chronic renal failure is associated with disorders of the extra and intracellular electrolyte homeostasis. The investigation of the intracellular electrolyte gives more important information on their level in the body (20). In our study there was highly significant decrease in serum sodium levels in patient group than the control.Sodium transport activity is regulated by many factors , including protein kinase-dependent phosphorylation ,which can increase both activity and channel numbers. Distal tubular Na+ and K+/H+ transport is regulated by the action of aldosterone , which increases the synthesis of apical Na+ and K+ channels, Na+-K+ ATPase , along with the activity of Na+-H+ exchange and the H+-ATPase .

In this study, there was highly significant difference in serum potassium between patient group and control. In advanced CRF ,fecal excretion of potassium increases to50% of the potassium load in comparison with 10 % in healthy subjects because its moves along a conc. gradient(21) .Serum potassium level is controlled tightly from 3.5 to5.5 meq/L.

Many patients with low plasma calcium have reduced activity of renal Cholecalciferol 1ά-hydroxylase and develop osteomalacia or rickets. A few patients show a third type of bone abnormality with increased bone density (osteosclerosis). It is not clear why any particular one of these various types of renal osteodystrophy should develop in an individual patient (22).

Phosphorus in the form of inorganic and organic phosphate is an important and widely distributed element in the human body. The present study showed highly significant increase of serum inorganic phosphate in CRF patients, which postulated that retention of phosphate in the extracellular space due to the decrease in GFR and the accompanying reduction in plasma, ionized calcium conc. was the primary event in the pathogenesis of secondary hyperparathyroidism (23 , 24).

Magnesium deficiency rarely occurs as an isolated phenomenon. Usually it is accompanied by disorders of potassium, calcium and phosphorus metabolism. It may therefore be difficult to identify signs and symptoms that can be specifically attributed to magnesium deficiency. However, muscular weakness, sometimes accompanied by tetany, cardiac arrhythmias and central nervous system abnormalities (e.g. convulsions), may all be due to magnesium deficiency.

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