Study of Several Anti oxidants , Total Acid Phosphatase, Prostatic Acid Phosphatase, Total and Free Prostate-Specific Antigen in Sera of Man with Chronic kidney failure

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ABSTRACT

Chronic kidney failure (CKF),is a progressive loss in renal function over a period of months or years. The symptoms of worsening kidney function are unspecific, and might include feeling generally unwell and experiencing a reduced appetite. Free radicals are formed in all living organisms during normal cell metabolism. Patients with chronic renal failure who are regularlydialyzed are candidates for free radical damage.The current study investigate possible links with total acid phosphatase, prostatic acid phosphatase, total and free prostate-specific antigen ,several Antioxidants and an increased risk of prostate disease present in males with chronic kidney failure (CKF). The present study is also to compare these features level among patients [chronic renal failure] undergoing haemodialysisand in control (age and sex matched)therefore, addressed this question by measuring prostatic markers in patients receiving long-term dialysis.Patients were chosen from the patients referred to the Medical City –Kidney Transplant Center, Iraq.The glomerular filtration rate (GFR)has been measure in 45 patients with CKF using haemodialysis method . Laboratory investigations including kidney function, serum urea, creatinine, albumin, S. calcium, S. Phosphorus, total protein, Uric acid, S. potassium, S. Sodium,Hb, in addition to serum total antioxidant capacity(TAC), lipid peroxidation(the level of lipid peroxidation expressed as malondialdehyde(MDA)) ,vitamin E, vitamin C and total acid phosphatase, prostatic acid phosphatase, total and free prostate-specific antigen had been measured in male with CKF . Blood samples were obtained from the patients and the control group consisted of 25age and sex matched normal healthy individuals who came to the hospital for health checkup.Hemoglobin, Serum urea,creatinine,GFR, S. calcium, potassium,PSA, fPSA, PAP,LH, TAC,MDA, uric acid , vitamin E, vitamin C showed significant difference between the patients and control group. There was a positive correlation in PSA[ng/ml] with TAC ($r=0.57$, $p<0.01$), MDA ($r=0.60$, $p<0.01$),While PSA correlated negatively with Vitamin E ($r=-0.65$, $p<0.01$), Vitamin C ($r=-0.57$, $p<0.01$) in the CKF patients while there was no significant correlation was observe in the control group. In this study, a

significantly negative association was observed between PAP[IU/L] with TAC (r=-0.63, p<0.01), MDA (r=-0.70, p<0.01),in the CKF patients while there was no significant correlation was observe in the control group. A significant positive correlation was found between PAP[IU/L] , and Vitamin E (r=0.61, p<0.01), Vitamin C (r=0.67, p<0.01).Hemodialysis leads to significant changes in the antioxidant system of the blood of patients with chronic renal failure. Despite an adverse metabolic environment in chronic renal insufficiency, Prostatic disease markers were useful in the routine screening of men receiving long-termdialysis, but the clinicians should be on alert when the dialysis duration increasesthe change in serum anti oxidants that accompanies decline in renal function.

دراسة بعض مضادات الأكسدة ،إنزيم الفوسفاتيز الحامضي الكلي والبروستاتيوالمستضد البروستاتي الكلي والحر في أمصال المرضى المصابين بالفشل الكلوي المزمن

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مفتاح الكلمات: الانتجين الخاص بالبروستات

الخلاصة :

يمثل الفشل الكلويالمزمن(CKF) الفقدان التدريجيلوظائف الكلىعلى مدىشهور أو سنوات. ان أعراضتدهوروظائف الكلى غير محددة، وربما تشمل الشعوربتعب وعموما تعاني منضعف الشهية. تتكونالجذور الحرة فيجميع الكائنات الحيةخلالعمليات ايض الخليةالعادية.إن مرضى الفشلالكلوي المزمنالذينيجري لهم غسيل كلوييانتظاماًأكثر عرضة للتعرض للضرر بالجذور الحرة.تهدف الدراسة الحالية بيان تأثير انخفاض وظائف الكلىعلى مستوى، أنزيم الفوسفاتيزالحمضيالكلي والحر وكذلك المستضد البروستاتي الكلي والحرإضافة لبعض مضادات الأكسدة .تهدف الدراسة الحالية أيضا إلى التحقق من وجود علاقة محتملة بينهما مستويات إنزيم الفوسفاتيزالحمضيالكلي والحر وكذلك المستضد البروستاتي الكلي والحرإضافة لبعض مضادات الأكسدة وزيادة خطر أمراضالبروستاتعند الذكورالمصابينبالفشل الكلويالمزمن (CKF). تضمنت هذه الدراسةأيضاإلمقارنة هذهالعوامل الكيميائي الحياتيةعند المرضى[الفشل الكلوي المزمن] مع مجموعة من الأصحاء .تم قياس معدل الترشيح الكبيبي (GFR)عند 45 مريضا مصابين بالفشل الكلوي المزمن اضافة لذلكالفحوص المختبريةلوظائف الكلى(اليورياوالكرياتينين) ،الالبومين ،الكالسيوم ،الفوسفات اللاعضوي، البروتين الكلي،حامض اليورك ،البوتاسيوم ،الصوديوم ونسبة الهيموكلوبين إضافةإلى قياس إجمالي مضادات الأكسدة(TAC)وMDAوفيتامين Eوفيتامين Cوكذلك تم قياس مستويات إنزيم الفوسفاتيزالحمضيالكلي والحر وكذلك المستضد البروستاتي الكلي والحر ومقارنتها بمجموعة الضبط المكونة من 25 شخص من الأصحاء.اشارت الدراسة الى وجود تغييرات معنوية في مستوى الهيموكلوبين و معدل الترشيح الكبيبي(GFR) اليوريا والكرياتنين، ،الكالسيوم ،البوتاسيوم،PSA،fPSA ،PAP،LH ،TAC،MDA،فيتامين E،فيتامينC ،حامض اليورك عند المرضى مقارنة بمجموعة الضبط.لقد أشارت الدراسة إلى وجود علاقة طردية بينPSA,TAC (r=0.57, p<0.01) وكذلك مع MDA(r=0.60, p<0.01)عند المرضى المصابين بالعجز الكلوي الزمن في حين لم تلاحظ هذه العلاقة عند الأصحاء ،وقد بينت الدراسة وجود علاقة عكسية بين PAPمع كل من TAC,MDAعند المصابين بالمرض في حين لم تلاحظ هذه العلاقة عند مجموعة الضبط ،في حين ارتبط PAP طرديا مع كل من فيتامين E،فيتامينC.ان عملية الديلزة تقود الى تغييرات معنوية في نظام مضادات الأكسدة عند المرضى المصابين بالفشل الكلوي المزمن .ان الدالات المرضية الخاصة بالبروستات مفيدة في الفحص الروتيني عند الرجال الذين

يخضعون لعملية غسيل الكلى بصورة دائمية ولكن على الطبيب ان يأخذ بنظر الاعتبار ان تغييرات كثيرة تحدث لمستوى مضادات الأكسدة في أمصال المرضى والتي قد تساهم بشك أوبآخر بتطور تدهور حالة الكلى.

Introduction

Chronic kidney failure [CKF] is a progressive loss of renal function over a period of months or years through five stages [1]. Each stage is progressive through an abnormally low and deteriorating glomerular filtration rate. Chronic kidney failure is defined as an estimated glomerular filtration rate <60ml/minute/1.73m^2 [2]. Renal failure causes alterations in electrolytes, acid-base, and water balance, and accumulation of substances normally excreted by the body. Such alteration can result in uremia, a toxic condition that effect all body systems[3].

Serum concentrations of total acid phosphatase(TAP), prostatic acid phosphatase (PAP),prostate-specific antigen (PSA) and free prostate-specificantigen (fPSA) are commonly used as a markerof prostatic disease[4,5].PSA is a 33000-dalton glycoproteinand as many other glycoprotein's are known toaccumulate in end-stage renal failure this might possiblycause on artefactual increase in prostatic diseasemarkers, with a high incidence of false positiveresults in patients with renal insufficiency[6].Totalacid phosphatase (TAP) and prostatic acid phosphatase (PAP) have also been used in the past to detect prostatic disease. [7] Since PSA was introduced into clinical practice in 1986, the early diagnosis and management of prostate cancer has been revolutionized and much has been learnt about the strengths and weaknesses of this assay[8] The PSA test is the most effective test currently available for the early detection, diagnosis and follow-up of prostate cancer in kidney disease (CKD) patients. [9-11]

Chronic renal failure causesmajor effects on the male reproductive system, notablyimpairment of spermatogenesis, steroidogenesis andsexual function, through effects at all levels of the hypothalamic–pituitary–testicular axis. Disturbances ofthe axis can be detected with only moderate reductionsin the glomerular filtration rate and progressively worse as the renal failure progresses. Approximately50% of uremic men complain of erectile dysfunctionwhile an even greater percentage complain ofdecreased libido and a marked decline in the frequencyof intercourse [12-13]. Plasma LH, andFSH -a levels are slightly elevated along with reduced circulating total and free testosterone levels and normal SHBG levels [14]. Although these changes are consistent with a primary defect in testicular function, there is also strong evidence for defective neuroendocrine regulation as an important functional aspect of the reproductive dysfunction in uremia. The increase in gonadotropins is largely explained by the significant reduction (70%) in renal filtration and whole body clearance rate of LH which, in the presence of decreased testosterone secretion, indicates significantly reduced LH secretion[15].

Patients with chronic renal failure, including those receiving regular long-term haemodialysis have a high incidence of premature cardiovascular disease.Oxidative stress which occurs when there is excessive free radical production or low antioxidant level, has recently been implicated as a causative factor in atherogenesis[16].Free radicals may cause lipid peroxidation and damage macromolcules and cellular structure of the organism, endothelium and erythrocytes. Some studies have shown that haemodialysis is connected with increased free radical production[17].Cardiovascular disease is one of the major cause of mortality in haemodialysed patients with chronic renal failure. Increased lipid peroxidation and depletion of antioxidant may contribute to increased risk of atherosclerosis[18].

The purpose of this study was to evaluate hemodialysis have any effect on prostatic disease markers such as PSA, fPSA, TAP, PAP and study any correlation between these factors and several antioxidants [TAC ,MDA ,VitC, Vit E, uric acid and albumine] which has never been discussed in the literature.We have therefore, addressed this question by measuring prostatic markers and several antioxidants in patients receiving long-term dialysis.

Forty five patients on chronic hemodialysis were included in the study. Patients were all men patients with average age (45.33±6.02).None of these patients received antioxidant medicines. Patients were chosen from the patients referred to the Medical City –Kidney Transplant Center, Iraq.For comparison, twenty five apparently healthy men who matched for age [n=25; age=42.44± 5.30 (years),None of these patients received antioxidant medicines.

All patients were subjected to a detailed history taking, thorough clinical examination, and laboratory investigations including kidney function, Hemoglobin, S. calcium, S. Phosphorus , S. potassium, S. Sodium, Total protein ,PSA, fPSA, TAP, PAP,LH,FSH , in addition to serum total antioxidant capacity(TAC), lipid peroxidation(the level of lipid peroxidation expressed as malondialdehyde(MDA)) ,uric acid , vitamin E, vitamin C and albumin had been measured in CKD patients. Blood samples were obtained from the patients on chronic hemodialysis and control group, Five ml were collected from each subject by vein puncture, centrifuged at 3000 rpm for 5 min after allowing the blood to clot at room temperature. The serum urea, creatinine, S. calcium, S. Phosphorus, total protein, Uric acid, Albumin, S. potassium, S. Sodium, level**s** were measured by spectrophotometeric methods supplied by Giesse Diagnostic. Plasma malondialdehyde was determined according to the modified method of Satoh[19]. Total antioxidant capacity(TAC) in serum samples was carried out according to Rice -Evans and Miller[20]. Ascorbic acid levels were estimated by the method of Tietz [21]. vitamin E levels were determined according to a modified of Hashim and Schuttringer[22]. Serum TAP and PAP levels were determined byenzymatic colorimetric methods (p-Nitrophenylphosphate,L-Tartarate; Pointine Scientific Inc., USA)on a TARGA 3000 autoanalyzer. Normal rangeof serum TAP 2.5- 11.7 U/L, serum PAP 0.2-3.5 U/L..The serum PSA, free PSA , LH, FSH were measured by Enzyme Linked Immunosorbent Assay (ELISA) (Biovender Laboratory Medicine, Brno,Czech Republic).The glomerular filtration rate[GFR] can be estimated using prediction equations that take into account the serum creatinine level and some or all of specific variables (age, sex, race, body size) [23,24] .The modification of Diet in Renal Disease (MDRD) study equation was used to estimate the GFR and as follow[25]:GFR (ml/min/1.73 m^2) =186×(Serum creatinine)$^{-1.154}$×(Age)$^{-0.203}$×(0.742 if female)×(1.210 if black).

All statistical analyses in studies were performed using SPSS version 17.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability P< 0.05= significant, P> 0.05 = non-significant.Correlation analysis was used to test the linear relationship between parameters.ANOVA test was used to show the differences between variables of differentiated groups.

Results And Discussion

In a CKF subjects, the mean urea and creatinine had significantly increased (p<0.001) and as shown in Table 1. The glomerular filtration rate[GFR] was significantly decreased(p<0.001) in patients when compared with control,Serum levels of calcium, total protein ,were significantlydecreased (p<0.001) and the Hemoglobin was significantly decreased, (p<0.001). Serum concentrations of sodium and phosphorus were not significantly different from the mean value for healthy subjectsas shown in Table 1

Chronic kidney disease is identified by a blood test for creatinine. Higher levels of creatinine indicate a falling glomerular filtration rate and as a result a decreased capability of the kidneys to excrete waste products. Renal failure is often complicated by elevations in potassium, phosphate, and decreases in sodium and calcium[26]. Hemoglobin showed a significant decreased(p<0.001) inpatients when compared to control group as shown in table 1 . This reduction in Hb occurs fora variety of reasons. Approximately90% of the hormone erythropoietin isproduced by the kidneys. Under normalphysiological conditions, hypoxiain the kidney leads to an increase inthe production of erythropoietin,which subsequently stimulates erythropoiesis[27]. The kidney, in turn, sensesincreased oxygenation because of theformation of the new erythrocytesand decreases

erythropoietin production.However, tubulointerstitial damageassociated with diabetes occursearly in the course of diabetes,even before a reduction in GFR oralbuminuria is noted[28]. As functionalrenal tissue declines in patients withCKD, the body is unable to produceadequate amounts of erythropoietinin response to hypoxia in the kidney[27].The meanserum PSA(ng/ml)[(mean ± SD)2.41±0.29],free PSA(ng/ml) [0.22±0.03],TAP(U/L) [5.02±1.21]in CRF were significantly decreased(p<0.001) when compared with control group ,while PAP LH,FSH levelswere not significantly different from the mean value for control as shown in Table 2.

PSA is a serine protease, which is produced by prostaticalveolar and ductal epithelial cells and correlatesclosely with tumor bulk and response to therapy for menwith prostate cancer. Its serum levels are reported to bestable in most patients with CRF and therefore it maybe useful in the diagnosis [29-32]. In this study, althoughPSA levels were found to be higher than that in thecontrol group, levels in all patients were within normallimits which were consistent with literature. These findings indicated that neither renal impairment nor chronic hemodialysis cause a art factual elevation of prostatic disease markers.

The measurement of PSA is a helpful tool in the diagnosis and follow up of patients. Although there are conflicting reports regarding the levels of PSA in hemodialysis patients, nevertheless, its levels are not affected significantly by either method of dialysis or type of membrane used during hemodialysis.[11,33,34]Some studies have determined that free PSA levels may be significantly higher in dialysis patients compared to PSA levels and should help in differentiating benign from malignant disease[11,34].The current study therefore is important to confirm the use of PSA for diagnosis of prostate cancer and benign prostatic hypertrophy in patients with CKD compared to controls. PAP was done due to its historic use, low cost and easy availability in most laboratories although it has never been proven to be a valid tumor marker for prostate.Chronic kidney disease and chronic renal failure causes major effects on the male reproductive system, notably impairment of spermatogenesis, steroidogenesis ,hypogonadism and sexual function, as well as psychological disturbances through effects at all levels of the hypothalamic–pituitary–testicular axis[35,36]. In this study plasma LH levels were significantly elevated compared to the reference group P< 0.01. LH level was increased due to diminished response of the hypothalamic-pituitary axis to lowered testosterone levels, and that the hypothalamic-pituitary axis in chronic renal failure is reset in such a way that it is more sensitive to the negative feedback inhibition of testosterone, impaired regulation of gonadotropin secretion.[37,39]. In our study plasma FSH levelis slightly elevated with no significance compared to the reference group. Many other authors who proved that mean FSHlevels in patients were not significantly different from those of control subjects [37,39] also approved this result.

Table 3 showed mean and standard deviation of serum TAC and MDAshowed significantlyincreased between CKF and control groups (P<0.001). Vitamin C and vitamin Ewere significantly decreased in the CKF group when compared with control group(P<0.001),Uric acid was significantly increased in CKF group when compared with control group(P<0.05) as shown in Table 3.

There are varying reports on changes in plasma lipid peroxidation and other antioxidants due to hemodialysis. Some of the studies showed an increase while some others showed a decrease. Some researchers[40,41], reported that the level of serum malondialdehyde in haemodialysis patients increased when compared with control groups. In the present study , results show a significant increase of plasma malondialdehyde in patients group and control group. Increased production of free radicals may cause lipid peroxidation and damage in macromolecules and cellular structure of the organism, endothelium and erythrocytes, i.e. - so called “oxidative stress reaction” [40,41],. A number of investigations have shown that oxidative stress is present in CRF and especially in dialysis patients [41,42].Oxidative damage can cause by the imbalance between the production of free radicals and the countering effect of the various antioxidant enzymes. Some studies show that activities of antioxidant enzymes change in hemodialysis patients due to the dialysis process .There are varying reports on the erythrocyte activities of enzymes glutathione peroxidase and superoxide dismutase. Inaddition, Vitamin C is a small, water-soluble molecule and is therefore likely to be lost

during dialysis. Vitamin C is generally considered to be a key aqueous-phase antioxidant [43], and Vitamin C deficiency may contribute significantly to oxidative stress in these patients.Decreased levels of Vitamin E in CRF patients as compared to healthy controls may be due to enhanced lipid peroxidation. There may also be impaired absorption of dietary Vitamin E due to altered lipid metabolism[44,45].

There was a positive correlation in PSA[ng/ml] with TAC ($r=0.57$, $p<0.01$), MDA ($r=0.60$, $p<0.01$),While PSA correlated negatively with Vitamin E ($r=-0.65$, $p<0.01$), Vitamin C ($r=-0.57$, $p<0.01$) in the CKF patients while there was no significant correlation was observe in the control group as shown in figure 1.

In this study, a significantly negative association was observed between PAP[IU/L] with TAC ($r=-0.63$, $p<0.01$), MDA ($r=-0.70$, $p<0.01$),in the CKF patients while there was no significant correlation was observe in the control group.A significant positive correlation was found between PAP[IU/L] , and Vitamin E ($r=0.61$, $p<0.01$), Vitamin C ($r=0.67$, $p<0.01$) as shown in figure 1.This pilot study is, to our knowledge, the first studyreported the correlation between level serum antioxidants and PSA ,PAP in CRFpatients.The result of the present study indicates that there is considerable oxidative stress in patients with CRF, which is further exacerbated by haemodialysis, as evidenced by increased lipid peroxidation and low antioxidant levels. Increased levels of Malondialdehyde (MDA) which is a reliable marker and a product of lipid peroxidation in CRF and dialysis patients, indicates the existence of oxidative stress.The decreased levels of non-enzymatic antioxidants like Vitamin E, and Vitamin C indicate the increase in oxidative stress. Exogenous supplementation of non-enzymatic antioxidants may decrease the damage to renal tissue by quenching and preventing the free radical action which are responsible for the disease process. PSA, free PSA, TAP and PAPcan be used to screen patients on dialysis, althoughthey should be used with caution in the diagnosis ofprostate cancer in long term chronic hemodialysispatients. The current results suggest more study in correlation between Prostatic disease markers and serum antioxidants to prevent any complication in prostatefor patients with CRF.

Table 1 : The mean and standard deviation of B. urea,Screatinine, GFR , Calcium ,Phosphorus,potassium, S. Sodium, total protein and Hb in patients group and control group

Characteristic	**Patientsmean±SD**	**Controlmean±SD**	**PValue**
Urea [mg/dl/]	118.08±35.39	32.04±6.08	<0.001
Creatinine[mg/dl/]	8.21±3.13	0.86±0.18	<0.001
GFR[ml/min/1.73m^2]	32.65±7.17	83.04±7.01	<0.001
S. Calcium[mg/dl/]	8.14±0.91	9.50±0.75	<0.001
S. Phosphorus[mg/dl/]	3.02±0.58	2.91±0.91	>0.05
S. Sodium [mEq/L]	136.98±6.93	137.84±2.08	>0.05
S. Potassium[mEq/L]	4.47±0.78	3.95±0.27	<0.01
Hb [gm/dl]	9.55±1.86	13.84± 0.93	<0.001
Total protein [gm/dl]	5.89±0.83	6.21±0.56	>0.05

Table 2:The mean and standard deviation of PSA ,free PSA,TAP,PAP,LH,FSH in CRF patients group and control group

Characteristic	Patientsmean±SD	Controlmean±SD	PValue
PSA[ng/ml]	2.34±0.29	1.61±0.35	<0.01
Free PSA[ng/ml]	0.27±0.05	0.24±0.03	<0.05
TAP [U/L]	5.02±1.21	6.08±0.76	>0.05
PAP [U/L]	0.91±0.29	1.60±0.27	<0.05
LH [U/L]	9.15±0.85	6.08±0.85	<0.01
FSH [U/L]	11.82±3.33	10.02±4.61	>0.05

Table 3 :Comparison of different parameters related to oxidative stress and antioxidant defenses systems incontrol and study group .

Characteristic	Patientsmean±SD	Controlmean±SD	PValue
TAC [µmol/L]	480.80±61.12	419.80±27.38	<0.001
MDA[µ mol/L]	3.76±0.72	1.42±0.18	<0.001
Uric acid [mg/dl/]	6.76±0.97	6.20±0.84	<0.05
Vitamin C [mg/dl]	0.80±0.12	1.65±0.32	<0.001
Vitamin E [mg/dl]	0.99±0.13	1.37±0.33	<0.001
Albumin [gm/dl]	3.56 ±0.28	3.66±0.16	>0.05

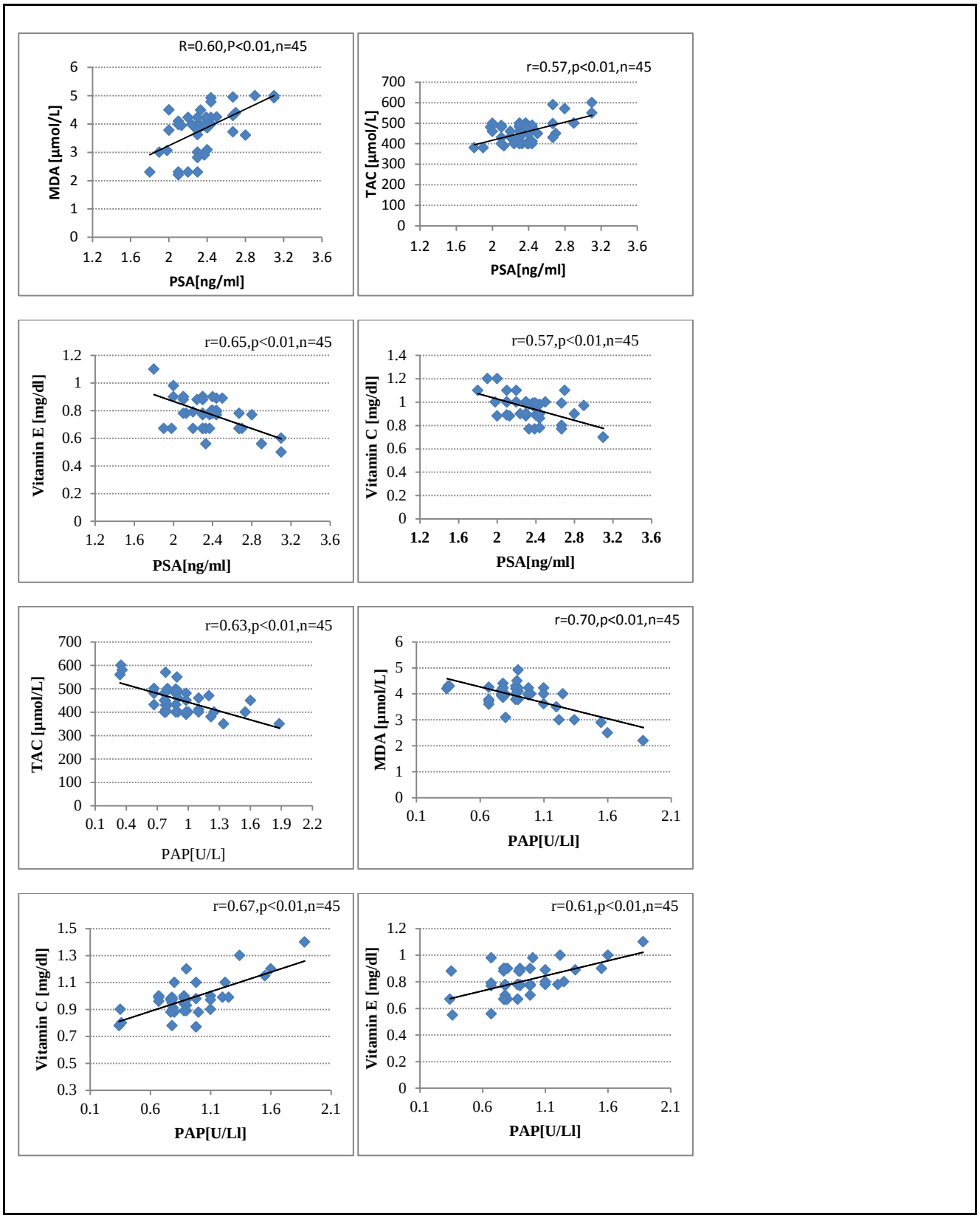

Figure 1:Correlation between PSA,PAP with TAC,MDA, Vitamin C, Vitamin E in CKF patients

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