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Detection of some Biochemical Indicators and Auto-Fluorescence Spectrophotometer of Kidney Disease and Renal Failure Patient's Urine

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ABSTRACT

The use of reliable biomarkers is becoming increasingly important for improved management of patients with acute and chronic kidney diseases. Recent developments have identified a number of these novel biomarkers in urine that can determine the potential risk of kidney damage. This research have elucidated that there are some biochemical enzymatic markers that have strong relationships with kidney disease and renal failure and could be used for diagnostic purposes, such as arginase and carbonic anhydrase that show a significant activity increase in both male and female patient's urine in all age stages at probability $P<0.001$ compared with normal one, this increase due to changes in kidney's histopathological changes such as apoptosis and inflammation in addition to the damage in renal's distal tubules.

Fluorescence spectrophotometric technique reveals that some natural urine's fluorophores intensities changed according to the clinical state of the persons, for this it can be utilized as a diagnostic indicator of some diseases such as kidney diseases and renal failure as shown in this research which indicated that the intensities of patient's urine emission fluorescence peaks have changed in both males and female patient's urine especially at 352, 353, 363 nm,401, 425,438,445 nm, 438-445nm and 703nm compared with healthy ones at fixed excitation wavelength 350-400 respectively.

We conclude from current research that urine could be utilized as an alternative to blood sample due to its easy to get beside many natural urine's metabolic fluorophores intensities and enzymes activities could be changed according to the clinical state of the persons, for this they can be utilized as indicators for kidney disease and renal failure.

Keywords: Arginase, Carbonic anhydrase, Auto-fluorescence spectrophotometer.

التحري عن بعض الدلائل الكيموحيوية والمطيافية الفلورنسية الذاتية لإدرار المصابين بأمراض الكلى والفشل الكلوي

الملخص

أكدت التطورات الحديثة ان استخدام بعض المؤشرات الكيموحيوية أهمية تشخيصية لأمراض الكلى والفـشل الكلـوي وهذه المؤشرات تعطي تمييزا لأنواع التلف الحاصل بالكلى ويمكن استخدامها لأغراض تشخيصية، بعض هـذه المؤشـرات تستخدم إما لوحدها أو مع عوامل إضافية أخرى لها علاقة بأمراض الكلى والفشل الكلوي وهي تعطي فكرة عن تقدم الأمراض الكلوية واستجابة المرضى للعلاج.

أوضحت نتائج البحث الحالي أن هناك بعض المؤشرات الكيموحيوية التي لها علاقة قوية بأمراض الكلى والفشل الكلوي والتي تستخدم لأغراض تشخيصية وتتضمن بعض الأنزيمات التي زادت فعاليتها معنويا في إدرار المرضى والتي من الممكـن استخدامها لأغراض تشخيصية وتضمنت كل من أنزيمي الارجنيز، الكاربونيك انهايدريز اللذان اظهرا زيـادة معنويـة فـي

الفعالية الأنزيمية عند مستوى الاحتمالية ($p<0.001$) في كل من إدرار مرضى الكلى والفشل الكلوي مقارنة بإدرار الأصحاء لكلا الجنسين. حيث ترتفع معدلات أنزيمي الارجنيز والكاربونيك انهايدريز وان هذا الارتفاع يعود إلى التغيرات المرضية النسجية كالموت المبرمج والتهاب خلايا الكلى وتلف النبيبات الكلوية البعيدة.

كما أوضحت النتائج أيضا أن استخدام تقنية المطيافية الفلورنسية يعد من التقنيات الرائدة التي من الممكن استخدامها لأغراض تشخيصية، وهذا يعزى إلى أن تركيز بعض الفلوروفورات (مركبات كيميائية مفلورة يمكنها إعادة بعث الضوء عند التحفيز الخفيف) المتواجدة طبيعيا في الإدرار تتغير تبعا لحالة الشخص السريرية ومن الممكن استخدامها كمؤشر تشخيصي لأمراض الكلى والفشل الكلوي حيث أظهرت هذه الدراسة تغيرا واضحا في كثافة القمم المطيافية للانبعاث عند الأطوال الموجية 352nm, 353nm, 363nm, 401nm, 425nm, 438nm, 445 nm, 703nm، وفي كلا الجنسين وعند تثبيت الأطوال الموجية للإثارة عند 350nm -400nm.

نستنتج من نتائج الدراسة الحالية انه من الممكن استخدام عينات الإدرار كبديل عن عينات الدم لسهولة الحصول عليها إضافة إلى إمكانية استخدام التغيير في كثافة المطيافية الفلورنسية الانبعاثية لبعض الفلوروفورات الايضية المتواجدة طبيعيا فيها تبعا لحالة الشخص السريرية كما أن التغيير في فعالية بعض الانزيمات المهمة يمكن من استخدامها كدلائل كيموحيوية على الأمراض الكلوية والفشل الكلوي.

الكلمات الدالة: الارجنييز، الكاربونك انهايديز، المطيافية الفلورنسية.

INTRODUCTION

Urine contains many organic compound which contain a number of natural fluorophores, most of these compounds are the result of Tryptophane, Riboflavine and Porphyrin and other metabolism (Krishnaraju *et al*., 2009). The change in urine's auto fluorescence with the succession in the condensation of these compounds is the result of both physiological and pathological changes that may occur due to a disorder of body metabolism, eating food, age, sex …etc. (Power, 2015; Patel *et al*., 2007), besides there are some enzymes that represent indicators for kidney disordors such as arginase and carbonic anhydrase. Magnesium Arginase EC 3.5.3.1 contains enzymes that stimulate the final reaction in Urea circle) which is a chain of chemo biological reaction, through which the body gets rid of harmful Ammonia (Perrone, 2011) between Arginine and water, to produce Orthinine and Urea, (Scaglia and Lee, 2016).

Many researchers believe that accumulation of Arginase stimulators such as (sulfhydryl compounds, heavy matals Fe or Cu, metabolic products ascorbic acid-Fe, methylglyoxal-Fe, alloxan-FE, Iron) increase kidney's Arginase expression, then stimulate kidney production of urea (Hezel *et al*., 2015; Fernandes *et al*., 2016).

In addition to this enzyme carbonic anhydrase EC 4.1.2.2 is one of the metal enzymes that contain zinc ion and stimulate the reverse analysis of CO_2 to HCO_3 (Supuran, 2008; Manthy *et al*., 2005).

There are 15 kinds of Carbonic anhydrases that exist in human being (Le Darz *et al* 2015, Del Prete *et al*., 2016).

Some kinds of human Carbonic anhydrase “CA” are (CA I, II, III, VII and XIII) CA I are cellular but others CA IV, IX, XII and XIV are membranous connected enzymes while two kinds of this enzymes exist in mitochondria, and there are other kinds CA VI exist in saliva (Supuran, 2008).

In the kidney, there are two kinds of this enzyme that play an important role in the process of Urinary acidification and reabsorbing bicarbonates (Ekinci *et al*., 2015).

MATERIALS AND METHODS

Determination of arginase activity in urine:

Enzyme activity have been estimated depending on Kocna *et al*., 1993 modified method of Konarska and Tomaszweski, 1986 which includes addition of 25µl of urine sample and 25µl of (5mmol/l triss-HCL) to 500µl of the reaction mixture that contain 35ml 1Mm Triss- HCL buffer pH 9.5 + 20ml 100 mM arginine + 0.009g $MnCL_2$ incubated for 2 h at 37^{C° then for 5 min in boiling water bath. 0.5 ml of ninhydrine detector and 1.5 ml of acetic acid 10M were added and left in boiling water bath for 60 minutes, and cooled to room temperature. Standard curve is prepared by using different concentrations of ornithine solution between (0-2 µmol/L). To each 0.5ml dilution, 0.5 ml of triss-HCL buffer, 0.5 ml of ninhydrine detector and 1.0 ml of (10M) acetic acid, have been added then left in boiling water bath for 60 minutes and then tubes were cooled at room temperature, Optical Density measured at 515 nm for all tests and standard.

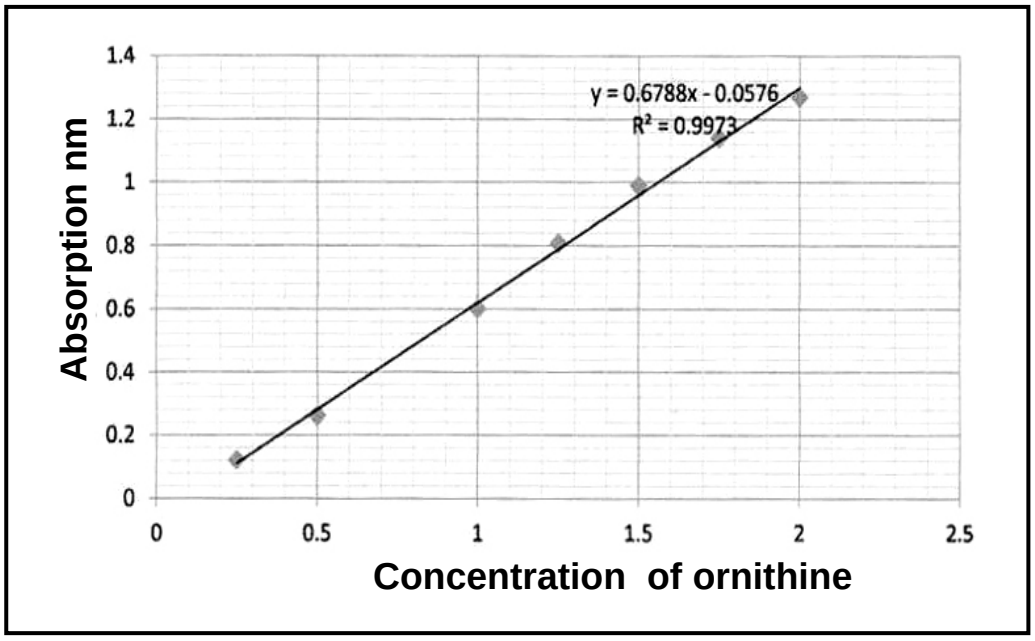

Fig. 1: Standard curve of Arginase enzyme

Determination of carbonic anhydrase in urine:

Carbonic anhydrase (CA) have been measured by MyBiosorce ELISA kit utilization. The stop solution changes the color from blue to yellow and the intensity of color is measured at 450 nm by a spectrophotometer. In order to measure the concentration of human CA in the sample, ELISA kit includes a set of calibration standard. These calibration standards assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus Human CA concentration. Human CA concentration in the samples is then determined by comparing the O.D of the samples to the standard curve.

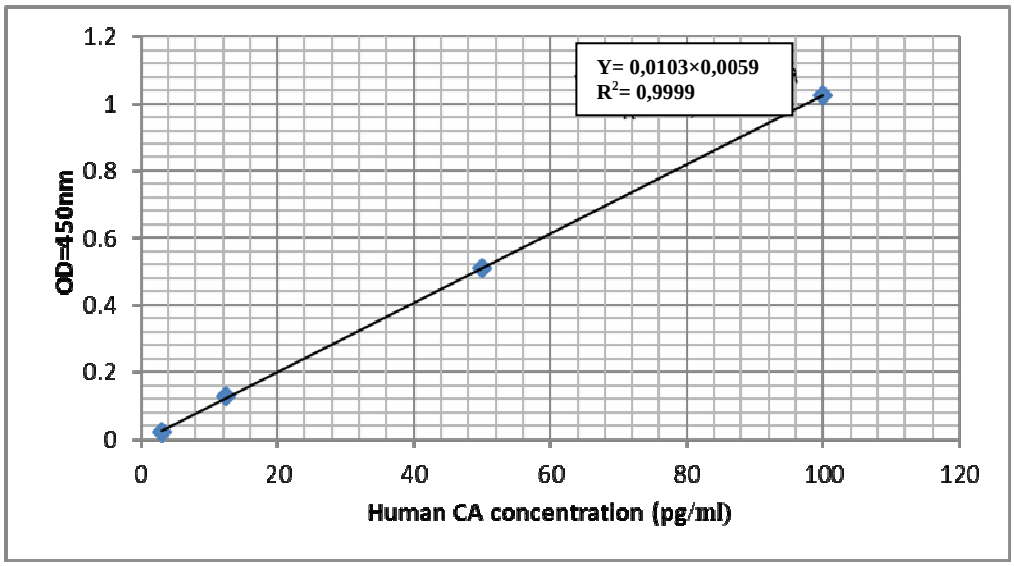

Fig. 2: Standard curve of Carbonic Anhydrase CA

Measurement of fluorescence intensity for urine:

Fluorescence intensity have been measured utilizing Japan, SHIMADZU, (RF-5301 PC) instrument, Urine samples have been diluted to 1:9 then special tube of the instrument have been used to measure the fluorescence emission peaks between 200-800 nm at fixed excitation wavelength 350-400 nm respectively.

RESULTS AND DISCUSSION

Arginase enzyme activity:

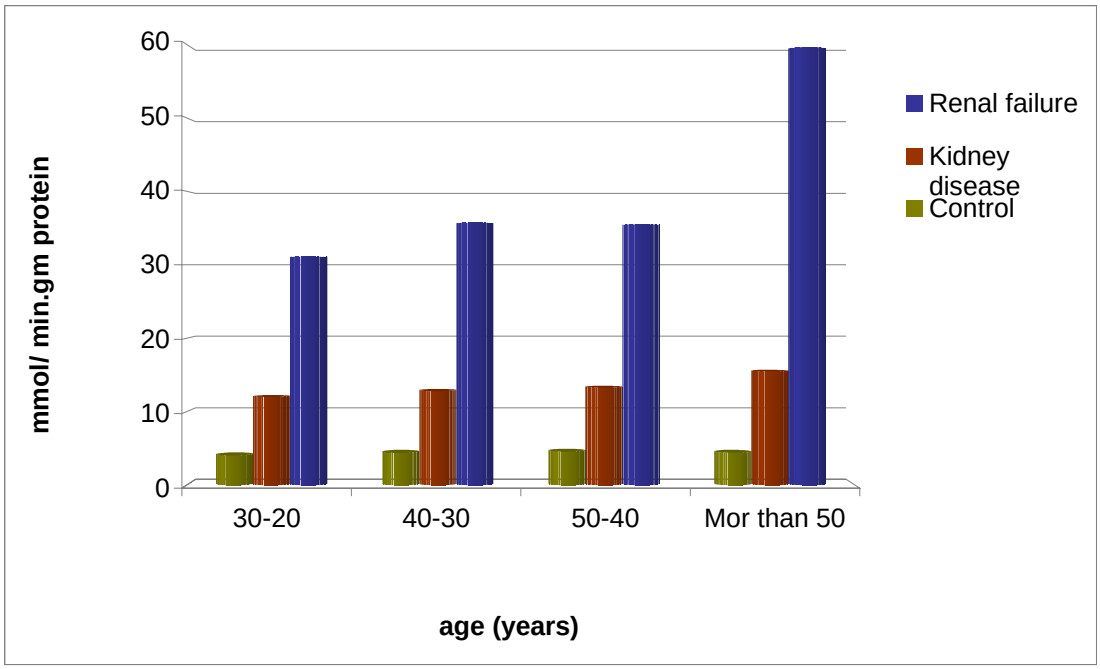

Fig. 3: Activities of arginase in male's urine of control, kidney disease and renal failure

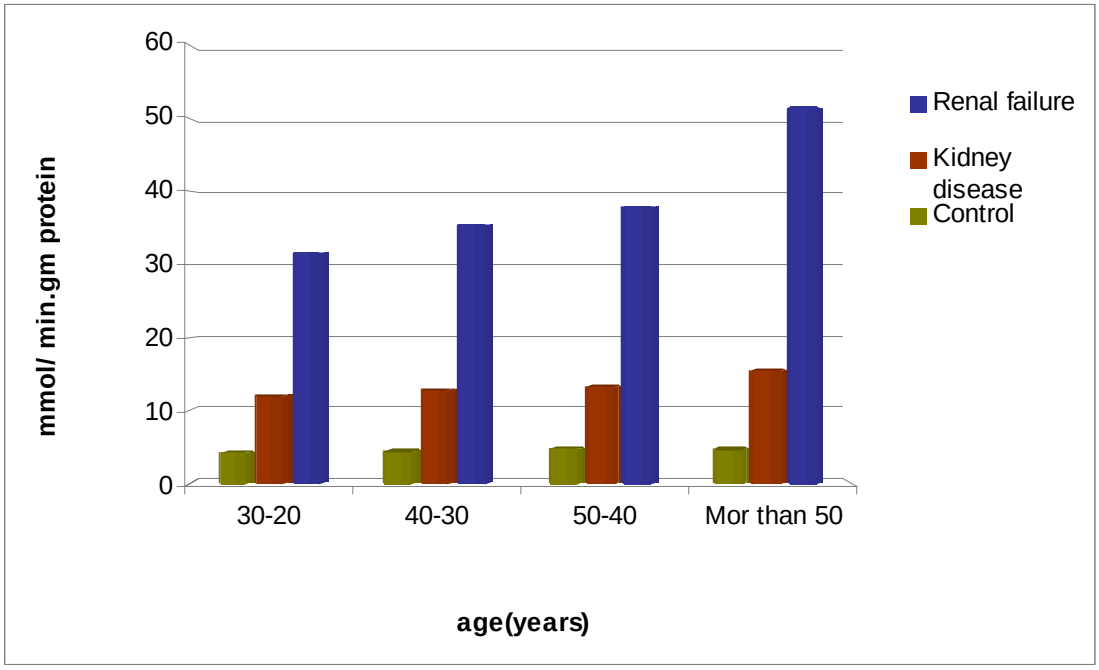

Fig. 4: Activities of arginase in female's urine of control, kidney disease, and renal failure

Fig. 3 and 4 clarified significant increase in arginase enzyme activities in male and female's urine of renal failure and kidney diseases groups compared with the control group at all age stages at probability level $P<0.001$ beside renal failure group showed higher activity compared with kidney diseases group kidney diseases include (tubular disease, urinary tract infection, urinary tract obstruction, renal calculi, cystic disease, vascular disease, tumors). This elevation had been accepted with other researchers such as (Scaglia, 2016), who postulated that this elevation caused by histological and pathological changes such as cell programmed death, infection of kidney and oxidation fatigue(a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, is discussed in relation to its possible role in the production of tissue damage) that increase the ranges of nitrogen oxide which prevent urea and creatinine crossing throughout the kidneys. Elevation of Arginase activity connected with the acute renal diseases, so it works to change the functions of the epithelial cells in heart diseases and renal diseases cases (Hezel *et al*., 2015).

Arginase reduce sodium excretion from kidney when little salt amounts had been taken but induce sodium excretion in high salt amounts case (Lakowicz, 2006). Also arginase increase NO excretion and circle guanosine mono phosphate in urine(arginase convert guanosine tri phosphate to guanosine mono phosphate therefore increased in urine (Lakowicz, 2006)

Arginase enzyme had a special importance, especially during disease period and while infecting with chronic diseases such as hypertension and renal diseases that induce enzyme activity (Scaglia and Lee, 2016).

carbonic anhydrase enzyme

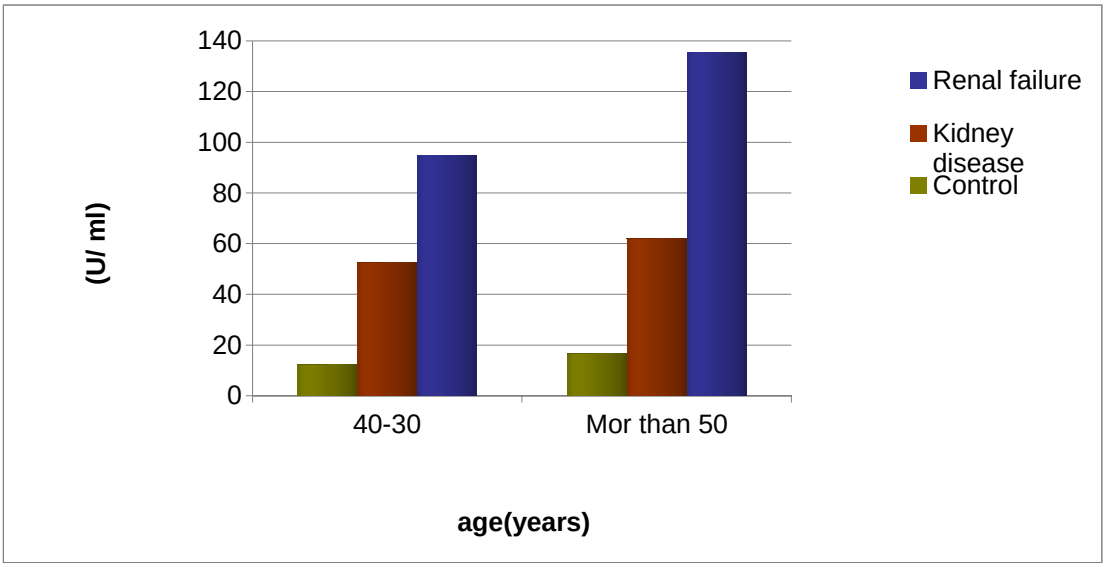

Fig. 5: Carbonic anhydrase activities in males' urine of control kidney disease, and renal failure

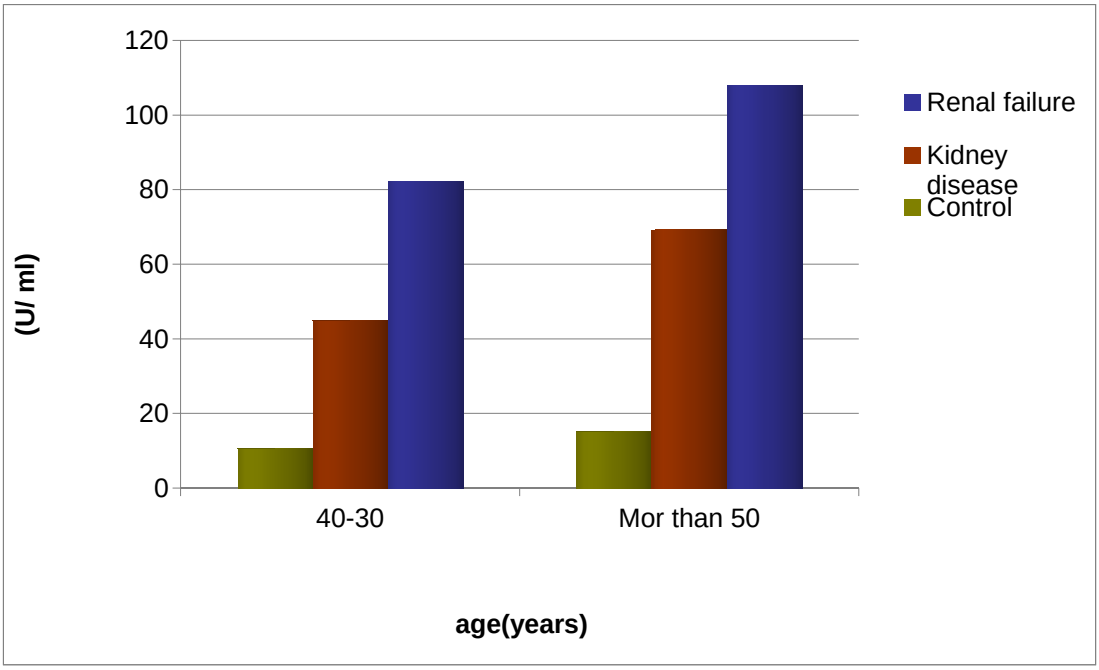

Fig. 6: Carbonic anhydrase activities in female's urine of control kidney disease, and renal failure

Fig. 5 and 6 revealed significant elevation of CA activities among renal failure and kidney diseases groups of all age stages and of both males and females comparing with control at the probability level $P<0.001$. Human CA is used as a guide to differentiate many kinds of cancers, and kidney cells and tissue's damage, and its existence in urine indicates occurrence of kidney's cell damage and disorder in distant tubule of kidneys.

Researches showed that kidneys are responsible of remaking the excreting materials that are lost such as hydrogen ions, and other materials of acid- alkaline balance by reabsorbing filtrated HCO3 and remaking it during metabolism. Kidneys are responsible of both processes excretion of hydrogen ion and reabsorbing of filtrated HCO3 then making new HCO_3 (Hoffmann *et al*., 2011).

Carbonic anhydrase CA plays an important role in the physiological functions of the kidney and stimulate bicarbonate absorbtion, CA inhibition work to prevent bicarbonate absorbing in the

proximal tubule and the high proportions of carbonic anhydrase indicates the occurrence of significant damage occurrence in proximal tubule which lead to non-reabsorption and excretion it with the urine, Researches also indicated that CA inhibitors in membrane act as significant reduction in water and bicarbonates absorption in proximal tubule (Waheed and Sly, 2017).

Intensity measurement of urine's fluorescence spectroscopy

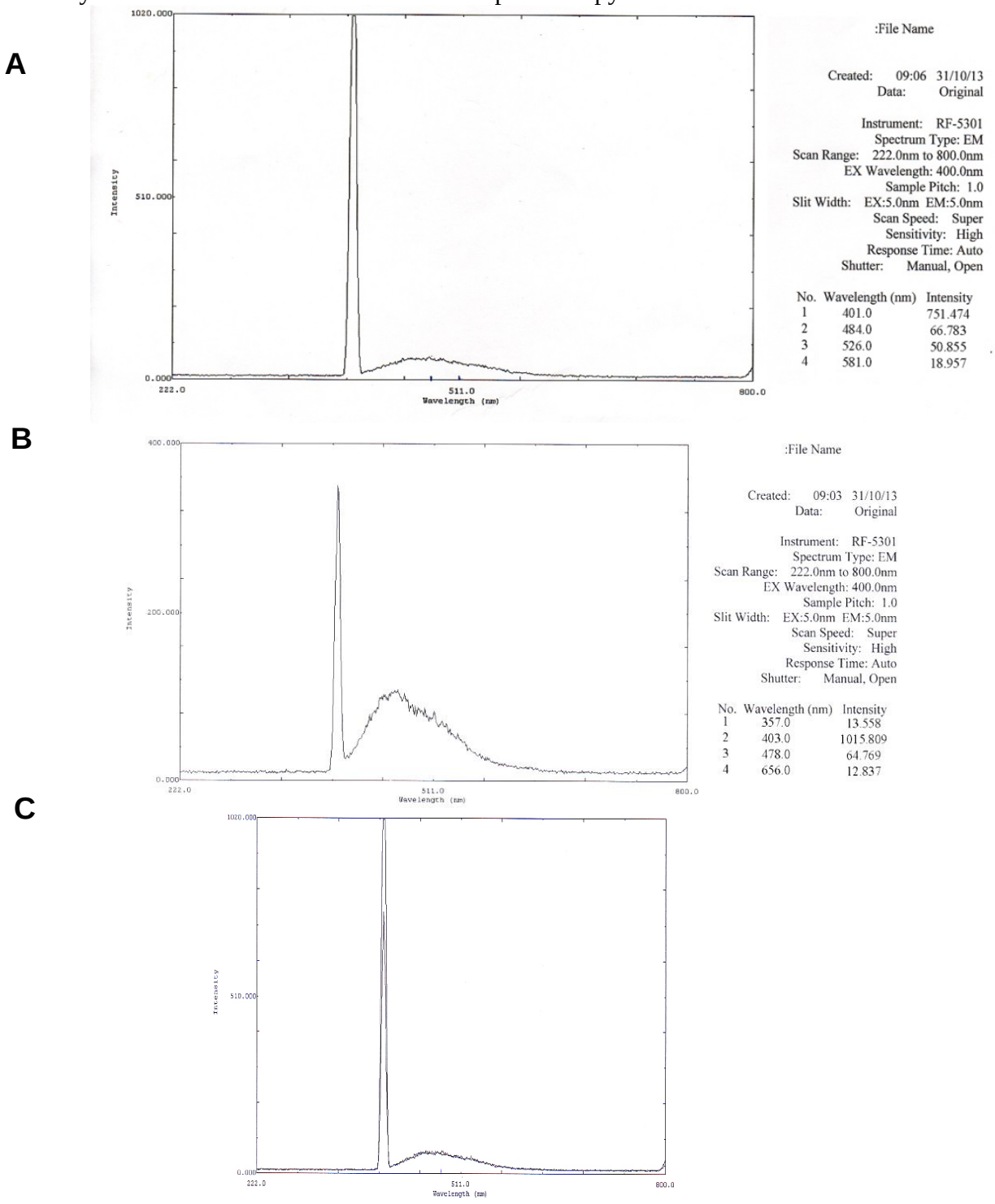

Fig. 7: Emission spectral peaks at fixed 400 nm of male and female urine A: normal male B: normal female C: normal male and female

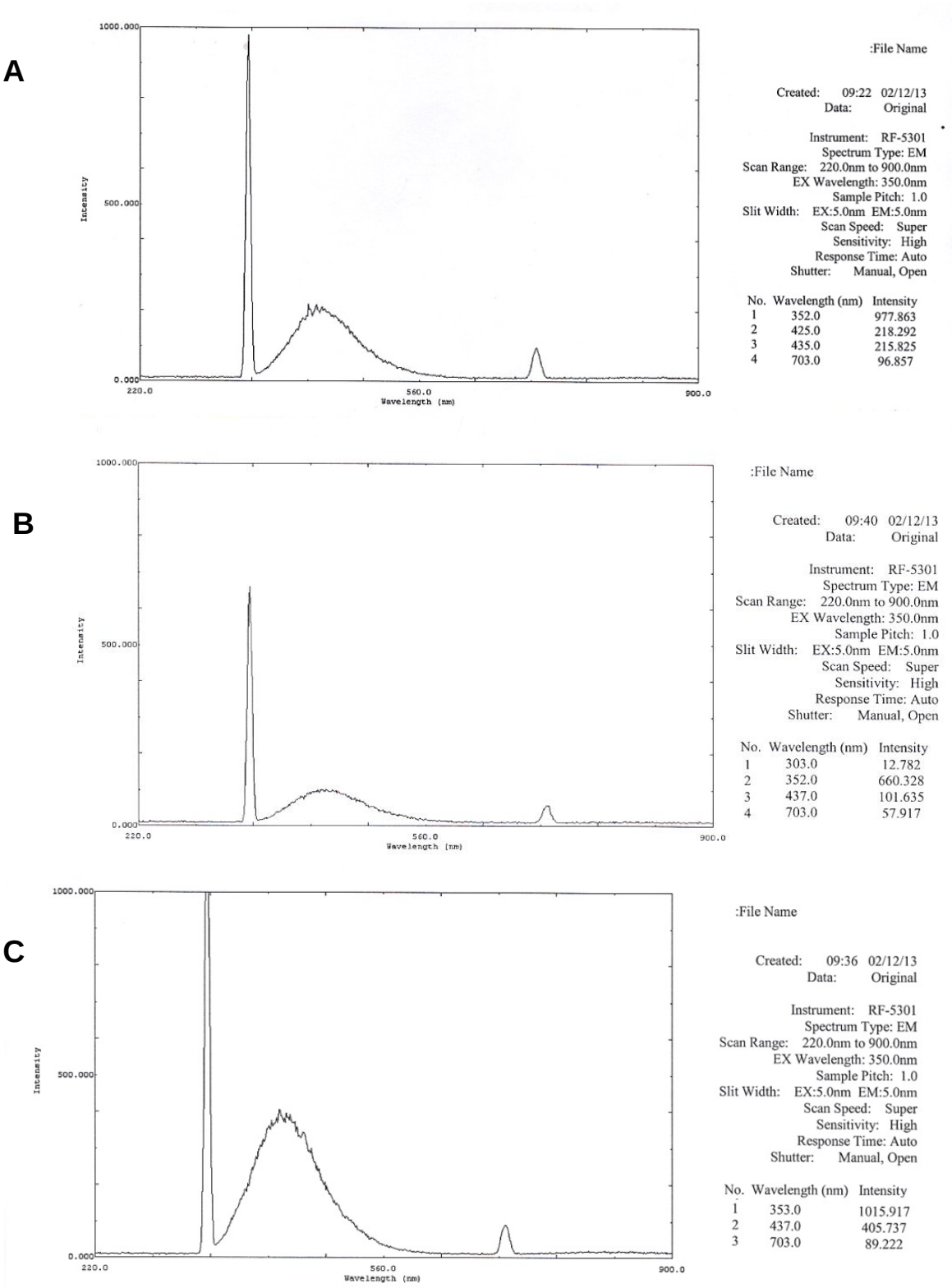

Fig. 8: Emmition spectral peaks at fixed 350nm of normal, kidney disease and renal failure male's urine

A: normal male b: kidney disease male c: renal failure

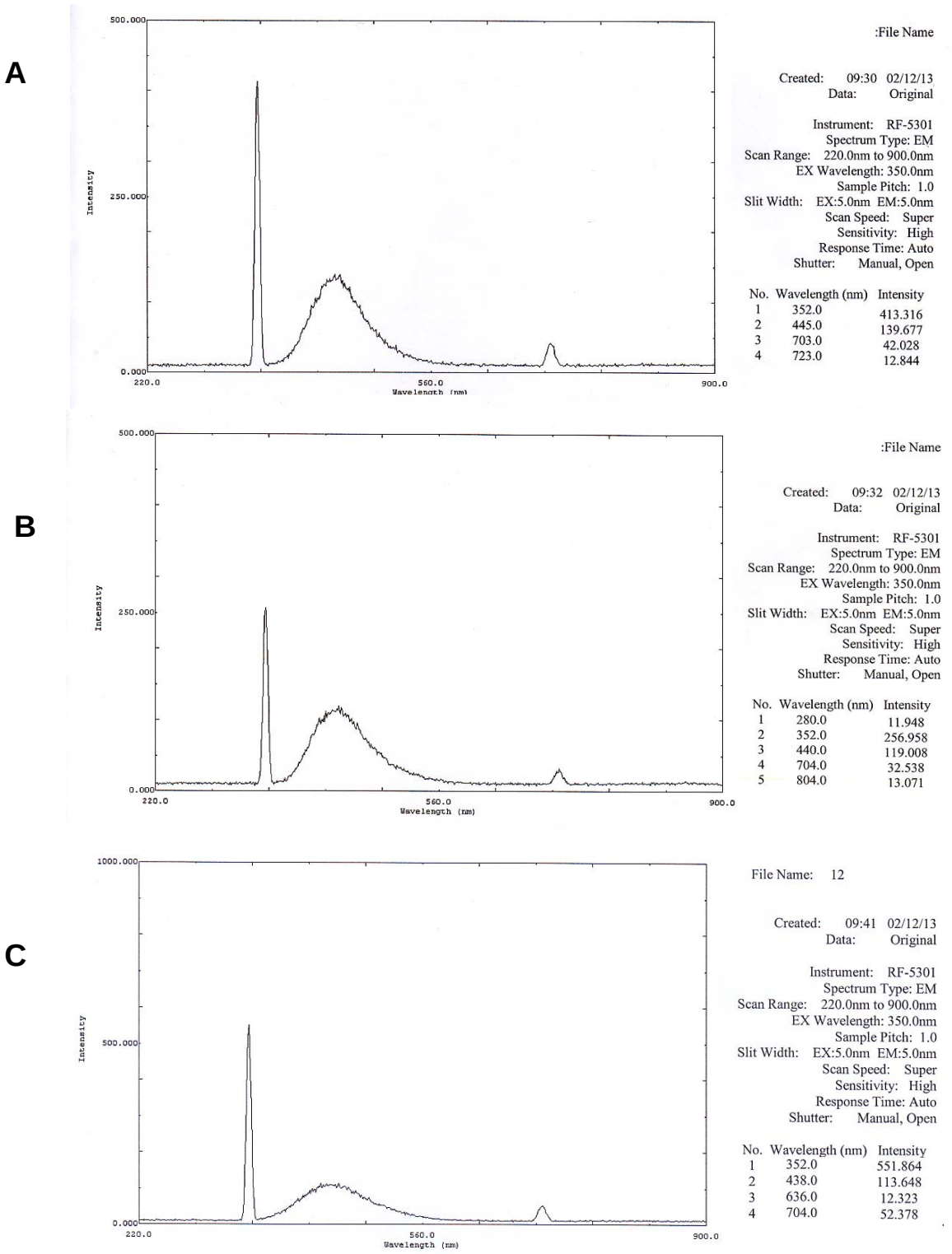

Fig. 9: Emission spectral peaks at fixed 350 nm of normal, kidney disease and renal failure female's urine

a: normal female's urine b:kidney disease female's urine c:renal failure female's urine

The results indicate that the emission fluorescence peaks of the most metabolic compounds showed intensities change between males and females healthy urine, especially at 352, 353, 363nm wavelength and at 425-427 nm when excitation wave lengths were fixed at 400nm as showed in figure (Fernandes *et al*., 2016).

While the changes in emission spectral intensity of male kidney and renal failure patients, at 350 nm fixed excitation wavelengths, revealed significant differences in emission intensity at 425-437 wavelengths, spectral peaks intensity increased to double in the kidneys diseases urine compared with healthy ones, but decreased to half at renal failure patients. Fig. (8)

Whereas in females, the emission wavelengths peaks at 438-445 nm decreased in kidney disease patient's urine and in renal failure cases compared with urine of the healthy people's urine as in Fig. (9).

Furthermore emission spectral peaks showed significant changes at 703 nm in males, so these peaks decreased within kidneys and renal failure patient's urine compared with healthy person's urine while females, intensity emission peaks showed a significant changes at 703-704 nm compared to healthy people when excitation wavelengths fixed at 350nm. These results had agreed with the research of Anwer, who revealed a decrease in fluorescence emission peaks intensity compared with the contaminated urin samples with bacteria (bacteriurea) when excitation wavelengths fixed at 290 nm whereas the excitation lengths 305,395,350 nm showed the highest emission spectral density (Power, 2015).

As well as, the results of this research agreed with the results of Zavarik *et al*. (2013) who confirmed that the emission fluorescence spectroscopy of the metabolic compounds in urine at 400-460 nm is one of the most change sites that had been registered at the patient's urine compared with the healthy persons. And these changes may be due to deficiency or existence of some metabolic compounds in urine such as pyridoxic acid and pteridin compounds that are decreased in the patients of kidney disease and renal failure related to deficiency of vitamin B6.

Also, Dubayova *et al*. (2003) confirmed that fluorescence spectral analysis technique regarded as an effective technique that revealed any changes occurred in metabolic materials exist in any vital system, so the increase of some fluorophores and their metabolic derivatives excreted into urine may happen at a specific illness situation (Patel *et al*., 2007).

As well as, Rejasecaran *et al*. (2013) indicated that urine was regarded as one of important vital diagnostic fluids that contianed different metabolic compounds and many of these compounds are natural fluorophores.

They also stated that emission spectrums intensities at 405 nm wavelength of person's urine infected with cancer showed an obvious difference comparing with healthy people's urine. The most important natural fluorophores of urine are NADH, flavine, derivatives of pyridoxine FAD and the protein connected with the metabolic processes.

Urine samples which have many natural metabolic fluorophors content that had its own emission spectral peaks measured at 305,325,350 nm wavelength and these emission spectral peaks differed between patients and healthy persons and these observed peaks wouldn't result by one metabolic content, but depend on many contents that differs according to the clinical situation of the person (Rana, 2008).

The results of this research came corresponded with the research of Leiner *et al*.(1987) which confirmed that emission fluorescence spectral peaks changes had been registered for some patients as a result of intensities change of some urine fluorophores. These fluorophores includ; indole derivatives that gave emission spectral peaks at 300-320 nm such as; 3-,5-hydroxyindol-3acetate, 4-pyridoxic acid, xanthine and hydroxyl anthranilic acid which have emission spectrum 345-435 nm.

We conclude from current research that urine's emission fluorescence spectroscopy measuring regarded as an effective method for revealing the differences of urine spontaneous metabolic compounds among health, kidney diseases and renal failure persons, and 350nm excitation wavelengths used in this research were effective for showing these differences.

REFRENCES

Del Prete, S.; Vullo, D.; De Luca, V. (2016). Cloning, expression, ourification; sulfonamide inhibition profile of the complete domain of the carbonic anhydrase from plasmodium falciparum. *Bioorg Med. Chem. Lett*., **26**,4184-4190.

Dubayova, K.; Kusnir, J.; Podracka, L. (2003)..Diagnostic monitoring of urine by means of synchronous fluorescence spectrum. *J. Biochem. and Biophys. Methods.* **55**, 111-119.

Ekinci, D.; AL-Rashida, M.; Abbas, G. (2015). Chromone containing sulfonamides as potent carbonic anhydrase inhibitors. *J. Enzyme Med Chem*., **27**,744-7.

Fernandes, HS.; Teixeira, CS.; Fernandes, PA.; Ramos, MJ.; Cerqueira, NM. (2016). "Amino Acid Deprivation Using Enzymes as a Targeted Therapy for Cancer and Viral Infections". Expert Opinion on Therapeutic Patents. pp. 1–15.

Hezel, MP.; Liu, M.; Schiffer, TA. (2015). Effects of long-term dietary nitrate supplementation in mice. *J. Redox Biol*. **5**, 234–242.

Hoffmann, K.M.; Samardzic, D.; VandenHeveer, K.; Rowlett, R.S. (2011). CO(II)-Substituted Haemophilus influenza b-carbonic anhydrase: Spectral evidence for allosteric regulation by PH and bicarbonate ion. *Archives Biochem. and Bioph.*, **511**(1-2), pp.80-87.

Kocna, P.; Fric, P.; Zavoral, M.; Benesova, A.; Pelech, T. (1993). Arginase new marker of mucosal proliferating activity in screening for colorectal neoplasia. *Clin. Biochem. Rev.*, **14**, 237.

Konarska, L.; Tomaszweski, L. (1986). A simple quantitative micromethod of arginase assay in blood spots dried on filterpaper. *Clin. Chim. Acta.*, **154**, 7-8.

Krishnaraju, V.; Rao, V.N.; Golakoti, T. (2009). In vitro and In vivo Antioxidant Activity of Aphanamixis polystachya Bark. *Amer. J. Infection Dis.* **5**(2), 60-67.

Lakowicz, J.R. (2006). "Principles of Fluorescence Spectroscopy". Springer Publisher. New York, chapter 10.

Le Darz, A.; Mingot, A.; Bouazza, F. (2015). Fluorinated pyrrolidines and piperidines incorporating tertiary benzenesulfonamide moieties are selective carbonic anhydrase II inhibitors. *J. Enzyme Inhib. Med Chem*., **30**, 737-45.

Leiner, J.P.; Hubmann, M.R.; Wolfbeis, O.S. (1987). The total fluorescence of human urine. *Analytica. Chimica. Acta*. **198**, 13-23.

Manthy, K.C.; Chew, Y.C.; Zempleni, J. (2005). Riboflavin deficiency impairs oxidative folding and secretion of apolipoproteine-B-100 in HepG2 cells, triggering stress respone system. *J. Nut.*, **135**(5), 978-982.

Patel, P.; Abraham, G.; Pratap, B.; Ramalaksmi, R. (2007). Clinical and biochemical parameters in chronic kidney disease with pulmonary hypertension. *India. J. Nephrol*., **17**,4-6.

Perrone, R.D. (2011). Cardiac magnetic resonance assessment of left ventricular mass in autosomal dominant polycystic kidney disease. *Clin. J. Am Soc. Nephrol*., **6**(10), 2508-15.

Power, H.J. (2015). Riboflavin (vitamin B-2) and health. *American J. Clin. Nutrition*. **77**(6), 1352-1360.

Rajasekaran, R.R.; Aruna, P.R.; Koteeswaran, D.; Padmanabha, L. (2013). Characterization and diagnosis of cancer by native fluorescence spectroscopy of human urine. Photochemistry and Photobiology. **89**(2), 483-91.

Rana, R. (2008). Primary diagnosis of abnormal urine via auto fluorescence process. *J. Laser A* **7**, 15-19.

Scaglia, F.; Lee, B. (2016). Clinical, biochemical; molecular spectrum of hyperargininemia due to arginase I deficiency. *Am J. Med Genet C Semin Med Genet*; **142**, 113-20.

Supuran, C.T. (2008). Carbonic anhydrase: novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug Discov*., **23**, 101-7.

Waheed, A.; Sly, W.S. (2017). Carbonic anhydrase XII function in health disease. **14**(20),10.

Zvarik, M.; Martinicky, D.; Hunakova, L.; Lajdova, I., Sikurova, L. (2013). Fluorescence characteristics of human urine from normal individuals and ovarian cancer patients. *Neoplasma*. **60**, 5.