UPM Inaugural Lecture Series

IN SEARCH OF AN EARLY INDICATOR OF KIDNEY DISEASE

Prof. Dr. Rasedee Abdullah

UPM
UNIVERSITI PUTRA MALAYSIA

Rasedee Abdullah

In Search of An Early Indicator of Kidney Disease

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INAUGURAL LECTURE

PROF. DR. RASEDEE ABDULLAH

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UNIVERSITI PUTRA MALAYSIA

RASEDEE ABDULLAH

Professor Rasedee Abdullah was born in Kelantan in 1947. He received his primary education at Sultan Ismail School and secondary education at Sultan Ismail College. Although he is a dedicated student he is also a keen sportsman, and represented the school and state in athletics and gymnastics. In 1967, he was inducted as a pioneer member of the first form six science class in a Kelantan school. Subsequently as a upper six form student he was elected the Head Prefect and School Captain. Recognising his potential, he was award the Colombo Plan scholarship to study Biochemistry at the University of Western Australia, Perth. This award was the first ever given to a student from a school in Kelantan. He graduated with B.Sc (Hons) in Pharmacology and Biochemistry(1973), immediately began serving UPM as a tutor. The Government of Malaysia and UPM provided him two more opportunities to further his studies. This he relishingly grabbed and eventually graduated with M.Sc at the University of New England (1975), PhD at the University of California, Davis (1989).

He was the Head of Department of Veterinary Pathology and Microbiology for 4 years (1991-1995) while serving as the coordinator of the Diagnostic Clinical Pathology Laboratory. During his tenure, he created the corporate plan for the department which became the model to the Faculty corporate plan. He was instrumental in creating among the members of the Department, a sense of belonging and allegiance to the Department, Faculty and University. He is was fully involved in improving the existing curriculum of the Faculty and developing new ones. He was a member of the original committee of three academic staffs which created the first Bachelor of Science (Biomedical Science) curriculum at UPM. In 2003 he mooted the idea of Bachelor of Sciences (Veterinary Technology) and almost single-handedly created the complete curriculum. He served in a number of Faculty and University committees and positions which included the Faculty and University research committees, University Senate member, Faculty Postgraduate and Computer Committee and several Conference Organising Committees. He also served as the Secretary of the Working Committee for the Establishment of the Veterinary Complex with a working budget of RM 50.5 million. The blue-prints are now ready, the building is expected to be built in the 9th Malaysia Plan. When the University aspired to obtain the ISO 9002 certification, he acted as the first Coordinator of the Faculty of Veterinary Science ISO 9002 certification Committee. Currently he is the Coordinator of the Hematology and Clinical Biochemistry Laboratory busy with diagnostic activities. He is also the Coordinator of the newly-formed Veterinary Laboratory Services Unit, Faculty of Veterinary Medicine, UPM which provides laboratory services to clients within and outside campus.

He was on the Editorial Board of Jurnal Veterinar Malaysia (Malaysian Veterinary Journal) for more than 10 years. He was the Editor of the Proceedings of the Conference on The Veterinary Profession and Education, 1993. Currently he is the Chairman of the committee responsible for the production of one volume of the Malaysian Encyclopaedia of Science and Technology on agriculture and forestry. The encyclopaedia is a collaborative effort between Universiti Teknologi Malaysia and Dewan Bahasa dan Pustaka. He also serves as a one of the Substantive Editors for the Encyclopaedia.

He strongly believes that Bahasa Kebangsaan Malaysia can be developed into a modern language of international standing. In 1981, he was invited as a consultant and expert advisor on veterinary science to Dewan Bahasa dan Pustaka, Malaysia, and has been contributing to the institute since then. In 2000, he chaired the regional (Malaysia-Brunei-Indonesia) committee to coordinate the use of veterinary terminologies in these countries. His contribution included 2 books on terminologies ("Istilah Veterinar" and "Istilah Perubatan) and one Dictionary of Veterinary Terminologies ("Kamus Istilah Praklinikal Veterinar").

Professor Rasedee is a prolific translator and has translated several text books, articles, and other manuscripts in veterinary and allied sciences. He has gained recognition as a translator and was invited to sit on the National Panel of Judges to determine the best Malaysian translation work (books) for 1995.

By training, he is a clinical pathologist, specialising in haematology and clinical biochemistry. In fact he is the only qualified Veterinary Clinical Pathologist in the country. As a teacher, he have always been open, always available for consultation and advice and in 2001 chosen as a role model to the Bachelor of Science (Biomedical Science) students by one the residential colleges of the University.

He has successfully supervised and co-supervised more than 10 PhD candidates, more than 30 M.S candidates, and more than 50 final student projects.

His early research was on acquisition of basic data, metabolic profiling, and basic haematological studies. Later his shifted his interest to coagulation disorders and eventually to experimental renal damage and equine medicine and reproduction. His work on coagulation abnormalities in nephrotic syndrome was generally recognised and as a result was invited to contribute an article to The Veterinary Clinics of North America. He then embarked on a search for an early plasma/serum indicator to renal damage which brought mixed findings. Currently, the focus of his study is on urine parameters in renal disease. He has more than 50 publications to his name, which he published internationally and locally.

Again, he has moved on, and currently his research is on the "Development and Production of Human Recombinant Erythropoietin", with an IRPA grant of RM 10.5 million. This is one of the five projects under the huge programme headed by Prof. Mohd Azmi of the Institute of Bioscience, UPM. His work on equine tendinitis which resulted in a PhD thesis awaiting review, is still in progress.

He was an avid squash player and now a competent golfer and represented the University in both sports. He coached squash to the UPM students and staff and in 1994 was appointed Manager of the Malaysian Squash Team to the ASEAN University Games.

In recognition for his dedication and contribution, each year Professor Rasedee has always been listed among the recipients of the Excellence Service Award given both by the Faculty of Veteinary Medicine and University.

"IN SEARCH OF AN EARLY INDICATOR OF KIDNEY DISEASE"

ABSTRACT

The incidence of kidney disease is on the rise worldwide and this health problem needs to be addressed urgently. Kidney diseases are often associated with diabetes mellitus and hypertension.

Nephrotic syndrome is a disease in which the glomerulus is damaged and this is manifested as proteinuria, hypoproteinaemia, lipidaemia, lipiduria and oedema. The syndrome is often complicated by haemostatic abnormalities. Our studies suggested that the haemostastic abnormalities in part can be due to a state of hypercoagulability of the blood, from platelet hyperaggregability and activation of the intrinsic coagulation pathway. Platelet hyperaggregability in nephrotic syndrome is mediated by thrombocytosis from increased platelet production by the bone marrow, increase serum fibronectin, and increase platelet to platelet interaction through platelet surface receptors to fibronectin.

Kidney disease can be detected through serum analysis and urinalysis. These parameters used in the diagnosis of kidney disease include blood urea nitrogen (BUN), serum and urine creatinine and their ratios. The kidneys have a high capacity for compensation thus making detection and diagnosis of early kidney diseases using these laboratory tests difficult. Although proteinuria is a constant feature in kidney disease, the detection of significant proteinuria would still suggest that the disease has progressed too far, making treatment difficult and expensive.

There is a need to discover an early indicator of renal diseases. Several urine enzymes, such (γ-glutamyltransferase (GGT), β-N-acetylglucosaminidase (NAG), β-glucuronidase (GRS), aspartate aminotransferase (AST) and alkaline phosphatase (AP) have been implicated as possible candidates. The studies have shown that GGT and AP are two urine enzymes that respond fastest in puromycin aminonucleoside (PAN) induced renal damage. A recent finding suggests there may be another candidate for an early indicator of renal damage. This protein, 11 kDa in molecular weight, seems to consistently increase in gentamicin-induced renal damage in rats.

INTRODUCTION

The incidence of renal disease has been estimated to gradually increase worldwide. This is an urgent public health concern that need to be addressed. Since the disease is not easily detectable at the early stages, the true incidence of renal disease is not known. In the United States alone the number of new cases of end-stage renal disease (ESRD) is projected to reach 650,000 by 2010 with an expected medicare expenditure of USD 28 billion (Xue *et al.* 2001). What was postulated to be the cause to the increasing incidence of renal disease include greater proliferation of environmental toxins (Port, 1992); increased use of nephrotoxic agents, such as analgesic medications (Muntner *et al.*, 2003); and the growing epidemic of diabetes mellitus. There are other unidentified and unknown factors that may also contribute to increasing incidence of renal disease.

Likewise the true incidence of renal disease in Malaysia is not known. Although in 2002, nephritis, nephrotic sydrome and nephrosis constitute 3.53% of the principal cause of death in Malaysia (Health facts 2002, 2004). Patients are often presented to the hospitals late, and only when symptomatic. As the data collected from the Department of Nephrology, General Hospital Kuala Lumpur in 1982 suggested, more than half of 174 patients with ESRD treated at the hospital presented for the first time as uraemic with no known renal disease in the past. The same study showed that the known causes of chronic renal failure in order of frequency were glomerulonephritis, diabetic nephropathy, obstructive uropathy, chronic pyelonephritis, lupus nephritis, malignant hypertension, gouty nephropathy and polycystic disease (D'Cruz and Chandrasekaran, 1990).

The Kidneys

The human kidneys are bean-shaped organs, about the size of the fist. These organs filter and remove metabolic waste and excess water from the body. The filtration process is performed by the units in the kidneys called nephrons. The kidneys have approximately a million nephrons and each nephron consists of the glomerulus, the proximal and distal tubules which drain into the collecting ducts. The glomerulus is a cluster of looping blood vessels intertwining with the tubules to allow filtration and exchange of water and waste materials between the urinary and circulatory systems.

Among the functions of the kidney is to regulate body fluid volume and electrolyte content especially, sodium, potassium, chloride, calcium, magnesium, phosphate, sulphate, and hydrogen, and acidity. The regulation of fluid volume helps to regulate blood pressure in the arteries. Thus kidney disease is the most common cause of secondary hypertension (high blood pressure). Even subtle disruptions in kidney function play a role in most cases of high blood pressure. Injury to the kidney can eventually cause malignant hypertension, stroke or even death.

The kidneys also release three important hormones:

- **Erythropoietin** or **EPO** which stimulate the bone marrow to produce erythrocytes (red blood cells). This hormone functions by recruiting progenitors cells, shortening erythrocyte maturation time, and increase erythrocyte release by the bone marrow hence facilitating erythrocyte production and release by the bone marrow (Figure 1).

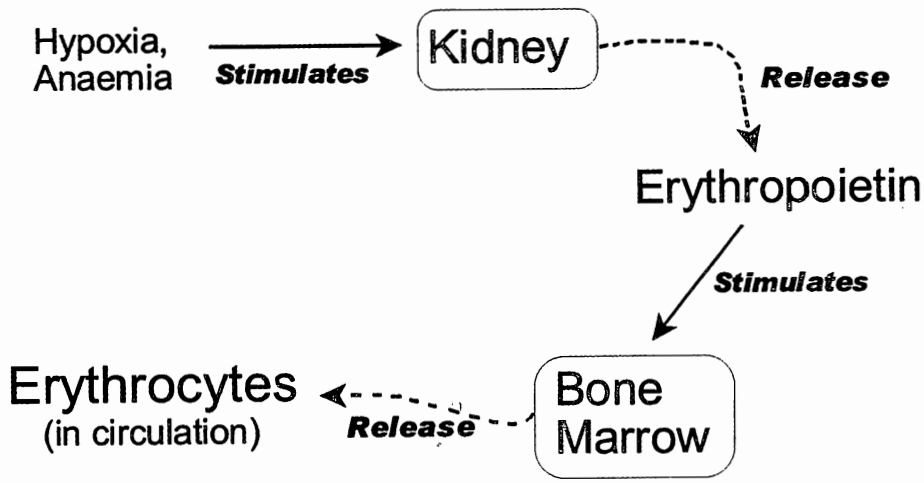

Figure 1. Erythropoietin in the erythrocyte production by the bone marrow

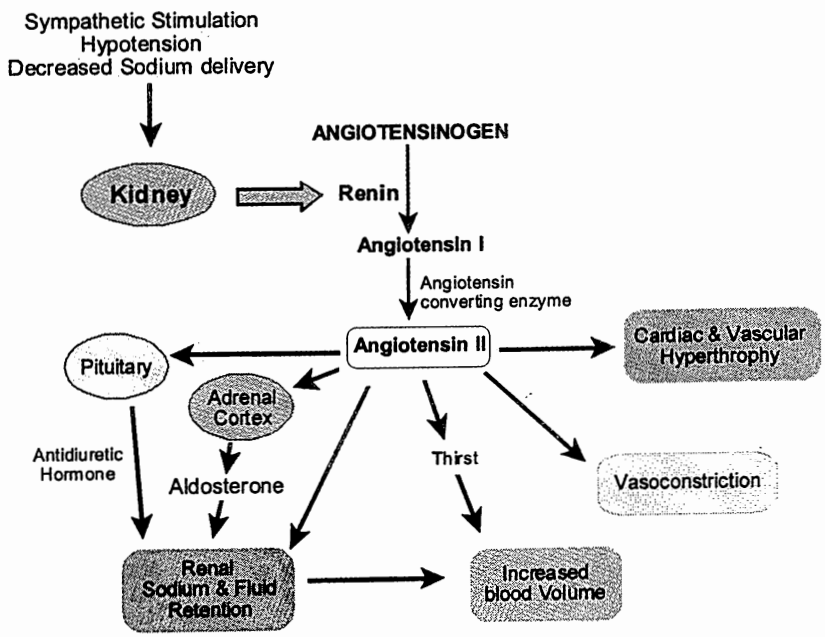

Figure 2. The renin-angiotensin system in the regulation of blood pressure.

- **Renin** which regulates blood pressure through the pathway in Figure 2.

- **Calcitriol** [1,25 dihydroxycholecalciferol], an active form of vitamin D which enhances absorption of both calcium and phosphate from the gastrointestinal tract, promotes release of calcium and phosphate from the bones where they are stored, and inhibits renal excretion of calcium and phosphate. In short, it increases the amount of calcium and phosphorus circulating in the bloodstream.

How do the kidneys fail?

Kidney disease results from damage to the nephrons causing them to lose their filtering capacity. Usually the damage occurs very gradually over years. Most kidney diseases occur in both kidneys simultaneously. There is no obvious symptom to kidney failure, thus it is often undetectable.

The most common causes of kidney disease are diabetes mellitus and high blood pressure with contribution from hereditary factors.

- **Diabetes mellitus**: In diabetes mellitus there is poor utilisation of glucose. Poor control in sugar intake would result in hyperglycaemia which is hazardous to the body. With diabetes, the small blood vessels are easily injured. The kidney function is totally reliant on blood perfusion. Damage blood vessels to the kidneys would render them unable to filter waste effectively. Diabetes may also cause damage to the nerves. This can cause difficulty in the emptying of the bladder. The pressure built-up from a full bladder can back up and injure the kidneys. In addition, prolonged urine retention in the bladder can result in infections from rapid bacteria growth in urine high in sugar.

- **High Blood Pressure**: High blood pressure can also damage the small blood vessels in the kidneys.

- **Hereditary**: Some kidney diseases result from hereditary factors. If the family has a history of any kind of kidney problems, there is a potential for kidney disease to develop. Polycystic kidney disease (PKD), for example, is a genetic disorder in which cysts grow in the kidneys. The PKD cysts can slowly replace some of the kidney tissue, reducing kidney function and eventually causing kidney failure

Other causes of kidney disease include poisons and drugs as follows:

Decrease renal perfusion
Non-steroidal anti-inflammatory drugs (NSAIDs), acetylcholinesterase (ACE) inhibitor, contrast media, amphotericin B, cyclosporin, tacrolimus

Tubular injury
Aminoglycosides, contrast media, amphotericin B, methotrexate, cipslatin, foscarnet, pentamidine, heavy metals, myoglobin, haemoglobin, intravenous immunoglobulin, HIV protease inhibitors

Tubular obstruction
Contrast media, methotrexate, acyclovir, sulphonamides, ethylene glycol, uric acid, cocaine, lovastatin

Inflammation (immune-mediated)
Penicillin, cephalosporins, allopurinol, NSAIDs, sulphonamides, diuretics, rifampin, ciprofloxan, cimetidine, tetracyclines, phenytoin

HAEMOSTATIC ABNORMALITIES IN RENAL DISEASES

Among renal diseases that manifest as significant proteinuria is nephrotic syndrome. Nephrotic syndrome is a disease in which the glomerulus is damaged causing loss of plasma protein into urine. In fact nephrotic syndrome has diverse causes and is manifested as proteinuria, hypoproteinaemia, lipidaemia, lipiduria, and oedema. Nephrotic syndrome is often complicated by haemostatic abnormalities, including thrombosis in the renal microvasculature or at more distal sites, such as mesenteric, pulmonary and coronary arteries (Rasedee, 1988).

Nephrotic syndrome has been considered to be a hypercoagulable state of the blood associated with a state of platelet (thrombocyte) hyperaggregability (Rasedee and Feldman, 1985) (Figure 3). Platelets are blood cells involved in blood coagulation. Activation of these cells results in aggregation and with the deposition of fibrin from activation of the coagulation cascade cause thrombosis and eventually partial or complete obstruction of the blood vessels. It was shown that in nephrotic syndrome induced by puromycin aminonucleoside (PAN) there is an increase in plasma and urine fibronection (Rasedee and Feldman, 1990) (Table 1). Fibronectin is an adhesive protein primarily involved in cell to cell and cell to surface interactions (Mosher, 1980). This glycoprotein is produced by vascular endothelial cells (Jaffe and Mosher, 1978), fibroblasts (Yamada and Olden, 1978), kidney and liver cells (Chen *et al.*, 1977) platelets (thrombocytes), and macrophages (Alitalo *et al.*, 1980). In primary glomerular disease there is a redistribution of this glycoprotein in the mesangial cell and the pericapillary regions of the kidneys(Ikeya *et al.*, 1985).

Table 1. Urine and plasma fibronectin concentrations in PAN-induced rat nephrotic syndrome

Days Post-PAN	Urine Fibronectin (μg/mL)	Plasma Fibronectin (μg/mL)
0	0	733.2 ± 10.6
2	0	501.0 ± 44.4
4	0	592.0 ± 25.6
6	146.6* ± 39.9	1278.0* ± 126.0
8	317.8* ± 14.4	1230.0* ± 206.6
10	337.4* ± 37.8	1377.0* ± 93.0

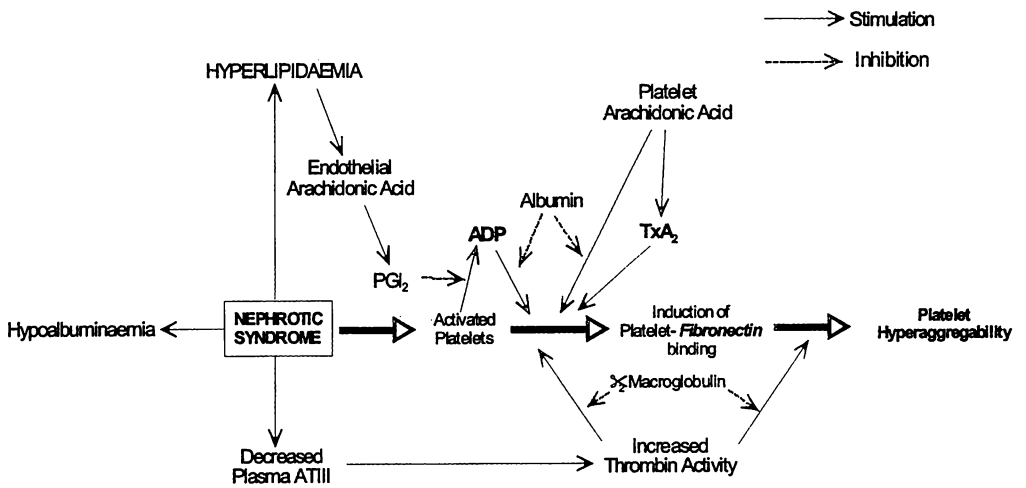

Figure 3. Mechanism of platelet hyperaggregability in nephrotic syndrome.

Table 2. Mean plasma concentration of coagulation factors in PAN-induced nephrotic syndrome in rats.

Coagulation Factor	Mean Plasma Coagulation Factor Concentration Post-PAN (% of pooled plasma)						
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	S.E.
II	93	110	92	133	141	153	12
V	99	77	65	197	261	310	27
VII	105	88	88	226	249	329	32
VIII	113	182	136	142	154	108	17
IX	92	103	92	81	82	52	10
X	119	220	144	282	264	292	29
XI	116	204	112	185	176	169	23
XII	102	91	75	54	64	40	10
Fibrinogen (I)	2	2	2	4	4	4	0
ATII	81	61	68	63	49	37	3
Plasminogen	100	77	66	30	20	18	4

In nephrotic syndrome platelet hyperaggregability was postulated to be the result of release of fibronectin and its expression on the platelet surface and causing platelet aggregation (Rasedee and Feldman, 1985; Rasedee, 1991b) (Figures 4 and 5). The coagulation factor XII, of the intrinsic coagulation pathway decreased while the coagulation factor VII of the extrinsic pathway increased with time in PAN-induced nephrotic syndrome (Table 2). This suggests that it is the intrinsic coagulation pathway that is primarily activated in PAN-induced nephrotic syndrome, and may be from blood vessel damage in the diseased kidneys (Rasedee, 1991a).

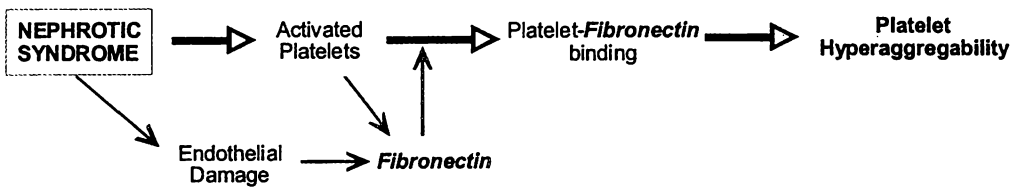

Figure 4. Proposed role of fibronectin in platelet hyperaggregability in nephrotic syndrome

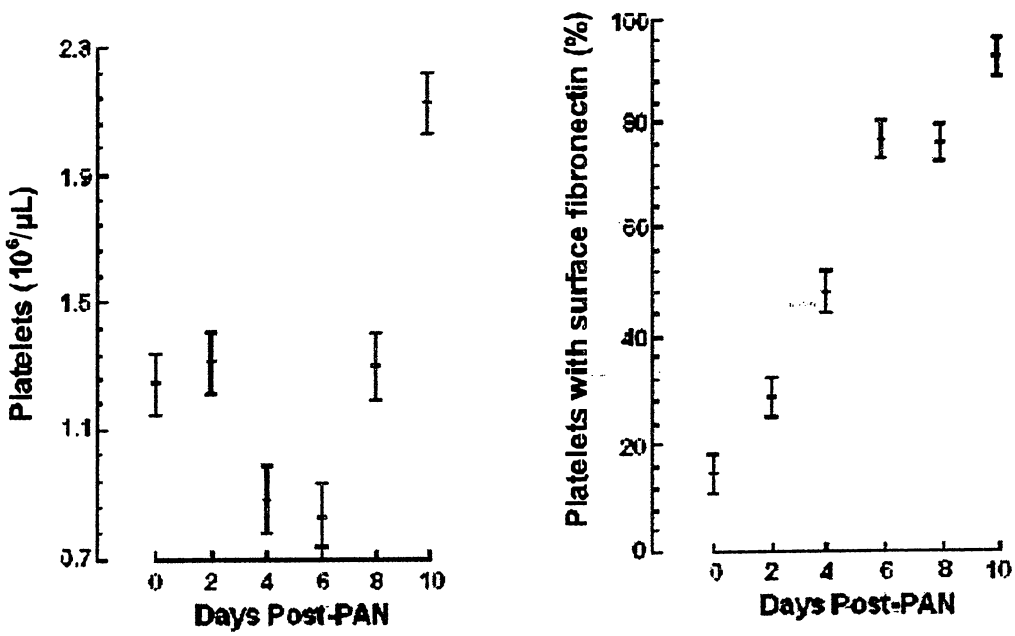

Figure 5. Platelet number and platelets with surface fibronectin in PAN-induced nephrotic syndrome

Although it was shown that thrombocytopaenia (decreased blood platelet number) occurred in early in nephrotic syndrome (Rasedee and Feldman, 1985) the platelet number began to increase later in the disease (Rasedee, 1991b). This was confirmed by the bone marrow response in the syndrome. The bone platelet precursors (megakaryoblasts and

megakryocytes) increased at the later stage of the disease suggesting stimulation of platelet production (Rasedee and Ragavan 1993) (Table 3). The study indicated that platelet response to nephrotic syndrome is biphasic, thrombocytopaenia in the early stage due to consumption and thrombocytosis (increased blood platelet number) in late stage of the disease due to bone marrow response to the disease.

Table 3. Percentage of bone marrow cells in puromycin aminonucleoside-induced nephritic syndrome.

Bone marrow Cells	**Mean percentage (%) of total cells counted**					
	Day 0	**Day 2**	**Day 4**	**Day 6**	**Day 8**	**Day 10**
Myeloblasts	0.1 ± 0.8	0.1 ± 0.1	0.8 ± 0.5	0.5 ± 0.4	0.7 ± 0.5	0.2 ± 0.1
Progranulocytes	1.2 ± 0.3	1.8 ± 0.5	3.8 ± 1.36	2.5 ± 0.6	3.5 ± 0.7	3.0 ± 0.6
Myelocytes	4.0 ± 1.4	7.9 ± 1.2	8.58 ± 1.4	11.8[a] ± 1.9	12.9[a] ± 0.8	6.6 ± 1.9
Metamyelocytes	18.5 ± 1.7	18.5 ± 2.2	12.9 ± 1.5	8.6[a] ± 1.4	12.6 ± 1.4	14.1 ± 2.5
Band neutrophils	8.1 ± 1.0	10.4 ± 1.4	6.0 ± 1.9	4.2[a] ± 0.7	5.5 ± 0.5	4.0[a] ± 0.8
Seg. Neutrophils	14.7 ± 2.0	13.6 ± 1.5	19.2 ± 1.2	3.1[a] ± 0.9	6.2[a] ± 0.8	10.6 ± 1.7
Eosinophils	2.6 ± 0.5	7.2[a] ± 1.7	4.2 ± 0.6	3.8 ± 0.7	5.2 ± 0.9	5.1 ± 0.6
Basophils	0.7 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.19
Lymphocytes	0.1 ± 0.1	0.2 ± 0.1	1.5 ± 1.0	0.7 ± 0.6	0.1 ± 0.1	0.35 ± 0.2
Monocytes	0.1 ± 0.1	0.7 ± 0.2	1.2 ± 0.6	0.7 ± 0.4	0.7 ± 0.2	0.7 ± 0.2
Plasma cells	0.1 ± 0.1	0.5 ± 0.4	0.5 ± 0.3	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
Rubriblasts	0.1 ± 0.1	0.1 ± 0.1	0.8 ± 0.4	1.2[a] ± 0.4	0.4 ± 0.2	0.5 ± 0.3
Prorubricytes	1.8 ± 0.6	2.3 ± 1.07	5.5 ± 0.8	3.7 ± 0.6	3.0 ± 1.1	5.5[a] ± 1.3
Rubricytes	33.6 ± 2.7	16.6[a] ± 5.0	11.2[a] ± 1.9	13.5[a] ± 1.9	16.1[a] ± 1.7	19.4 ± 2.6
Metarubricytes	14.3 ± 2.7	19.9 ± 5.1	23.8 ± 4.1	45.5[a] ± 2.8	32.9[a] ± 5.3	29.6[a] ± 4.1
Megakaryoblasts/ Megakaryocytes (cells/lpf*)	21.2 ± 2.4	19.4 ± 5.0	17.4 ± 3.1	66.6[a] ± 7.8	53.0[a] ± 6.2	65.4[a] ± 7.8
Myeloid/Erythroid Ratio (M:E)	1.0 ± 0.1	1.5[a] ± 0.2	1.4[a] ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.1

The bone marrow response in renal disease is not limited to the production of platelets. The myeloid series in the bone marrow, that is the precursors to the circulating leucocytes, also increased dramatically in the disease, while cells of the erythroid series decreased (Rasedee and Ragavan, 1993) (Table 3). Stimulation of erythrocyte production occurs through stimulation by EPO produced by the normal kidneys (Figure 2). In renal disease the production of EPO would be affected resulting in inhibition of erythrocyte production by the bone marrow and eventually anaemia (decrease in circulating erythrocytes).

It is a normal practice, to provide anaemic patients with iron supplement. If the anaemia is not due to kidney disease, iron supplement if used judiciously will eventually alleviate anaemia. However, in anaemia of renal disease, the cause is not intrinsic to the bone marrow. The marrow is usually normal, except stimulus to erythrocyte production is deficient or absent. In a study using the rat with induced renal disease as a model, iron supplement had an adverse effect on the bone marrow. There was increase in leucocyte production by the bone marrow but the erythrocyte production was relatively inhibited compared to those not treated with iron (Nooraini and Rasedee, 2001). What complicates the issue is that, anaemia may manifest without kidney function tests showing any abnormality, causing a misdiagnosis. Subsequently instituting iron supplement therapy for the anaemia can produce a deleterious rather than therapeutic effect.

Rats with renal damage were also treated with furosemide. Furosemide is a diuretic agent, used to encourage water loss through the kidneys, often used in patients with hypertension. In nephrotoxicity, furosemide administration seems to decrease all blood cells. This was clearly shown by the decrease in bone marrow precursors to both the leucocytes and erythrocytes and simultaneous decreasing the bone marrow myeloid:erythroid (Haiyatul Hanim and Rasedee, 2001).

LABORATORY DIAGNOSIS OF KIDNEY DISEASE

Serum and Urine Biochemistry

Two traditional serum parameters used in the assessment of kidney function and detection of abnormalities are blood urea nitrogen and serum creatinine.

Blood urea nitrogen (BUN)

Urea is a by-product of protein metabolism. Urea is formed in the liver and carried by the blood to the kidneys for excretion. Because urea is cleared from the bloodstream by the kidneys, a test measuring how much urea nitrogen remains in the blood is used as a test of renal function. BUN analysis measures the amount of nitrogen in the urea. BUN is filtered through the glomerulus and a small amount is reabsorbed by the tubules into circulation. Thus, a high BUN concentration would suggest kidney dysfunction as a result of damage or disease. However, there are many factors besides renal disease that can cause BUN alterations, including high protein diet, protein breakdown, hydration status, and liver failure.

Increase in BUN is termed azotaemia. Azotaemia may be of prerenal, renal, or postrenal causes. Prerenal azotaemia is usually reflected by mild increase in BUN. This can be the result of increase in protein metabolism in intestinal haemorrhage, tissue necrosis, infections, fever, and corticosteroid administration. Circulatory disorders as in

cardiovascular diseases, shock, and dehydration may also result in prerenal azotaemia. Renal causes produce the most severe azotaemia. However the manifestation of renal azotaemia is delayed, usually at the late or end-stage of kidney disease. In renal azotaemia, clinical signs of uraemia correlates directly with BUN and serum creatinine concentrations. In postrenal azotaemia, the cause is usually partial or complete obstruction of the urinary tract. This can be due to presence of kidney stones lodged either in the pelvis, ureter, and/ or urethra. In case of postrenal azotaemia, the BUN returns to normal several days after relief of obstruction.

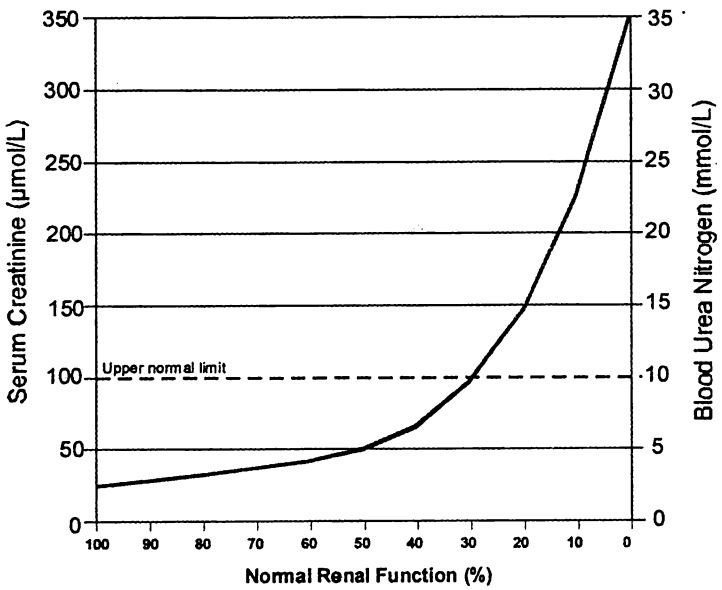

Figure 6. Serum creatinine and blood urea nitrogen changes in kidney disease.

Serum creatinine (S_{Cr})

Creatine is synthesised in the body from glycine, arginine and methionine (Murray *et al.*, 2000). The muscle holds more than 90% of total body creatine. Creatinine is spontaneously formed from creatine and phosphocreatine in the muscle. The amount of creatinine in the plasma and also in urine is proportional to the amount of creatine and phosphocreatine present in the body and hence the muscle mass (Hoberman, *et al.*, 1948).

Unlike BUN, creatinine is neither reabsorbed nor excreted by the renal tubules. Diet does not significantly affect serum creatinine. Thus this allows the serum concentration to remain largely constant. Increase in serum creatinine concentration may suggest renal problems. In fact, serum creatinine has always been used and is a better marker of glomerular filtration rate (GFR) than BUN. However, large reductions in GFR initially produce only small increases in S_{Cr} concentration. Therefore, even small increases in S_{Cr} should be carefully evaluated (Anderson and Barry, 2004).

Both the BUN and S_{CRs} are relative insensitive as indicator of renal damage with measurable increase above normal range occurring only after the kidneys have lost at least 75% of its function (Figure 6)

BUN:S_{Cr}

Typically the BUN:S_{Cr} is approximately 15:1. In complete absence of glomerular filtration, BUN and S_{Cr} increase by 10 to 15 mg/dL and 1.0 to 1.5 dL per day, respectively. Divergence from this proportion can occur in the conditions or diseases listed in Table 4 (Anderson and Barry, 2004).

Table 4. The BUN:S_{Cr} in several diseases and conditions

BUN:S_{Cr}	
Greater than 15	**Less than 15**
Increased urea formation	Decreased urea formation
High intake of protein	Starvation
Catabolic states	Advanced liver disease
Fever	Deficiency of urea cycle enzymes
Tissue necrosis	Post-dialysis
Corticosteroids	Rhabdomyolysis
Teteracyclines	Decreased creatinine secretion
Sepsis	Cimetidine
Decrease elimination of urea	Trimethoprim
Decreased cardiac output	Pyrimethamine
Obstructive uropathy	Interference of assay by other compounds
	Ketones
	Cefoxitin
	Ascorbic acid
	methyldopa
	Flucytosine
	Barbiturates

Serum early indicator of renal damage

In our earlier studies, several attempts were made to identify the early indicator of renal damage in serum. If there is an early indicator, a serum parameter cleared mainly by the kidney would be the logical candidate since it would reflect renal function. Although, our studies indicated that a protein compound may increase in experimental renal damage, the appearance of this protein was inconsistent (Rasedee, unpublished data) and would be unreliable as a marker for renal disease. Because of lack of consistency, the search of an early marker of kidney disease in blood was temporarily shelved.

URINE IN THE DIAGNOSIS OF RENAL DISEASE

Urinalysis

Among the easiest test which can be used in the diagnosis of renal disease is urinalysis or examination of urine. Urinalysis provides a variety of clinical information regarding not only the state of the kidneys but systemic diseases as well occurring elsewhere in the body. Although this test is simple, if carefully conducted it can provide a lot of information about the disease, its causes, and prognosis. Besides being a rapid test it can be conducted without fear, pain, danger, or distress. There are two major components of urinalysis.

Macroscopic examination: This is an examination of urine physical characteristics and properties and the chemical tests. In most modern laboratories, the chemical test uses commercially available reagent strips. Among the routine parameters measured are colour, turbidity/transparency, specific gravity, pH, proteins, glucose, ketone, biliubin, urobilinogen and blood.

Microscopic examination: This a microscopic examination of urinary sediments. This includes the examination for the presence of erythrocytes, leucocytes, epithelial cells, microorganisms, parasites, casts, crystals and fat.

Some expected findings in urinalysis in certain diseases and conditions are as in Table 5.

Table 5. Expected findings in urinalysis in several diseases and conditions.

Disease/Condition	Urinalysis
Kidney disease	Abnormal specific gravity,proteinuria, casts, leucocytes, and erythrocytes
Bladder infections/injury	Proteinuria, squamous and transitional epithelial cells, leucocytes, erythrocytes, and bacteria
Liver disease	Bilirubinuria, urobilinogenuria, bilirubin crystals
Haemolytic anaemia	Haemoglobinuria and bilirubinuria
Diabetes mellitus	Glucosuria, ketonuria, and proteinuria
Diabetes insipidus	Low specific gravity
Acidosis	Low urine pH
Alkalosis	High urine pH
Neoplasia of urinary tract	Neoplastic cells and haematuria.

Examination of the Urine:

Urinalysis done on the patient with acute renal failure can provide both diagnostic as well as prognostic information of the patient.

Proteinuria

Increased presence of protein in urine or proteinuria is an abnormal finding. A dipstick test result of 3+ or greater (500 mg/L or greater) for protein suggests intrinsic renal disease with glomerular damage. Prerenal azotemia, obstruction, and acute tubular necrosis tend to be associated with lesser proteinuria (trace to 2+; up to 100 mg/L) than glomerular diseases. If there is proteinuria it should be confirmed using a 24-hour urine collection sample.

Proteinuria usually reflects an increase in glomerular permeability of plasma macromolecules such as albumin. The normal protein content of an adult 24-hour urine sample is approximately 150 mg. The dipstick can detect urine protein concentration at as low as 30mg/L, and this is concentration-dependent. Thus in oliguria (low urine volume: < 400 ml/24 hours in humans) from any cause, the urine may erroneously show proteinuria even when the protein content of urine is normal. In a 24-hour sample, a protein content of greater than 300 mg most likely suggest a glomerular rather than a vascular or interstitial abnormality.

The dipstick method primarily detects albumin. Thus other proteins such as urine immunoglobulins and their light chains may not be detectable. In fact the presence of immunoglobulins may give a negative or weakly positive reaction with the dipstick method. In this case, the sulfosalicylic acid precipitation method is more useful, since it can detect all kinds of protein.

By definition, a 24-hour urine protein content of < 30 mg is normal; 30 to 299 mg indicates microalbuminuria; > 300 mg is clinical proteinuria.

Urine protein: creatinine

Although 24-hour urine protein concentration has been the "gold standard" for quantitative evaluation of proteinuria, this method is cumbersome, inconvenient and difficult to perform. On the other hand, the random urine (spot urine) sample may not reflect the true proteinuria because of the inconsistencies in diurnal urine excretion. Urine creatinine excretion is fairly constant. Both the urine protein and creatinine are volume dependent. Thus the urine protein:creatinine (P/C ratio) has been suggested as an alternative parameter for assessment of renal function. This ratio can be calculated from:

$$\text{Urine P/C ratio} = \frac{\text{Urine Protein (mg/dl) x Urine Volume (ml)}}{\text{Urine Creatinine (mg/dl) x Urine Volume (ml)}}$$

$$= \frac{\text{Urine Protein}}{\text{Urine Creatinine}}$$

The above formula shows that the urine P/C eliminates the effect of urine volume, thus spot urine samples can be used in the analysis. The P/C ratio is usually <0.5, and any value >1.0 is obviously abnormal. The P/C on spot urine samples, preferably the first morning sample, is a good alternative to urine protein concentration estimation (Gan Xin *et al.*, 2004).

Blood, haemoglobin and myoglobin

A dipstick positive for blood indicates the presence of erythrocytes (> 5 cell/hpf) (haematuria). If no erythrocytes are present, then a positive test can be due to the presence of myoglobin or hemoglobin in the urine. This would require confirmation by microscopic examination since a dipstick test often gives false-positive results for erythrocytes and leucocytes. Haematuria, usually suggest urinary tract injury, while myoglobin (muscle protein) in urine (myoglobinuria) would suggest muscle damage, and haemoglobin in urine (haemoglobinuria) suggests either intravascular haemolysis or breakdown of erythrocytes in urine.

Specific Gravity

Urine specific gravity can easily be determined by refractometry. In urinalysis, urine specific gravity is probably the most important test of kidney function. Among the functions of the nephrons are to concentrate and dilute urine. During the process of concentration and dilution of urine, the specific changes accordingly. In high water intake and cold weather for example, there is excessive body water. In this case the urine volume increases and specific gravity of urine becomes low. In a hot environment or low water intake the kidneys function to conserve water and subsequently the urine volume is low and specific gravity high. This concentrating and diluting ability of the kidney is dependent on intact nephrons.

The specific gravity of the glomerular filtrate is 1.008 to 1.010, which is equivalent to the specific gravity of protein-free plasma. If the urine specific gravity is higher or lower than this range, it would suggest that the kidneys have ability to alter the concentration of the glomerular filtrate. The normal range of urine specific gravity is 1.005 to 1.030. Generally a urine specific gravity of greater than 1.020 suggests good kidney function. A urine specific gravity of approximately 1.010 which is isosthenuria, suggests severe kidney disease or end-stage kidney disease. However, this needs to be confirmed by other kidney function tests.

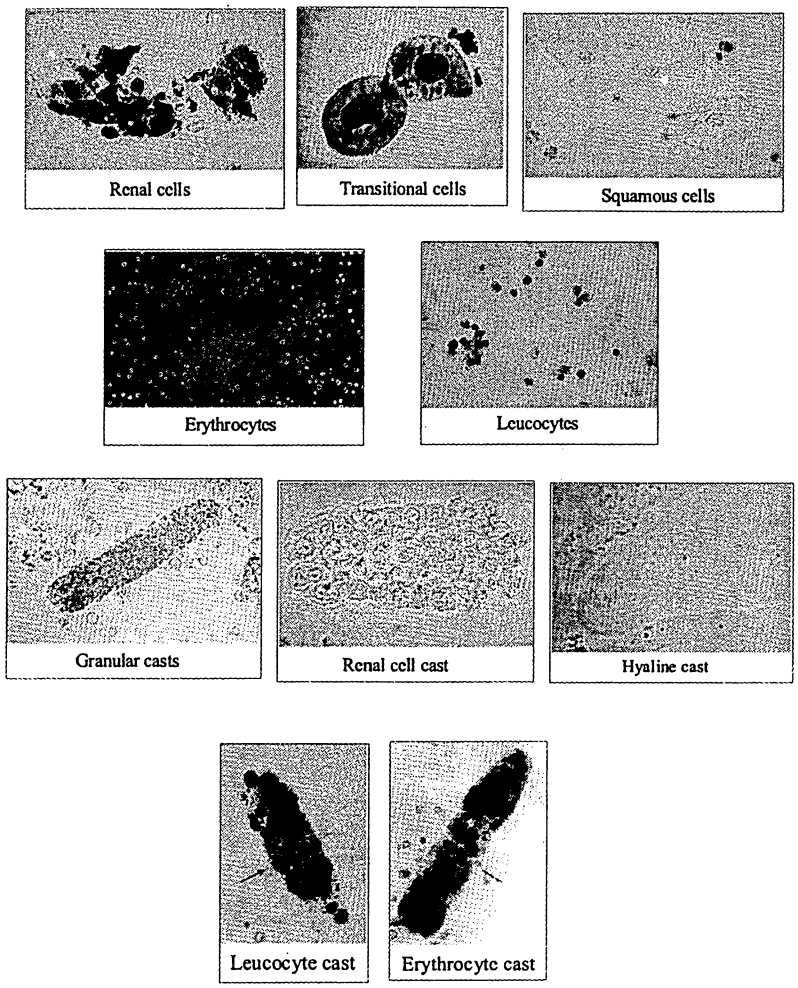

Figure 7. Urine Sediments

Urine sediments

In most cases the most significant information obtained from urinalysis comes from the examination of the sediments in centrifuged urine samples. The slide preparation of the sediment, stained or unstainded, can be examined under high power for erythrocytes, leucocytes, renal tubular epithelial cells, casts, fat bodies, bacteria, and crystals (Figure 7). The presence of some of these insoluble components of urine may suggest urinary tract disease.

Casts are formed from urinary Tamm-Horsfall protein produced by the tubular epithelial cells. This protein tends to gel in conditions of high concentration and when mixed with red cells, tubular cells, or cellular debris. Thus, the composition of casts reflect the contents

of the tubule and rate of urine flow. Hyaline casts are those that are devoid of contents, and are seen with dehydration, after exercise, or in association with glomerular proteinuria. Erythrocyte and erythrocytes casts indicate glomerular haematuria, as seen with glomerulonephritis. Leucocyte and leucocyte casts are seen in acute interstitial nephritis and renal parencymal inflammation. The finding of eosinophils in a Wright-stained urine sediment has been suggested as an indication of drug-induced acute interstitial nephritis. Granular casts are composed of cellular remnants and debris. Fatty casts are usually associated with heavy proteinuria and the nephrotic syndrome.

Prerenal, renal and post-renal azotaemia

Generally changes in urine parameters in kidney diseases of different causes are reflected by Table 6.

Table 6. Changes in urine parameters in prerenal, renal and post-renal azotaemia

Causes	Urinary Sediment	Proteinuria	Specific Gravity
Prerenal			
	Generally benign sediment: few hyaline or granular casts	trace	increased
Renal			
Acute glomerulonephritis	RBC with casts, WBC with casts, granular casts, fatty cast, renal cells	4+	increased
Acute tubular nephritis	cast, course granular cast	2+	1.010 - 1.012
Acute interstitial nephritis	RBC,WBC, granular cast	2+	1.010 - 1.012
Post-renal			
	Usually benign urine sediment	trace	early: high Later 1.010 - 1.012

Kidney Stones

Kidney stones is a stone-like mass that can form in one or both kidneys from the crystallisation of excreted substances in the urine (Figure 8). The stone may remain in the kidney or break loose and move down the urinary tract where the larger stones can get lodged in the ureter, the bladder or the urethra. They can cause bleeding and may block the flow of urine. The kidney stones can cause pressure, pain and infection. Depending on the size and position of the stones, if left untreated could cause permanent damage to the kidneys. The kidney stones are also known as renal calculi, urinary calculi, nephroliths, uroliths, and ureteroliths. The size of the stone may vary from as small as grains of sand to as big as a golf ball.

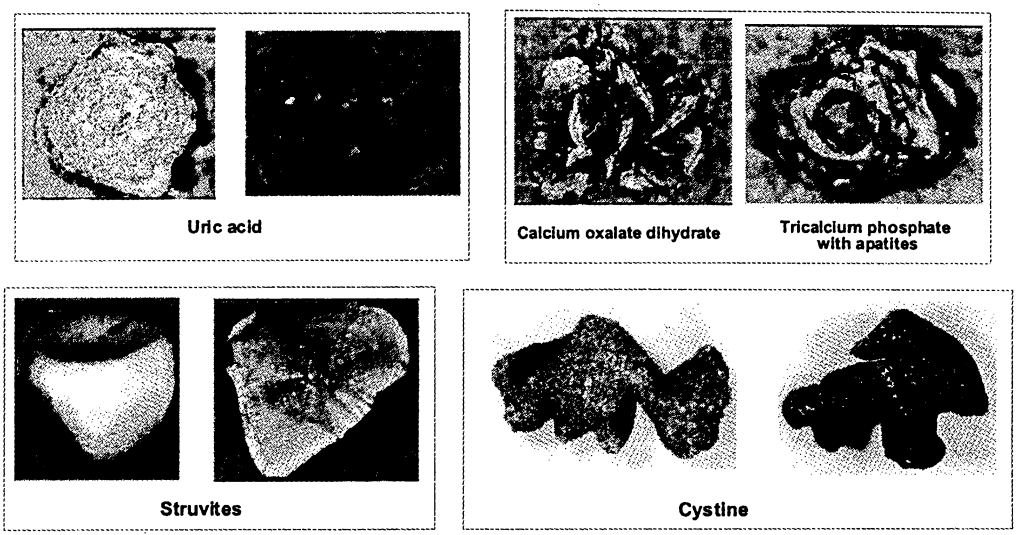

Figure 8. Kidney stones (For each type of stone, the size, colour, and consistency are variable)

It is unclear as to the cause of kidney stone development. However, it as been suggested kidney stone deposition is the result of breakdown in the balance of liquids, salts and minerals in urine. However there are a number of contributing factors to stone formation including:

- *Age* (more common during middle age)
- *Sex* (three times more common in males than females)
- *Activity level* (more common in immobilised individuals)
- *Excess calcium, phosphate, oxalate and uric acid in the urine*
- *Insufficient amounts of stone inhibitors in the urine*
- *Certain medications* (e.g diuretics, antacids and thyroid medications)
- *Low fluid intake*: Resulting in concentrated urine and crystal formation
- *Recurrent urinary tract infections*
- *A family history of stone formation*

There are four major types of kidney stones.

Calcium stones
These are the most common types of stones. They are made of calcium oxalate and calcium phosphate. These are chemicals found naturally in certain foods.

Uric acid stones
Excessive amount of uric acid in urine can be caused by too much meat in the diet. Patients with gout and undergoing chemotherapy are especially prone to uric acid stones.

Struvite stones
These stones contain magnesium and ammonia. They may form after urinary tract infections.

Cystine stones
Cystine is one of the building blocks of muscles, nerves and other parts of the body. Cystinuria runs in families and is a rare disorder.

Diagnosis of kidney stones

In addition to a complete medical history and physical examination, diagnostic procedures for kidney stone include the following:

Urinalysis: Laboratory examination of a 24-hour urine sample may show the presence of blood cells (erythrocytes and leucocytes), excessive proteins, and crystals.

Blood tests: The purpose of these tests is to identify the presence of excessive amounts of chemicals which may promote stone formation.

Intravenous urogram (IVU): This a series of X-rays which involves the intravenous injection of a contrast dye. This technique can detect kidney stones, obstructions, tumours, and assess renal blood flow.

Ultrasound scan: This is a noninvasive test in which a tranducer producing sound waves is passed over the kidney. The picture (ultrasonograph) can be used to determine the size of the kidney and detect the presence or absence of kidney stones, masses, cysts, and other abnormal structures.

Urine Enzymes

The dire need for a sensitive and specific indicator for renal disease has turned research towards the search of novel urine markers. Over the last decade the search has turned to urine enzymes as indicators of renal damage (Walter and Fred, 1999). Among the requirements for enzymes to be good indicators of renal disease are; (i) The molecular weight of the enzyme must be large enough that it cannot be normally filtered from blood through the glomerulus. Thus any change in urine enzymes would be a reflection of abnormalities in the nephrons only: (ii) The enzyme must be found in large enough concentration in urine of damaged kidneys to be easily detectable. More than 40 enzymes have been evaluated. However, It has been shown that (γ-glutamyltransferase (GGT), β-N-acetylglucosaminidase (NAG), β-glucuronidase (GRS), aspartate aminotransferase (AST) and alkaline phosphatase (AP) meet the requirements. GGT and AP also found in blood, are sensitive indicators of liver disease. In the kidneys these enzymes are localised in the proximal tubules (Guder and Ross, 1984). They are either intracellularly located or

membrane-bound. Their presence in urine can be detected in increased kidney cell activity, degeneration and damage to the kidney cells (Deborah, *et al.* 1985; Walter and Fred, 1999). These urine enzymes, particularly GGT, have been suggested to be reliable biomarkers of nephrotoxicity and very useful as early indicators of renal tubular damage. In fact, a four-fold increase in in excretion of NAG in urine was also observed in gentamicin-treated rats (Cojocel *et al.*, 1983). In several studies, GGT as been suggested to be among the best urine enzyme as an early marker of renal tubular damage (Deborah *et al.*,1985; Salvatore *et al.*, 2002).

IN SEARCH OF AN EARLY INDICATOR OF RENAL DAMAGE

The traditional kidney function parameters are relatively insensitive markers for kidney disease because they increase significantly only after considerable kidney damage has occurred (Price, 1982). Urine enzyme is an indication of renal tubular abnormality, leakage or necrosis. Although there is a dirth of data on the urine activity of enzymes in early renal disease, they are potential early indicators (Deborah et al. 1985).

Urine GGT to Creatinine ratio

The potential diagnostic utility of the urine GGT to creatinine ratio (GGT:creatinine) was evaluated in an experimental canine model of gentamicin-induced nephroxicity. A therapeutic dosage of gentamicin resulted in a 2-fold increase in the mean urine GGT:creatinine that was not associated with clinically significant nephrotoxicity. The increase in mean urine GGT:creatinine ratios approximately three times baseline values preceded clinically significant abnormalities in S_{Cr}, urine specific gravity, and urine protein to creatinine ratio. The urine GGT:creatinine appears superior to S_{Cr} as an indicator of puromycin aminoglycoside-induced nephrotoxicity. (Rivers *et al.*, 1996).

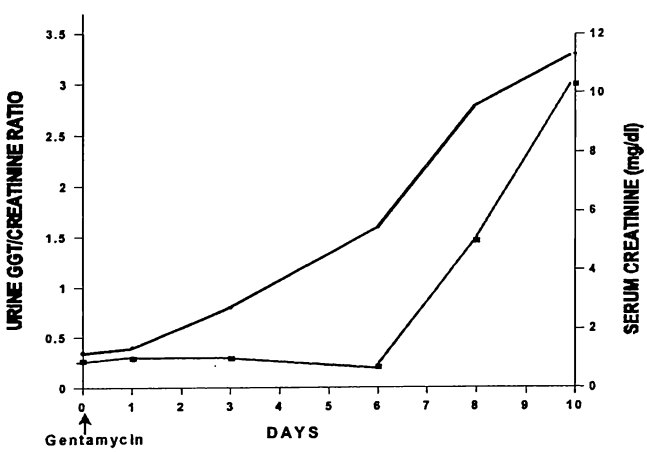

Figure 9. Urine GGT:creatinine and serum creatinine in gentamicin nephrotoxicity.

Although a 24-hour urine sample would be the most accurate presentation of kidney function, it is still inconvenient to collect. A correlation between 24-hour and spot urine samples were determined using dogs with gentamicin-induced nephrotoxicosis (Grauer *et al.*, 1995). The same day spot urine NAG and GGT:creatinine seems to be significantly correlated with the 24-hour urine NAG and GGT. In gentamicin-induced nephrotoxicity, the urine GGT:creatinine is more sensitive than urine creatinine concentration, that is it responded earlier to induced kidney damage than the urine creatinine concentration (Rasedee, 1998) (Figures 9).

The experiment was repeated. This time tissue kidney tissue samples were also obtained from damage rat kidneys induced by gentamicin. The results showed that urine GGT and AST activities peaked at approximately 2 days post-gentamicin administration which reflects the development of kidney tissue damage (Sairah *et al.*2004) (Figures 11 and 12). The most surprising finding was the urine AP activity, which seems to increase earlier than even the urine GGT. The activity of AP in normal urine is very low compared to GGT. Increase in AP was recorded as 20-fold compared to increase in GGT which was only 3- to 4-fold. However, peak urine GGT was 3 times higher than peak urine AP concentration.

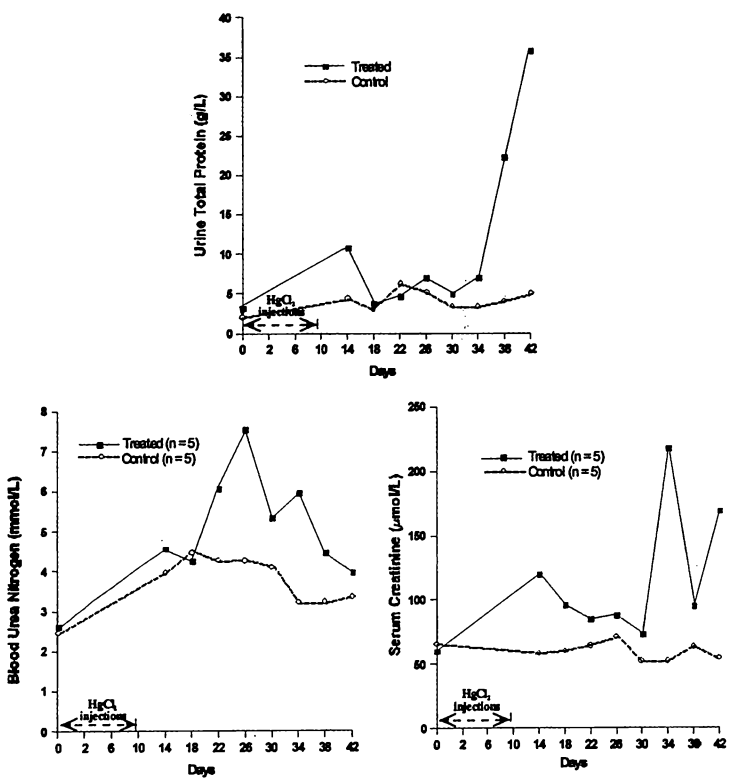

Figure 10. Urine total protein, blood urea nitrogen, and serum creatinine changes in mercuric chloride induced renal damage in rats.

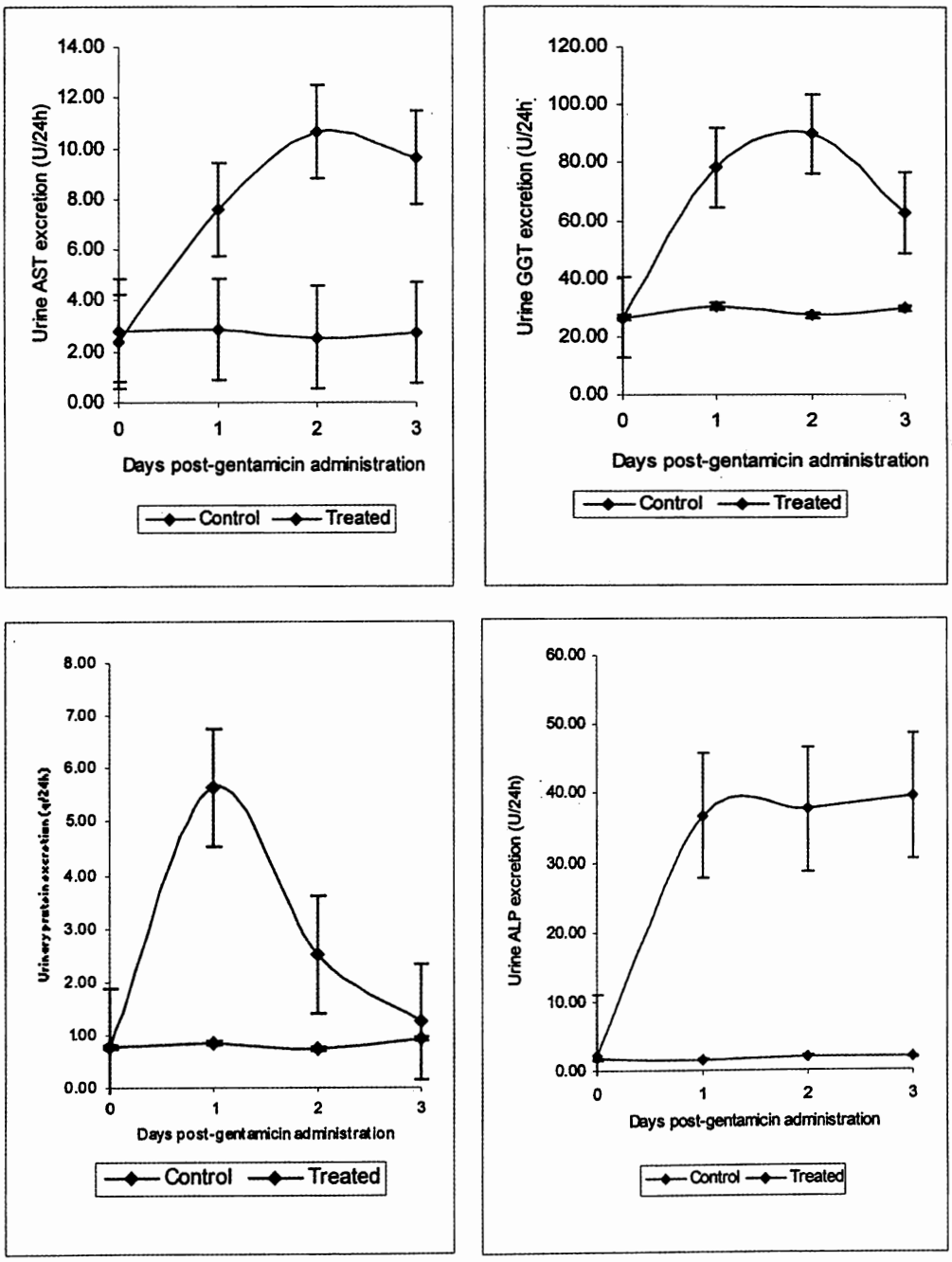

Figure 11. Urine enzymes and protein in gentamicin induced renal damage in rats

Proteinuria in kidney damage develops by either one or both of the following mechanisms: (a) Loss of size and charge selectivity of the glomerular membrane, resulting in increase passage of macromolecules through the membrane and (b) Distubance in reabsorption of proteins by the tubules producing proteinuria from low molecular weight proteins. Thus urine protein concentration is very useful in the diagnosis of renal disease. As indicated earlier, if proteinuria is primarily due to high molecular weight proteins, especially albumin, glomerular disease is indicated. Appearance of low molecular weight proteins in urine would suggest tubular disorders. In our series of studies, using rats induced to develop renal damage, proteinuria was also the consistent feature. In mercuric chloride-induced renal damage, in which the period of study was relatively long, development of proteinuria was shown to be biphasic (Rasedee *et al.*, 1997) (Figure 10). However, in shorter gentamicin nephrotoxicity study (Sairah *et al.* 2004), the biphasic response was not evident but the development of proteinuria was relatively faster (Figure 11). This may due to the fact that the gentamicin causes renal damage within a shorter period compared to heavy metal compounds. The effects of most nephrotoxic drugs on the kidneys are evident after just a few days.

In an attempt to identify the proteins in proteinuric urine, the SDS-PAGE technique was employed (Sairah *et al.* 2005). All urine samples from nephrotoxic rats showed an extra protein band of approximately 11 kDa molecular weight which seems to increase in intensity with time post-gentamicin (Figure 13). This band, yet to be characterised, may be a good candidate of early renal damage and progressive tubular insufficiency.

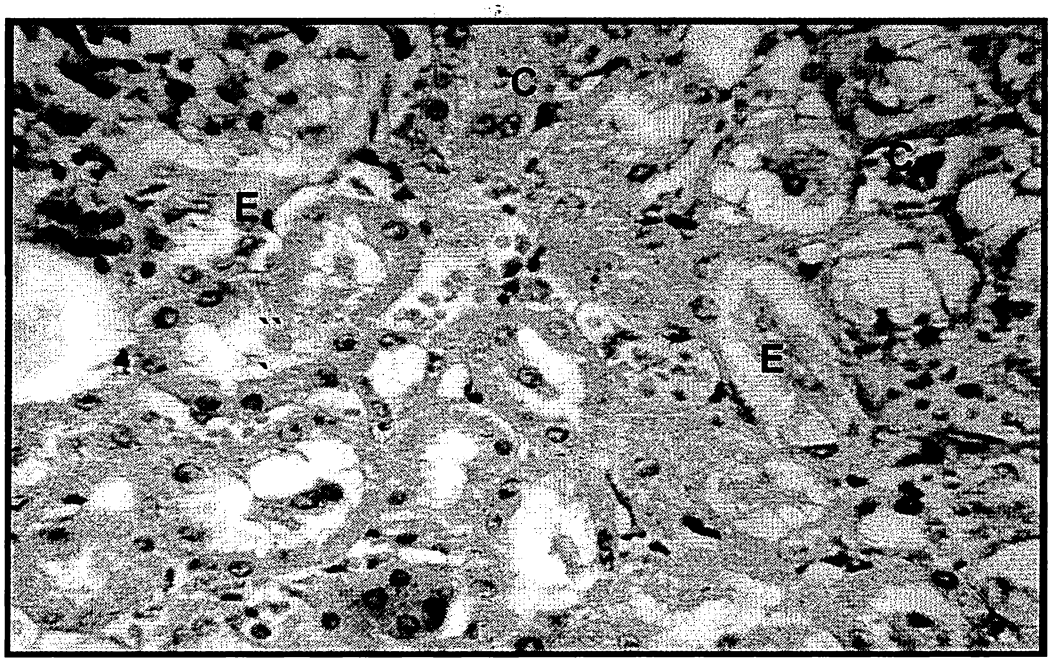

Figure 12. Kidney section from gentamicin-treated rat. Necrotic cells (N), eosinophilic debris (E) and congestion (C) were noted (H&E, x200).

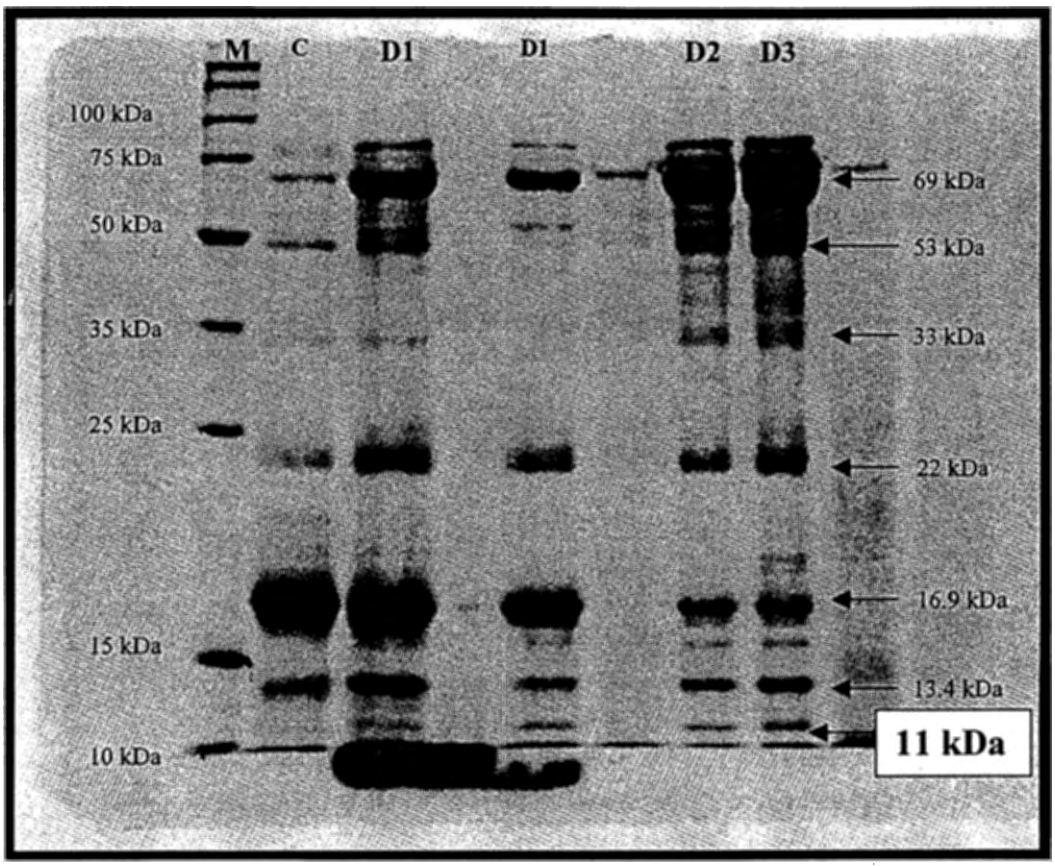

Figure 13. Urine protein pattern in gentamicin-induced renal damage in rats (M: standard marker, C:Control, D1, D2, D3: Days 1, 2, and 3 post-gentamicin administration respectively).

CONCLUSION

Malaysia is fast approaching the status of a developed nation. In such a modern society, life is fast and hectic. Often because of the intense pursuit of wealth and a good life, short-cuts are taken to obtain good health. Consumption of drugs, local concoctions, vitamins, food supplements from disreputable suppliers may be practiced without fear of long-term effects. Many of the components of the so-called food supplements may in fact be hazardous to health.

The kidneys and the liver are two organs that function to clean the body of toxic compounds which can cause tissue damage. In the performance of their functions, these organs themselves become damaged in the process. The kidney for example is very susceptible to damage from accumulation of toxins although they have tremendous ability to compensate for lost of function. Because of this compensatory ability too, minimal or even moderate damage to the kidneys due to disease or toxic compounds may not be

detectable. As a result, the disease may progress without the patient or physician being aware of failing kidney function. By the time clinical kidney disease becomes evident, it would have been too late, and the solution lies only in dialysis or kidney replacement. Management of kidney failure patients is time consuming and/or expensive. Currently in the United States at least 70,000 kidney failure patients are awaiting suitable kidneys.

Currents laboratory diagnostic techniques cannot detect early renal damage. With the advent of sophisticated technologies, it is not inconceivable to assume that any day now a method of detection early renal disease will surface. However, discoveries will not come without sweat and concerted efforts. The search for the elusive early indicator of renal disease must go on. Although the detection of early renal disease may prove not to be fool-proof after all, at least it would serve as a flag of caution for the patients and physicians alike to take precautionary measures in ensuring that, in case there is kidney disease, its progress could be curtailed at the earliest stage.

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ALLAH is GREAT

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