GP Handbook v8.2

Laboratory Tests

ENDOCRINE TESTS

Assay Methods

The quantity of hormones is low when compared with other analytes like proteins and electrolytes, and the bulk of circulating hormone is protein-bound. Immunoassay is the main method involved, although use of mass spectrometry in hormone analysis has gained popularity and the outcome is promising. Immunoassay is a headache in endocrine investigation because hormone may be present in several isoforms and metabolites, which could have altered cross-reactivities in various analysers. The immunoreactivity is not necessarily correlated with the in-vivo bioactivity of the hormones. Furthermore, a legion of interferents have the potential to invalidate the immunoassay results, examples include commonly encountered proteins like fibrinogen, and unexpected substances like human against mouse antibody, which is developed in subjects with history of contacts with animals or animal products. It is therefore common to observe discrepant results if the same specimen is analyzed by different immunoassays. To improve method comparability, standardization of hormone assays is in active progress.

Adrenocorticotrophic Hormone (ACTH)

The secretion of ACTH and cortisol are also episodic and the circadian rhythmicity is well-known. Dynamic function tests like short synacthen stimulation test (SST) and insulin tolerance test (ITT) are considered more reliable for investigation of adrenal insufficiency than a spot level. However, the dynamic function tests are time consuming, troublesome and potentially dangerous. It has been shown that morning cortisol < 100 nmol/L and > 550 nmol/L are highly correlated with the SST findings and has been recommended as a screening test. Morning cortisol < 100 nmol/L is highly suggestive of cortisol insufficiency whereas level > 550 nmol/L reflects adequate adrenal function. Levels between 100 and 550 nmol/L should be confirmed by SST or ITT.

Growth Hormone (GH)

The secretion of GH, like other pituitary hormone, is pulsatile. In acromegaly, the episodic secretion is preserved despite an increase in number, duration and amplitude of pulses. Very high random GH level may be diagnostic of acromegaly, but about 10% of patients with acromegaly have random GH level falling within the reference interval. An oral glucose tolerance test is viewed as gold standard and a non-suppressible GH is diagnostic of acromegaly. Random insulin-like growth factor-1 (IGF-1) is considered an accurate reflection of integrated GH production, and has been recommended as the initial test for suspected acromegaly.

Human Chorionic Gonadotrophin (hCG)

Urine pregnancy test usually has a detection limit of 25 IU/L and comparable serum level could achieve a positive result if concentrated urine (ie morning urine) is used. Urine pregnancy test is considered one of the most reliable laboratory test but it is prudent to test the specimen with another lot or brand of test kit to confirm the true negativity. Phantom hCG immunoreactivity is a notorious interferent of serum hCG assay and results in a false positive result. It can be caused by trypsin-like molecules, cholera toxin, transforming growth factor- and certain bacteria. The unawareness of this condition may lead to unnecessary operation and chemotherapy if malignancy is suspected. Phantom hCG can be confirmed by distorted stoichiometric relationship upon dilution, abnormal recovery with PEG precipitation and discordant result on another analyser.

Parathyroid Hormone (PTH)

PTH level is negatively controlled by ionized calcium level. The relationship of serum calcium and PTH is steeply sigmoidal and a depressed PTH is thus expected even in case of mild hypercalcaemia. In a patient with primary hyperparathyroidism and a sestamibi scan can be used to look for any parathyroid adenoma.

Prolactin (PRL)

In most individuals with increased prolactin, 60-90% of prolactin is monomeric form, 10-30% is "big" PRL, and 0-10% is "big-big" PRL. It has been shown that latter 2 types of prolactin may aggregate with immunoglobulin G and the renal clearance is thus prolonged. The "big-big" PRL is also known as macroprolactin and is biologically inactive in vivo. The macroprolactin has been found to be the dominant form of immunoreactive PRL in some subjects without any evident cause and features of hyperprolactinaemia. Clinically the presence of macroprolactin is difficult to confirm since response to dopamine agonist may be noted as well, and radiological abnormality is probably present as pituitary incidentaloma is not uncommon. Currently, macroprolactinaemia can be detected using screening method of polyethylene glycol (PEG) pretreatment and it has become a routine practice of some laboratories to re-analyze all hyperprolactinaemic samples with PEG pretreatment. A more cost-effective approach is to request PEG pretreatment for the first sample if no specific acromegalic features (eg galactorrhoea) has been noted, and obvious causes like drug-induced hyperprolactinaemia has been excluded.

HEAVY METALS

Heavy metal poisoning can be associated with accidental over-exposure from industrial, dietary, drug-related, or even cosmetics-related sources. The effect of heavy metal toxicity can vary from gastrointestinal upset to severe neurological damage. The mechanism of this wide range of toxicity is due to the covalent binding of heavy metals to sulphydryl groups at the active site of important enzymes in various organ systems causing loss of function.

The screening of heavy metals using a correct sample type is also of prime importance to the subsequent clinical diagnosis and management. Hair is no doubt a convenient sample that may reflect recent exposure. However, contamination of hair samples with air particles, dust, wave and colouring treatments is commonly encountered. Moreover, the measurement errors contributed by weighing the hair samples will be enormous if only a few roots are collected for analysis. In general, laboratory diagnosis of heavy metal poisoning should rely on properly collected blood and urine samples.

Workers at risk of contacting toxic metals are recommended to have regular and appropriate medical check-up including chest radiograph, lung function, audiometry, blood, and urine tests for the assessment of industrial over-exposure and early detection of complications.

Clinical & Laboratory Approach

Identification of possible source(s) of exposure
Occupational, drug and dietary histories as well as personal habits are important information to note. If possible, obtain the remains of the suspicious drug, cosmetics, and food for future analysis.

Laboratory diagnosis
Owing to the difficulty in clinical diagnosis, laboratory screening for heavy metals is commonly requested for the diagnostic work-up of patients suspected of poisoning. If necessary, analysis of the offending drug, cosmetics or food can also be performed. However, contamination is a major challenge to laboratories performing trace element analysis. Quality results require good pre-analytical preparation. Obtaining instructions for sample collection and certified specimen bottles from a reputable laboratory that has participated in regular quality assurance programme for trace element analysis is essential.

Assessment of complications
Renal toxicity with tubular cell damage resulting in significant proteinuria is common. Urine retinol binding protein or b-2-microglobulin normalised to creatinine can be requested as an objective assessment of tubular proteinuria. Central nervous system is another common site affected by heavy metals such as lead and mercury after they have crossed the blood-brain-barrier. Nerve conduction study can be requested as a non-invasive but objective measurement of the neurotoxic effects of heavy metal deposition.

Chelation therapy
This depends very much on the severity, toxic signs and symptoms. Advantages and disadvantages of the chelation therapy have to be considered thoroughly and discussed with the patient since chelation therapy may have deleterious side effects.

Heavy Metals in Cosmetics

Consumers should be careful and selective in purchasing beauty creams and other cosmetics. They should prefer products that are manufactured in developed countries, where regulations for quality control and product labelling are more stringent before export. Doctors should be aware that patients might be exposed to widely available and easily purchased cosmetics and other products that are adulterated or contaminated with heavy metals such as mercury, lead, cadmium, and arsenic, and be alert to the possibility of chronic heavy metal poisoning with vague and non-specific signs and symptoms. In suspected cases, they should refer the patients for heavy metal analysis and other appropriate laboratory investigations including a complete blood count and renal function test.

Aluminium

Aluminium is widely distributed in our environment. Food and beverages contain a small amount of aluminium so that our daily dietary intake is estimated at about 5mg/kg of body weight. In some developed countries, the problem of acid rain may increase the solution of aluminium from soil, thereby contaminating the underground drinking water. Fortunately, gastrointestinal absorption of this metal is less than 1% in healthy individuals. Any absorbed aluminium will be excreted in the urine except in the lung tissue, where the macrophages retain aluminium-containing particles by phagocytosis. Upon absorption aluminium is transported to every part of our body by transferrin in the systemic circulation. After crossing the blood-brain barrier, it acts as a neurotoxin. Exposure of aluminium is best diagnosed by measuring its concentration in serum or blood since it is equally distributed in plasma and erythrocytes.

In a specialist trace element laboratory, proper blood collection precautions and acid-washed specimen bottles are issued to ensure the quality of the blood sample, as environmental contamination of aluminium using non-acid-washed specimen bottles is very common.

Arsenic

Arsenic is a very toxic chemical in its trioxide form with an oral lethal dose of about 3mg/kg of body weight when taken acutely. Exposure to arsenic compounds is usually industrial as arsenic is used in the manufacture of glass, pigment, wood preservative, and semiconductors. Sometimes it is used in homicidal attempts. Arsenic is also used therapeutically in both traditional Chinese medicine in the form of realgar (雄黃, arsenic sulphide) for the treatment of peptic ulcer, as an antidote for snake or scorpion bite via external application, and in western medicine for the treatment of acute promyelocytic leukaemia.

Acute exposure can give symptoms of headache, nausea, and severe gastrointestinal upset accompanied with intense abdominal pain, vomiting, and diarrhoea. If left untreated, dehydration followed by oliguria and circulatory collapse with encephalopathy and death will supervene. Chronic exposure causes gastrointestinal discomfort, thick erythematous areas of skin and pruritic pinpoint dermatitis, alopecia, and peripheral neuropathy. Patients with chronic exposure have an increased risk of developing carcinoma of the skin and lungs. After initial exposure, arsenic quickly enters and leaves the systemic circulation. Therefore, exposure is best diagnosed and monitored by its urinary excretion rate if a timed urine collection is available or its urine concentration normalised to the creatinine concentration.

In general, elevated urinary arsenic concentration is either occupational or dietary. Non-toxic organic compounds of arsenic (arsenobetaine and arsenocholine) are well absorbed in the gut and abundant in seafood, particularly fish and shellfish. After a seafood meal, urine output of arsenic may increase to 50 times above normal values for the first day before the organic arsenic is completely excreted in the second day without any toxic effects. The proper pre-analytical precaution is therefore abstinence from seafood for 5 days before urine collection. When this patient preparation is not practicable, such as for urgent investigation of a suspected case of acute poisoning, or forensic

examination of a post mortem sample, speciation of inorganic and organic arsenic is available from specialist centres.

Cadmium

The processes of smelting and refining of zinc and lead ores produce dust and vapour laden with cadmium. Electroplating, soldering, brazing and the disposal of industrial waste also generate cadmium-rich vapour and dust. Other sources of cadmium are from diet including shellfish, animal kidneys, and tobacco. The gastrointestinal absorption of cadmium is variable depending on the intake of other divalent nutritional metals as they share the same carrier for absorption. Once absorbed, cadmium is transported within the plasma to liver where it is combined with metallothionein which will then be re-distributed to the renal cortex slowly via glomerular filtration and tubular reabsorption. Whole blood cadmium concentration is therefore a better index of exposure as urine cadmium excretion is usually normal until renal damage has occurred.

Cobalt

Exposure of inorganic cobalt can occur during production of tungsten carbide materials, manufacture of alloys and pigments, and corrosion of cobalt-alloy joint prosthesis. Cobalt can cause cardiomyopathy resulting in fulminating heart failure and polycythaemia. Hard metal interstitial lung disease can also occur following occupational exposure of cobalt powder or vapour. Unlike cadmium, inorganic cobalt exposure requires urinary assessment as it is rapidly excreted in urine within 2-3 days after initial exposure.

Lead

The daily intake of lead is about 1mmol via drinking water and 1mmol from food. Once absorbed, lead rapidly accumulates in erythrocytes (95%) with a half-life of 35 days before it is passed into the urine or is transferred to soft tissues including hair, nail, alimentary secretion, and finally to bone tissue which contains 99% of the total body lead burden. Therefore, the laboratory investigation of choice is whole blood lead while urine lead excretion rate should be used for monitoring chelation therapy using 2,3-dimercaptosuccinic acid (DMSA).

Mercury

Three forms of mercury exist naturally: elemental, inorganic, and organic, with methyl mercury representing the prototype of the organic form of mercury compounds. Mercury poisoning in fungicide-users is an example of occupational risk. Over-exposure can also occur with industrial accidents resulting in pneumonitis due to its volatile nature. A number of Chinese medicinal compounds contain inorganic mercury salts. The inorganic form can exist in more than 30 kinds of mineral and medicinal compounds as well as mixtures of compounds.

In western medicine, all species of mercury are considered toxic. Organic mercury is readily absorbed through our diet from contaminated seafood, and is the most common source of contacting mercury in daily life. Exposure to inorganic and elemental mercury is usually by accident either in the industry, hospital, or through deliberate self-harm.

After absorption, elemental mercury is converted to the active inorganic mercury ions (Hg^{2+}) that bind avidly to the sulphydryl group of most proteins and enzymes and render them inactive. Mercuric ions also displace other divalent metal ions such as copper ion from active sites thereby inhibiting the oxidative-reductive reactions. Organic and elemental forms of mercury are even more

toxic as they readily passes through the blood-brain-barrier as well as placenta. Therefore, the foetus, babies, and developing children are at an increased risk of mental side effects. The acute load of mercury will be handled by our renal system resulting in renal tubular damage.

Acute poisoning with elemental mercury will cause severe pneumonitis and adult respiratory distress syndrome. For acute poisoning with inorganic mercury, mucous membrane ulceration, excessive salivation, nausea, vomiting, abdominal pain, diarrhoea, lethargy, oliguria or even anuria may be resulted. In chronic poisoning with organic mercury and other mercury species, the signs and symptoms are less well demarcated. Mild symptoms like back and joint pain are very common. Neuropathy, mental disturbance, tunnel vision, etc, represent late and ominous signs of chronic intoxication. The best marker for the laboratory diagnosis of acute and chronic mercury poisoning is whole blood mercury collected in certified specimen bottles.

A widely available Chinese medicinal mouth spray named "Watermelon Frost (西瓜霜) is believed to be useful in controlling pain and healing mucosal wounds. The mercury content of the spray was 878 ppm (normal allowable limit <1), and the mercury species inside was largely inorganic (98%).

IRON STATUS

Iron plays a vital role in many metabolic functions, especially in oxygen transport and energy metabolism. Iron is distributed into a number of physiological compartments, namely in the red blood cells (RBCs) in the form of haemoglobin, as a component of many oxidative enzymes, in muscles in the form of myoglobin and stored in cells in the form of ferritin. The iron status of the body can be measured using haematological and biochemical indices.

Haematological indices
Hematological indices such as haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH), haematocrit (HCT), and red-cell distribution width (RDW), are useful in the assessment of the severity of iron deficiency. However, these markers are insensitive to early changes of iron status, and therefore, a normal complete blood picture (CBP) does not rule out iron deficiency, but reflects the absence of functional haematological consequences at that stage.

Biochemical markers
These are the direct indices of the body iron status and include serum iron, serum ferritin, total iron binding capacity (TIBC) or serum transferrin, the derived transferrin saturation percentage (Sat%) and the more recently introduced soluble transferrin receptor (STFR).

Iron deficiency is the most common nutritional deficiency worldwide. About 10% of pre-menopausal women has early iron deficiency without anaemia. In patients with obvious cause of excess iron loss or increased body iron requirement (eg pregnancy and lactation), iron status should be assessed even in the presence of a normal blood picture.

Serum Ferritin

During the development of chronic iron deficiency, ferritin stores would gradually deplete followed by an elevation in TIBC with consequential reduction in the Sat%. Eventually, there would be inadequate supply of iron for haemoglobin synthesis, resulting in anaemia. Absence of iron staining in bone marrow is the gold standard test for iron deficiency. However, this procedure at the bone marrow is too invasive for routine practice. The alternative is a therapeutic trial of oral iron for 3 months.

Ferritin is the major iron storage protein in tissues. A small amount is present in the circulation. Serum ferritin concentration varies directly with the body iron stores. Therefore, serum ferritin is the most sensitive marker of iron deficiency in metabolically stable patients. It is the first marker to fall in early iron deficiency when all other markers are still normal. ***The only reason for a low serum ferritin is iron deficiency.***

Acute phase reaction
Serum ferritin is a positive acute phase reactant and its concentration rises significantly in acute illness and acute liver pathology. On the other hand, serum iron and transferrin are negative acute phase reactants and therefore serum iron and TIBC, which is an indirect measure of transferrin concentration, may decrease in the event of acute illness. Therefore, isolated high elevation of ferritin usually means acute phase reaction without iron deficiency.

Anaemia of chronic disease (ACD)
In ACD (eg chronic infections, inflammatory conditions, neoplastic diseases) without iron deficiency, the serum iron, transferrin, TIBC are reduced, and ferritin, ESR, and C-reactive protein are raised. This is due to acute phase reaction as explained above. Patients with ACD in fact have adequate body iron stores which are compartmentalised in the reticuloendothelial system and made

unavailable for erythropoiesis.

ACD and iron deficiency can coexist in some patients with chronic diseases. These may pose a diagnostic confusion because of the inflammatory effects exerting on the biochemical iron markers. In general, co-existing iron deficiency is likely when serum ferritin < 225 pmol/L (100 mg/L) in the presence of ACD.

Reference ranges

When establishing a reference range for serum ferritin, it is difficult to exclude all asymptomatic subjects with early iron deficiency from a reference sample. There is also considerable overlap between serum ferritin concentrations of normal and iron deficient subjects. In general, serum ferritin < 34 pmol/L (15 mg/L) is diagnostic of iron deficiency in patients without anaemia. For those with anaemia, a cut-off at 68 pmol/L (30 mg/L) seems more appropriate.

In pregnant women, serum ferritin can normally fall < 45 pmol/L (20 mg/L) during the second and third trimesters. Moreover, pregnancy and oral contraceptives can increase the TIBC up to 90 mmol/L. An increased TIBC can result in apparently low transferrin saturation, which does not necessarily indicate iron deficiency. Iron deficiency is considered unlikely when Hb > 11.0 g/dL and serum ferritin > 68 pmol/L (30 mg/L) in the first trimester; Hb > 10.5 g/dL and serum ferritin > 45 pmol/L (20 mg/L) in the second trimester.

Serum ferritin concentrations rise remarkably after the age of 65. Only 55% of iron deficient elderly have serum ferritin < 40 pmol/L (18 mg/L).

Paediatric values of serum ferritin vary a lot from adult range. For 1 - 6 months old infants, the reference ranges are:

- Iron 4.5 - 22.6 mmol/L
- Ferritin 81 – 740 pmol/L
- TIBC 24.7 - 65.3 mmol/L
- Sat% 7 - 44

Iron Overload

The determination of the iron saturation of serum transferrin is useful in screening iron overload. It has a higher diagnostic predictive value and is more sensitive than that of serum ferritin. Sat% > 45 in female and > 55 in male and postmenopausal women indicate iron overload despite normal serum ferritin. It is important that fasting morning samples should be used to minimize the effects of dietary iron and diurnal variation. Serum ferritin levels > 450 pmol/L (200 mg/L) in women and 675 pmol/L (300 mg/L) in men indicate increased iron stores. Hypothyroidism and Vitamin C deficiency decrease the serum ferritin concentrations and may mask iron overload. Hereditary haemochromatosis should be considered first in Caucasians patients with prevalence of about 1 in 300 of the Australian population.

Serun Iron, TIBC & Transferrin Saturation

Although its importance is often underestimated, iron status should be assessed in patients at high risk of iron deficiency, eg menstruating females, people on diet, pregnant women, lactating mothers, vegetarian, infants, toddlers, preschool children, adolescents in rapid growth phases, and people who do not eat red-meat.

Care is required in interpreting serum iron results because they are influenced by a variety of physiological and pathological factors. Men have serum iron 10-20% higher than women. Morning

levels are higher than from afternoon samples. The diurnal variation can fluctuate as much as 50%. Since Sat% is derived from dividing serum iron by TIBC and multiplying by 100, an apparently low serum iron in any evening samples can result in a low Sat% despite a normal TIBC. In contrast, both serum ferritin and transferrin do not show significant circadian rhythm. Serum iron is also sensitive to day-to-day fluctuations of dietary iron intake. To avoid confusion, fasting morning samples should be taken for iron profile whenever possible. In addition, very low values may be seen immediately prior to, and during, menstruation. In summary, serum iron on its own provides no useful information and it should be interpreted in conjunction with serum TIBC or transferrin. One should not rely on a single serum iron or transferrin saturation result to diagnose iron deficiency. In uncomplicated iron deficiency, low Sat% must be accompanied with a high TIBC.

Soluble Transferrin Receptor (STFR)

This is a good marker to differentiate ACD from iron deficiency in concurrent inflammation. STFR is the circulatory extracellular part of transferrin receptor which is expressed on the surface of human cells that require iron. Its concentration reflects cellular iron status. When the iron store is depleted, transferrin receptors are up-regulated to enable the cell to compete more effectively for iron. High STFR concentrations are therefore associated with iron deficiency. The merit of STFR is that it is not affected by acute or chronic inflammatory conditions. Compared with ferritin, STFR is normal in patients with ACD and during inflammatory conditions. The use of STFR/log ferritin index is found to have a better diagnostic efficiency than STFR or ferritin alone. One of the limitations of STFR is that it is also elevated in any causes of increased effective or ineffective erythropoiesis, eg in haemoglobinopathies.

LIVER FUNCTION TEST (LFT)

LFTs are among the most frequently requested panels of clinical biochemistry tests. A liver function panel usually refers to a profile of biochemical parameters which include: total bilirubin, alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), total protein and albumin. These parameters are useful for the diagnosis of hepatobiliary pathology when considered COLLECTIVELY. However, the key to appropriate interpretation of LFT lies in the understanding that when considered individually, none of the members of the LFT panel is specific for liver pathology.

Alkaline Phosphatase (ALP)

There are several isoenzymes of ALP. They are present in liver, bone, intestine and placenta. Liver or bone pathologies are the most common causes of elevated serum ALP. ALP in the liver and GGT are found on the membranes of the bile canaliculi and ducts. If there is any doubt, ALP isoenzyme testing could be performed to distinguish between bone and liver origin of an elevated serum ALP.

Bilirubin

Plasma total bilirubin consists of conjugated and unconjugated forms. Unconjugated bilirubin is a metabolic by-product of haem metabolism. Thus, haemolytic disorders are associated with unconjugated hyperbilirubinaemia. Unconjugated bilirubin is normally conjugated by the liver to facilitate biliary excretion of bilirubin. Therefore, most liver pathologies are associated with conjugated hyperbilirubinaemia, except when there are enzymatic deficiencies in the bilirubin conjugation pathway, eg Gilbert's disease.

Gilbert's disease is a common benign condition which is seen mole in male and affects 1-5% of the population. Bilirubin levels typically are <50 mmol/L and fluctuate with mild illness, infections and periods of starvation, leading to mild jaundice occasionally. Bilirubinuria (urine darkening on standing) is typically lacking, as bilirubinuria is a sign of conjugated hyperbilirubinaemia. When a haemolytic condition is excluded or unlikely, mild fluctuating unconjugated hyperbilirubinaemia is often due to Gilbert's disease.

Patterns of Abnormalities

When considered collectively, certain patterns of the LFT reflect particular types of liver pathology. Such patterns can be broadly categorised as:

Hepatocellular pattern
This is associated with a predominant increase in serum ALT / AST but normal or only mildly elevated ALP / GGT. This is because both ALT and AST are intracellular enzymes in hepatocytes. Therefore, significant damage to hepatocytes results in the release of ALT and AST into the circulation. Causes: viral hepatitis, drugs (eg paracetamol), circulatory shock and liver congestion from cardiac failure.

Cholestatic pattern
This is associated with a predominant increase in serum ALP / GGT and conjugated bilirubin, with normal or only mild increase in ALT / AST. Both ALP and GGT are present on the membranes of bile canaliculi. Therefore, bile flow obstruction would result in induced production of GGT and

increased release of ALP into the circulation. Causes: gall stones, cholangitis, cholangiocarcinoma, carcinoma of the head of pancreas, liver metastasis, primary biliary cirrhosis, liver cirrhosis and drugs (eg flucloxacillin, augmentin).

Mixed pattern

This is usually associated with raised ALT, AST, ALP, GGT and bilirubin. Such a pattern is often evident when the liver insult is prolonged, eg prolonged biliary obstruction leading to significant liver cell destruction (due to back pressure), and vice versa (acute hepatitis may give rise to severe cholestasis due to hepatic congestion).

Chronic Liver Disease

As chronic liver disease develops insidiously with adequate opportunity for compensatory mechanisms to adapt, the biochemical profile is often normal except when in advanced stage, particularly when cirrhotic nodules obstruct biliary drainage. Hypoalbuminaemia is usually associated with chronic liver disease due both to reduced hepatic albumin synthesis and haemodilution consequent to generalised oedema. Prolongation of prothrombin time is often considered a marker reflective of the severity of the chronic liver disease as the clotting factors II, VII, IX and X are produced by the liver via a Vitamin K dependent pathway. However, Vitamin K deficiency due to fat malabsorption, eg due to biliary obstruction, could also result in prolongation of prothrombin time. Plasma ammonia is measured in assessment of hepatic encephalopathy as ammonia is normally detoxified by the liver and excreted as urea. Accumulation of ammonia in a patient with liver disease is a sign of hepatic failure.

RENAL FUNCTION TEST (RFT)

RFT is a test profile that usually includes urea, creatinine, sodium, potassium and occasionally chloride and bicarbonate. Sodium and potassium are the two most abundant cations in our body. Their changes can be the results of numerous diseases, while a renal disease is just one of these. Similarly, the levels of chloride and bicarbonate are disturbed in acid-base disorders, which can originate from the kidneys or other organ systems in the body. Therefore, any changes in serum (or plasma) levels of sodium, potassium, chloride or bicarbonate, as reflected in the results of a RFT, are not necessarily due to renal problems.

Urea & Creatinine

Urea and creatinine are the most commonly used serum markers to screen for renal disease. Both analytes are metabolic end products and are excreted almost exclusively by the kidneys. Factors that increase the measured serum concentrations of urea and creatinine are as follows:

Urea

- decreased GFR
- dehydration or low urine flow
- gastrointestinal bleeding
- hypercatabolic state

Urea is derived from the breakdown of dietary and endogenous proteins. It is freely filtered at the glomerulus and undergoes 50% reabsorption at the renal tubules. Low urea levels are seen in people on low protein diets.

Creatinine

- decreased GFR
- drugs that reduce tubular secretion (eg cimetidine and trimethoprim)
- high protein (eg meat) intake
- false positive (eg ketone bodies and intravenous cephalosporins)

Creatinine is mainly derived from the metabolism of creatine in skeletal muscles. Its serum level is proportional to the total muscle mass. Lower levels are seen in infants and children, the elderly, and in female than in male.

It is very common for laboratories to provide a single reference interval for serum creatinine (usually 60 - 120 mmol/L) for all subjects. Underestimation of renal impairment may result if this reference interval is applied to all individuals without consideration of other physiological factors that can affect the serum creatinine level. Enzymatic assays for creatinine measurement are associated with less interference but are more costly than routine assays.

An important point to note is that serum creatinine is not a sensitive marker for mild renal impairment. GFR has to drop to at least 50% of normal before we can see a rise in serum creatinine level above the upper reference limit.

A relatively greater increase in urea than creatinine suggests prerenal renal failure.
Urea-to-creatinine ratio is a useful marker in this clinical situation. In normal individuals, the ratio is around 0.04 - 0.06 (mmol:mmol). Increased values suggest either an increase in urea production or an increase in tubular reabsorption of urea due to prolonged tubular transit from marked dehydration or reduced renal perfusion.

Glomerular Filtration Rate (GFR)

Estimates of GFR are the best indices of the level of renal function. Chronic renal failure is preceded by a progressive decline in GFR in most forms of renal diseases. Estimation of GFR also helps to determine the proper dosages of renal excreted drugs. Conventionally, GFR is corrected for body surface area, which in the average adult is approximately 1.73m. The corrected GFR for healthy adults is 80 - 120 ml/min/1.73m and this value decreases with age at a variable rate.

In routine clinical practice, the most commonly used parameter of GFR estimation is creatinine clearance (CrCl). Calculation of CrCl usually involves the collection of 24-hour urine and a random serum for creatinine measurements. Most of the inaccuracy of CrCl determination in 24-hour urine comes from improper urine collection.

Abnormal RFT

When a patient is found to have deranged renal function, further investigations are always required to delineate the underlying cause as numerous disease processes and pharmaceutical agents have primary or secondary effects on the kidneys. Some simple laboratory tests (eg calcium, urate, glucose, complete blood count, urine microscopy, etc) and radiological examinations (eg KUB, ultrasound) can give valuable clues in most circumstances. More specialised tests should follow if necessary.

In general, changes in potassium, bicarbonate, phosphate and calcium levels do not become evident until the GFR falls to below 20 - 25 ml/min.

Severe hypernatraemia is commonly due to dehydration especially in the elderly, which was the result of poor oral intake and increased insensible water loss (eg fever).

Micro- & Macroalbuminuria

Diabetic nephropathy is one of the most common causes of end-stage renal disease and its first manifestation is increased urine albumin excretion. Albuminuria of diabetic nephropathy is divided into 2 stages:-

- Microalbuminuria: 30-300 mg/24hr, OR 2.5-25 mg/mmol creatinine
- Macroalbuminuria: > 300 mg/24hr, OR >25 mg/mmol creatinine

The use of 24-hour urine collection is the traditional way for diagnosis of microalbuminuria. However, spot urine sample is now recommended for urine albumin measurement and the result is expressed as albumin-to-creatinine ratio (ACR) (units: mg/mmol creatinine).

The use of creatinine in ACR is to correct for variations caused by hydration. Interpretation of results may not be straight-forward when in the presence of the following conditions that may increase urine albumin excretion: urinary tract infection, haematuria, acute febrile illness, vigorous exercise, short-term pronounced hyperglycaemia, uncontrolled hypertension and congestive heart failure.

All type 2 diabetic patients should be screened for diabetic nephropathy at initial assessment. A spot urine sample should be first checked for the presence of protein using standard urine dipstick. If it is negative, a test for either ACR (spot urine) or urine albumin excretion rate (UAER, 24-hour or timed urine) should follow. On the other hand, if a spot urine is tested positive for protein by standard urine dipstick, overt nephropathy is present. Then urine total protein should be measured.