The pre-analytic phase is an important component of total laboratory quality. A wide range of variables that affect the result for a patient from whom a specimen of blood or body fluid has been collected, including the procedure for collection, handling, and processing before analysis, constitute the pre-analytic phase. Physiologic variables, such as lifestyle, age, sex, conditions such as pregnancy and menstruation, are some of the pre-analytic phase factors. Endogenous variables such as drugs or circulating antibodies might interact with a specific method to yield spurious analytic results. The pre-analytic phase variables affect a wide range of laboratory disciplines. In recent years, considerable attention has been given to the influence of pre-analytic effects on laboratory results. Pre-analytic variables can be grouped broadly under 3 categories: physiologic, specimen collection, and influence or interference factors.

**PHYSIOLOGIC VARIABLES**

The effects of age, sex, time, season, and altitude, conditions such as menstruation, pregnancy and lifestyle are some of the physiologic variables that affect laboratory results.

**Age**

The effect of age on laboratory results has been well recognized to the point that separate reference intervals have been used to distinguish paediatric, adolescent, adult and geriatric populations. Bone growth and development in a healthy growing child is characterized by increased activity of bone-forming cells, the osteoblasts, which secrete the enzyme, alkaline phosphatase. Hence, the activity of this enzyme is approximately 3 times higher in a growing child compared with the activity in a healthy adult.

A substantial increase in the RBC count in the neonates compared with that in adults is the reason glucose is metabolized very rapidly in neonates. Increased arterial oxygen content within a few days of birth leads to an increase in haemoglobin levels owing to the destruction of the RBCs. Serum bilirubin levels then increase, because the immature liver lacks enzymes to convert bilirubin to the water-soluble bilirubin diglucuronide. A substantial decrease in serum uric acid levels is noted between days 1 and 6 after birth.

Secondary risk factors, such as genetics and obesity, may have a role in the decrease in glucose tolerance with aging. Homeostatic control by the hypothalamic-pituitary-adrenal axis is compromised because of aging. Hence, there is an increase in plasma corticotropin and corticosteroid levels due to aging.

**Sex Differences**

Between 15 and 55 years of age, the levels of total and low-density lipoprotein cholesterol (LDL-C) increase progressively, with levels slightly higher in premenopausal females compared with the levels in males. Thus, in female subjects, a change in cholesterol value from 4.50 mmol/L at age 25 years to 5.51 mg/dL at 55 years has been reported. In the corresponding age span for men, cholesterol values have been reported to range from 4.29 mmol/L at age 25
years to 6.00 mmol/L at 55 years. In contrast, the high-density lipoprotein cholesterol (HDL-C) level does not fluctuate in either sex between the ages of 15 and 55 years; levels are slightly higher in females, presumably owing to the stimulating effect of estrogens. Analytes with levels that depend on muscle mass, such as creatinine and creatine kinase (CK), are generally at a higher concentration and activity, respectively, in males compared with females. However, when increased muscle mass is acquired as a consequence of strenuous athletic activities in female subjects, the sex difference in levels of creatinine and CK is abolished.

Time
There are time-related fluctuations in the level of some analytes. Circadian rhythm is responsible for the diurnal changes seen in the circulating levels of some analytes. A classic example of an analyte subject to diurnal variation is cortisol, which generally peaks at around 6:00 AM, with levels becoming lower toward the evening and midnight. Glucose values obtained during an oral glucose tolerance test tend to be higher when the test is performed in the afternoon than when the test is performed in the morning. The cortisol rhythm apparently may be responsible for the discrepancy noted in the results of an oral glucose tolerance test performed in the afternoon.

Seasonal Changes
Vitamin D levels tend to be higher during the summer, apparently due to prolonged exposure to sunlight. A slight increase in the total cholesterol level (average, 2.5%) has been observed during the winter compared with values measured during the summer. The decrease in the level of triglycerides in serum from summer to winter is more striking than the extent of decline noted between spring and autumn months when the temperature tends to be milder.

Altitude
Changes in levels of some of the constituents in blood occur when measured at sea level as opposed to measurement at a higher altitude. A 65% increase in C-reactive protein has been reported at 3,600 m. Concentrations of some analytes, such as plasma renin, serum transferrin, urinary creatinine, estriol and the creatinine clearance rate decrease with increasing altitude.

Menstruation
At the onset of menstruation, a low estrogen level triggers the release of follicle stimulating hormone from the pituitary gland. The ovaries thus are stimulated to produce estrogen, and the level begins to increase noticeably from the sixth or seventh day after menstruation; the peak level is reached on approximately the 13th day. A day later, a burst of luteinizing hormone released from the pituitary gland signals ovulation. With the onset of ovulation, the progesterone level continues to rise until it decreases together with the estrogen level, just before the commencement of the next menstrual cycle. Thus, the reference intervals for estradiol, follicle stimulating hormone, luteinizing hormone, and progesterone are influenced by the stages of menstrual cycle (i.e., follicular, midcycle and luteal phases) Coincident with ovulation, serum cholesterol levels are lower than at any other phase of the menstrual cycle. During midcycle or the luteal phase, the aldosterone concentration is approximately 2-fold higher compared with values during the follicular phase. Renin activity may increase during the luteal phase of the cycle. In contrast; serum phosphate and iron levels decrease during menstruation.

Pregnancy
A dilutional effect is observed due to an increase in the mean plasma volume, which in turn causes haemodilution. The effect is more pronounced when measuring trace constituents such as trace elements in serum. During pregnancy, there is a considerable increase in the glomerular filtration rate. Hence, the creatinine clearance rate is increased by 50% or more over the normal rate. During the third trimester, the urine volume may increase by approximately 25%. During the second half of pregnancy, the placenta progressively begins to produce hormones antagonistic to insulin. As a result, the levels of hormones, such as estrogen, progesterone, and human placental lactogen increase, leading to the onset of gestational diabetes. An increased metabolic requirement during pregnancy leads to a deficiency of iron and the depletion of iron stores is reflected by a decrease in ferritin.
Lifestyle
Diet influences the results obtained for some analytes. Differences between vegetarians and non-vegetarians consuming a high-protein or a high-purine diet are well known. Non-vegetarians tend to have increased blood levels of uric acid, urea, and ammonia compared with those for their vegetarian counterparts. Diets rich in saturated fatty acids in general are lipogenic; the effect, however, varies depending on the fatty acid. A diet rich in fish oils lowers serum triglyceride and very low-density lipoprotein (VLDL) levels, presumably because of the ability of fish oils to inhibit the synthesis of VLDL triglycerides. Sustained consumption of ethanol can, by enzyme induction, lead to the increase in the activity of liver enzymes, such as gamma-glutamyl transferase.

SPECIMEN COLLECTION VARIABLES
The preparation of subjects for blood specimen collection, such as the duration of overnight fast; time of specimen collection and posture during blood sampling; the effects of the duration of tourniquet application; infusion and exercise; the effects of anticoagulants and stabilizing additives used for blood collection; the anticoagulant/blood ratio; specimen handling and processing; the relative merits of anticoagulants and other variables need to be delineated and standardized to minimize pre-analytic error.

INTERFERENCE FACTORS
Drug Administration has many effects on clinical laboratory tests results. It is therefore impossible to describe all potential sources of difficulties here. It is important to realize that there are however very complete lists of such effects available. The effects of drugs are of two different types; drugs or their metabolites may interfere with the analytical method used by the lab, or may lead to physiological effects which cause changes in clinical laboratory test results.

OTHER FACTORS
Many specimens are taken from patients who have diseases which cause levels of analytes in body fluids to be different from the apparently healthy. Such analytes may cause difficulties in interpretation of test results. Examples include bilirubin present in sera from jaundiced patients which can cause interference in a number of lab test procedures such as a false low serum calcium result found when using semi-automated fluorimetric techniques.

SUMMARY
Each patient on whom clinical lab tests are performed is unique and much must be known about the individual and the lifestyle adopted by the individual before laboratory test results can be interpreted correctly.

References
2. Interpretation of Clinical Chemistry Laboratory Data - CG Fraser

Questions
1. Discuss the physiological variables which affect lab results.
2. What factors need to be taken into consideration to minimise pre-analytical error when a blood sample is taken?
3. How can drug administration affect the outcome of patients’ lab results?