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Pre–Analytical Considerations in Clinical Chemistry*

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Introduction

Control of pre–analytical variables are important to assure the overall quality of laboratory results. Pre–analytical variables can be generally classified as specimen collection variables, physiological variables and drug induced variables. These variables will be addressed in this paper.

Specimen Collection Variables

These variables include preparation of patient prior to blood collection such as posture during sampling, duration of tourniquet application, the time of blood collection, duration of fasting, the effect of exercise, the effect of additives and anticoagulants for specimen collection, and the effect of infusion.[1,2]

Posture during blood sampling

Postural change from supine to erect or sitting position results in shift of body water from intravascular to interstitial compartment. This results in the concentration of large molecules such as proteins or small molecules bound to proteins. Small molecules such as glucose are readily taken up from the interstitial space and thus their concentration is unaffected. There is a significant change in the concentration of renin and aldosterone when going from a supine to an erect position.

Postural change from an upright to a supine position has a dilutional effect on proteins and substances bound to proteins or lipoproteins such as

cholesterol and triglycerides bound to respective lipoproteins.

For outpatient blood collection, the posture should be standardized by instructing the subject to sit calmly in a chair for 10 minutes to stabilize postural condition before drawing blood.

Duration of tourniquet application

There is a greater hemoconcentration at 3 minutes of tourniquet application as compared to 1 minute. In the former, venous stasis results in increased anaerobic glycolysis and a decrease in blood pH with the accumulation of lactate. The hypoxic effect leads to release of potassium from cells. There is an increase in ionic calcium and ionic magnesium levels and the free drug concentration with the drop in blood pH.

Ideally the tourniquet should be released as soon as the needle enters the vein.

The time of blood collection

Due to changes depending on the time of day when blood is collected, the concentration of some of the analytes may vary. Cortisol levels are higher in the early morning hours (8 a.m.) and lower towards evening and midnight. Similar changes are seen for serum iron

and even glucose. Results for glucose tolerance are higher in the afternoon as compared to the morning hours. Growth hormone secretion during walking hours is minimal. Reproductive hormones such as LH, FSH and testosterone are released in bursts lasting just 2 minutes. Hence the need for multiple blood specimens collected at closely spaced intervals in order to accurately estimate the concentration of these hormones in circulation.

Duration of fasting prior to blood collection

Prolonged for a period of 48 hours reduces the hepatic clearance of bilirubin, thus increasing the serum bilirubin value, and decrease the concentration of specific proteins such as C3 component of complement, albumin, prealbumin and transferrin. Even during a 1 day fast there is an increase in acetoacetic acid concentration in an obese individual. Subjects should be instructed to fast overnight for at least 12 hours prior to specimen collection, since serum triglyceride levels can be affected by a meal consumed 9 hours before blood collection[3].

Effect of exercise

Strenous exercise will deplete the

concentration of the energy yielding compound adenosine triphosphate (ATP) from muscle cells, thus changing cell membrane parameability and release of enzymes from within the cells. Creatine kinase (CK) levels can be very high with release of CK–MB fraction, although CK–MB as a percent of total CK would be normal after strenous exercise. There is increase in plasma lactate and consumption of haptoglobin by hemoglobin released during intravascular hemolysis. Ionic calcium and ionic magnesium levels increase due to drop in blood pH. Subjects should be instructed to refrain from any strenous activity at least the night before and not exert themselves prior to blood collection.

Additives and anticoagulants for specimen collection

Glycolytic inhibitors such as fluoride or iodoacetate may be needed for long term preservation of glucose unless the serum or plasma can be promptly separated from cells. In newborns in whom glucose is consumed rapidly, even in presence of glycolytic inhibitors some glucose is metabolized. Lithium heparin at a concentration of 14.3 units/mL of blood gives true electrolyte and total protein values and is generally used as an anticoagulant for plasma chemistry determinations. Even at the concentration of 14.3 units of heparin/mL of blood statistically significant changes have been noted for ionic calcium. Hence it is important that evacuated blood collection tubes be filled to the nominal draw volume in order to maintain the proper anticoagulant to blood ratio. EDTA is the ideal anticoagulant for maintain the stability of lipids since it prevents oxidation of lipids by chelating divalent cations. EDTA together with a proteolytic enzyme inhibitor such as aprotinin stabilizes labile polypeptide hormones such as glucagon and ACTH.

Effect of infusion

Extremely elevated glucose values can be obtained by collecting blood from the same arm which is also receiving an infusion of glucose. Hence blood should always be collected from the arm opposite to the one which is receiving the infusion.

Physiological Variables

These include aspects of life style, age and gender related differences.

Life style

Alcohol consumption – Secondary changes are induced by sustained

consumption of ethanol, reflected by an increase in serum gammaglutamyl transferase (GGT) level. A marked intra and inter individual variation in GGT levels was noted on 3 day ethanol challenge (0.75 g/Kg of body weight). Long term consumption (225 g/day for a month) cause skeletal muscle changes and release of cellular enzymes[2].

Caffeine consumption – Caffeine inhibits phosphodiesterase. As such, cyclic AMP (cAMP) is not inactivated to 5'-AMP. Glycolysis and lipolysis increase, the latter causes a 3-fold increase in plasma free fatty acid level. Free fatty acids displace drug or hormone bound to albumin thus affecting their measurements. Decrease in pH causes release of calcium bound to albumin[4].

Smoking – Cigarette smoke contains carbon monoxide thus increasing carboxyhemoglobin levels in smokers, since the affinity of carbon monoxide for hemoglobin is much greater than of oxygen. As a result, long term smoking causes an increase in hemoglobin concentration, the red blood cell count, the mean corpuscular volume (MCV) and the white blood cell count. Cadmium levels are also higher in smokers.

Physiological conditions

Age – Alkaline phosphatase activity in a healthy child reflecting bone growth is three fold higher when compared to a healthy adult. Due to increased hematocrit in the newborn, glucose is metabolized rapidly[4].

Creatinine clearance decreases with each decade[5]. Decrease in glucose tolerance with aging could also be related to secondary risk factors such as genetics and obesity.

Gender or sex–related differences – Differences in muscle mass and organ specific and endocrine differences, as well as stages of menstrual cycle and pregnancy may explain differences in reference values for some analytes between male and female subjects.

The stage of menstrual cycle influences the reference intervals of hormones such as estradiol, LH and FSH[4].

Pregnancy has a dilutional effect with the level of trace constituents such as trace elements in serum being decreased[4].

Insulin resistance develops during the second half of pregnancy due to production by placenta of hormones antagonistic to insulin.

There is a considerable increase in glomerular filtration rate during pregnancy[5]. Alkaline phosphatase, alpha-fetoprotein and hormone binding proteins such as thyroxine binding globulin increase during pregnancy. There is increased mobilization of lipid due to the metabolic demand during pregnancy.

Drug induced variables

To the extent that a drug alters the levels of an analyte without actually interfering in the analytical procedure, the interference could be regarded as pre-analytical. Diuretics such as thiazides are known to alter serum lipid profiles[3].

Oral contraceptives by altering the level of binding proteins cause an increase in the level of bound hormones or drugs.

In conclusion awareness of pre-analytical variables and where possible controlling and minimizing them can lead to improved quality of laboratory results.

References

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