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Women's Health and Laboratory Testing

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Abstract

Laboratory testing of body fluids and tissues is an essential part of diagnostic work-up and monitoring for health and healthcare. About 70% of clinical decisions are driven by laboratory data [1,2]. Most of the time laboratory testing is done to screen for a disorder, diagnose or rule out a disorder, monitor the progress of treatment and disease. This narrative addresses the usual laboratory tests that may be done to monitor the health of the person and is not geared towards screening or diagnosis of specific entities. This issue has gained importance since the availability of laboratory results to patients, via patient portals. Patients/lay persons, may over-react to results labeled as abnormal. At the same time patients may not realize the importance of results with a narrative report, e.g., presence of a monoclonal immunoglobulin on serum protein electrophoresis [3,4]. Laboratory testing results usually include a range of normal values, reference range, in laboratory parlance. A reasonable question is, "Where do normal values/reference ranges, come from?" Classically, at least 120 normal, healthy people, of a given age, gender, ethnicity and geographic area, are tested for the chemical/cell type/ analyte under consideration. The lowest 2.5% and highest 2.5% of the results are discarded and the central 95% results are adopted as the reference range/normal value. Under ideal conditions, the specimen is collected in post-absorptive, recumbent state. At one time, metabolic units in academic centers, admitted healthy people who were kept on a prescribed diet and activity. Body fluid, usually blood was collected on waking up for testing to establish normal values [2]. There are a number of issues with the ideal state of specimen collection and what takes place in real life. Some of the issues are addressed below.

Common Laboratory Tests and Health

Healthy individuals

In 1948, the World Health Organization adopted this definition of health**,** "A **state of complete physical, mental and social well-being** and not merely the absence of disease or infirmity." Being that it would be impractical to find "healthy" people, apparently healthy people, free of disease of the organ system affecting or being affected by a given analyte, are selected to determine the reference range. Blood donors are the usual surrogates for "healthy" people [5]. Reference ranges derived from such a population may not be relevant for a given patient population, e.g., hemoglobin levels during pregnancy, serum free light chain levels in older patients, skeletal muscle enzyme levels in ambulatory patients, and liver enzymes in "social" drinkers [6-8].

Central 95%

By taking the central 95% of the values of a healthy population, by definition 5% of healthy people will have an abnormal test result for the analyte

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[2]. Such minor variations from the reference range may cause concern and anxiety in patients and lead to unwarranted repeat testing, additional tests or imaging studies. For some analytes only one end of the range is relevant, e.g., an elevated serum creatinine value indicates renal insufficiency, however, a low serum creatinine does not imply kidney hyperfunction, however, it may still be important as an indicator of low muscle mass. For troponin levels, there is no abnormal low value. To be considered abnormal a troponin value has to be greater than the 99th percentile of a normal range.

Other issues affecting reference ranges

The reference ranges vary by age, gender and ethnicity [2, 9]. While the variations by age and gender are well accepted, and standard texts do list different ranges by age and sex. There is controversy about using ethnicity/race as the later has been used in discriminatory practices. However, past discriminatory practices and effort to eliminate discrimination do not negate the valid biological differences in different ethnic groups/races. For example, WBC and neutrophil counts are lower in black subjects compared to white subjects and have relevance for diagnosis of neutropenia [10]. Vitamin D levels are lower in blacks due to genetically determined lower levels of vitamin D binding protein [11]. Obese individuals have lower vitamin D levels and require larger doses of vitamin D to see the same level of rise in the vitamin D level as compared to healthy weight individuals [12,13]. Blacks tend to have higher A1C levels than whites despite being normoglycemic.[14] Hemoglobinopathies have strong ethnic correlation, e.g., sickle cell disease in blacks; thalassemia is more prevalent in Mediterranean, through the Middle East, and into Southeast Asia; hemoglobin E disease is present selectively in Southeast Asian population [15]. Alcohol intolerance due to deficiency of acetaldehyde dehydrogenase is a feature of the east Asian individuals [16].

Table 1: Different reference ranges for women and men among commonly used laboratory tests. (Endocrine tests were not included in the survey due to too many variables.)

Reference ranges by gender	Female	Male
Hemoglobin *	12-16 g/dL	14-18 g/dL
Ferritin	12-150 ng/mL	15-200 ng/mL
Serum creatinine ≠	0.59 to 1.04 mg/dL	0.74 to 1.35 mg/dL
Creatine Kinase	30-135 U/L	55-170 U/L
Uric acid	2.7-7.3 mg/dL	4-8.5 mg/dL
HDL ±	>50.0 mg/dL	>40 mg/dL

*Women can deliver sufficient oxygen to their tissues with 2.0 gm/dL lower hemoglobin, than men, due to their hormonally driven higher levels of 2,3 Diphosphoglyceric Acid (2,3 DPG). 2,3 DPG facilitates release of oxygen from hemoglobin. The lower levels are noted in healthy women, with sufficient iron stores and menstrual blood loss is an unlikely explanation of the lower hemoglobin in healthy women.

≠ The lower levels of serum creatinine and creatine kinase reflect lower muscle mass in women.

± The higher HDL levels in adult women tend to decrease following climacteric.

As shown in table 1, reference values are usually higher in men, particularly for hemoglobin. In addition to the analytes listed in table 1 values of pCO2, and liver enzymes are slightly higher in men. HDL is about the only commonly measured entity for which women have higher levels [17].

Expert opinion

The distribution of laboratory test results in apparently healthy people may not be accepted as the reference/normal value and an expert opinion may be overlaid on the observed value. Serum cholesterol, body mass index (BMI), blood pressure, and vitamin D levels are examples of such parameters where expert opinion mandates different levels than those seen in an apparently healthy population [18,19].

Paradox between reference ranges and empirical values

The normal range for serum albumin is generally taken to be 3.4 to 5.0 gm/dL, however, insurance company data reveals a 150% risk threshold for people with serum albumin lower than 3.8 gm/dL [20]. Similarly, serum cholesterol levels and BMI values higher than the recommended levels are associated with lower mortality. The "normal" upper level of total cholesterol is cited as 200 mg/dL; however, longevity data suggests that 150-200 mg/dL is the optimum level. The graph of cholesterol vs mortality data has a "U" shaped curve with the lowest risk around blood cholesterol level of 150-200 mg/dL. The normal range for BMI is cited at 18.5-25, however, people with BMI of 25-30 have been observed to have better mortality data [19]. To add to this paradox, obese people have better outcomes from acute illnesses such as myocardial infarction, ICU admission and stroke, than normal weight people. This observation is referred to as the obesity paradox. Such paradoxes remain unresolved and are a cause of confusion among patients as well as providers [21,22]. A seemingly obtuse observation shows higher mortality in patients with higher red cell distribution width [23].

Mis-reading of laboratory results

Laboratory values need to be interpreted in the context of the whole patient and not taken in isolation. A person with beta thalassemia trait, or a hemoglobin variant with lower oxygen binding affinity will have lower than normal hemoglobin levels without any ill effects and need not be subjected to unneeded investigation or treatment [24]. Abnormal ratio of serum free light chains has been erroneously used to diagnose light chain monoclonal gammopathy, without any evidence of a monoclonal lesion and subjected to unwarranted investigations, including bone marrow biopsy. Patients with incorrectly diagnosed monoclonal gammopathy

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of undetermined significance suffer the same level of mental anguish and trauma as a patient with a diagnosis of multiple myeloma. Even worse, the so-called early diagnosis provides no benefit [25,26]. Borderline "abnormal" results may be monitored and if stable and not associated with any health issues, could be that person's normal and need not be investigated further [25,26].

Countervailing arguments for iron

Iron deficiency has been documented in about 40% adult women in US and iron deficiency anemia occurs in about 13%. This data would argue for routine iron supplementation in adolescent and adult women [27,28]. The current ferritin ranges could be underdiagnosing deficiency in women. Using ranges based on statistical distributions represents the population norms but can camouflage the prevalence of low iron stores in women [29]. The commonest pathogenic gene abnormality in people of northern European extraction are the genes for susceptibility for hemochromatosis, though the penetrance of the gene is low. The prevalence of high iron in blood donor women was 0.003 making the risk of routine iron supplementation a miniscule amount [30, 31]. The cost of testing for serum iron, iron binding capacity and ferritin is about $60.00. The cost for a year's supply of supplemental iron is about $2.00. Iron sufficiency may require adequate iron stores and not just prevention of iron deficiency anemia.

Potentially inappropriate reference ranges and practices

The reference ranges and recommended daily allowance (RDA) for essential nutrients are not geared toward optimal nutrition and health but rather for meeting minimum daily requirements. The RDA is meant to prevent disease in 98% of otherwise healthy people, not to obtain the optimum level of health. Two examples of this issue are folic acid and vitamin D.

Folic Acid/Folate

According to the NIH website "a value above 3 ng/mL indicates adequacy". (Folate - Health Professional Fact Sheet). A serum folate level of 3.0 ng/ml is sufficient to prevent megaloblastic anemia in an otherwise healthy person. Even though 3.0 ng/ml is supposedly an adequate level, many subjects, with this level, have elevated levels of homocysteine. In responsive individuals, hyper-homocystinemia is corrected with sufficient supplementation with folic acid that raises the serum levels to 7.0 ng/mL [32].

Mandatory fortification of folic acid, a synthetic, easily bioavailable form, in processed cereals and cereal products has been implemented in the US since 1 January 1998, to reduce the risk of neural tube defects (NTD) in newborn children [33]. However, the level of supplementation is inadequate to raise serum folate levels to 13.0 ng/ml, the minimum level for optimal effective prevention of NTD. Therefore, all women of reproductive age, including adolescents, should take supplemental folic acid. Except in the presence of vitamin B12 deficiency, there is no toxicity to taking 1.0 mg of folate daily [34,35]. Up to 5.0 mg/day may be safe but there are theoretical risks at higher doses. As in the case of iron, cost of testing may exceed the cost of supplementation.

Vitamin D

Unlike some other essential nutrients, humans can synthesize vitamin D in skin on exposure to sun light. 7-hydroxycholesterol is converted to cholecalciferol, vitamin D3, on exposure of skin to UVB light (Wave length of 290 to 320 nm) [36]. Sun exposure can provide sufficient vitamin D for optimal health and exposure to sun does not result in vitamin D toxicity. Almost all through human history, people worked outdoors all day and extant populations with similar life style have serum vitamin D levels of 50-80 ng/mL. A serum level of 12.0 ng/mL may be considered sufficient as levels above this are sufficient to prevent rickets and osteomalacia. However, patients have elevated levels of parathyroid hormone at vitamin D levels below 20 ng/mL and administering vitamin D corrects that. A level of 30ng/mL or higher is considered normal. There is controversy about benefits of vitamin D administration. Vitamin D supplementation has been cited to have beneficial effects in preventing falls and fractures in the elderly, reducing musculoskeletal pains, cancer, type 2 diabetes, depression, cardiovascular disease, and multiple sclerosis [12,13].

Given that about 80% of adult US population has vitamin D serum levels below 30 ng/mL. it may be prudent to supplement the diet with vitamin D and calcium, especially in adolescents to ensure optimal bone formation [37]. This opinion is not based on randomized controlled trial results. In patients, much larger doses of vitamin D are needed, than the recommended daily allowance, to correct deficiency and maintain sufficiency [35,36].

Alcohol

While not a laboratory test, alcohol intake is more deleterious for women than it is for men. Women have lower levels of alcohol dehydrogenase in the stomach, as compared to men. Therefore, more alcohol reaches intestine and women have higher levels of blood alcohol than men for a similar intake of alcohol.[38] The smaller lean body mass also contributes to higher blood alcohol levels. A single ill-timed drink in early pregnancy may cause fetal alcohol syndrome and associated neurological deficits in the fetus/potential human [39].

Laboratory testing at transitional states of life

Some of the issues addressed here are beyond the realm of usual laboratory diagnostic testing, however, these issues are important for consumers and providers to be aware of for promoting health.

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Pre-implantation testing

Testing of blastocyst stage embryos may be used in preventing illness in the potential human, e.g., Lesch-Nyhan Syndrome, cystic fibrosis, and Sickle cell disease etc. [40,41]. Embryo selection is a controversial issue; however, prospective parents should have the option to use this technology to avoid preventable defects in their potential offspring.

Antenatal testing

Triple and quad screening tests on maternal blood are used to screen for fetal health. The results require expert processing to normalize the finding by considering maternal age and health issues, e.g. diabetes, obesity and fetal age [42]. Assay for fetal lung maturity testing has undergone changes with the change in methods for amniotic fluid testing. Amniotic fluid is also tested to monitor for hemolysis in the fetus. Cells in the amniotic fluid and chorionic villus sampling may be used for any number of genetic tests [43]. Fetal blood may be sampled by umbilical vessel cannulation and the resulting samples could be tested for a whole range of chemicals and nucleic acids [43].

Cell free DNA in maternal plasma is partly of fetal origin and after 9 weeks of pregnancy may be tested for fetal disorders, especially trisomies, and paternity determination [44]. Such testing has occasionally revealed abnormalities in maternal health, e.g. presence of undiagnosed tumors.

Newborn testing

Starting with phenyl ketonuria testing, states have mandated testing of newborn infants for multiple disorders. Dried blood spots on Guthrie cards are screened and confirmatory testing is done by a different method, usually by mass spectrometry. Most of these tests are done in specialized laboratories [45]. However, testing the newborns for bilirubin is also mandated by states and this testing is done usually at the local laboratory to assess infants at risk of hyperbilirubinemia and kernicterus. Hemolyzed specimens are a frequent cause of error and meticulous attention to specimen collection is paramount. Newer transcutaneous measurements can overcome the specimen collection issues; however, transcutaneous measurement is not as accurate and serum may be needed to settle borderline results from transcutaneous testing [46].

Childhood to adolescence

No specific testing is needed in this age group, except for nutritional deficiencies. Increasing prevalence of obesity may require testing for diabetes.

Adolescence

Adolescents are at risk of eating disorders and substance abuse and may require related laboratory testing [47]. Urine drug testing is usually done by immunological methods and may have both false positives and false negatives. Confirmation by a different method, usually mass spectrometry is generally carried out in case of positive screening by immunological methods. Pregnancy test is relevant for all women of reproductive age, including adolescents.

Pregnancy test: Home pregnancy tests and point of care pregnancy tests are generally accurate. A false negative result is usually due to testing too early in pregnancy. Very dilute urine due to high water consumption may produce a false negative result. Very high levels of beta HCG, especially the presence of degraded hormone, may produce a false negative result. False positive results may be due to a missed or incomplete abortion. Other medical causes of false positive results include: hydatidiform mole, choriocarcinoma, germ cell tumors pituitary disorders, urinary tract infection, blood contamination of urine, cancers of the ovary, bladder, kidney, liver, lung, colon, breast, stomach, and ovarian cysts. Other causes of false positive urine results include, exogenous hCG preparations for "weight loss", assisted reproduction, and doping. False results on testing blood may be due to factors listed above and the presence of heterophile antibodies. It is imperative that the testing instructions for home tests be followed closely, including the time interval within which the test must be read. Even though the pregnancy tests in use are generally accurate, given the large number of tests done even a small error rate will produce a large number of false results [48].

Pregnancy

This physiological state is associated with changes in blood volume and biochemistry. There is decline in hemoglobin and platelet counts. Iron, folate and supplementation of other nutrients is appropriate. Pregnancy results in a hypercoagulative state, though in a normal pregnancy intervention is not needed. In addition to changes in reproductive hormones, endocrine changes in thyroid and glucose tolerance are common. Total T4 and T3 are elevated due to increase in thyroxine binding protein, though free T4 and free T3 levels remain near normal. TSH levels decrease due to thyrotrophic activity of hCG. Iodine supplementation may be considered along with other nutritional supplements [49]. Glucose intolerance is usually tested by an oral glucose tolerance test generally administered between 24 and 28 weeks of pregnancy, but may be done earlier for women with risk factors.

Along with weight, hemoglobin, and blood pressure, urine is usually tested for protein to screen for preeclampsia, Urinary protein excretion is considered abnormal in pregnant women when it exceeds 300 mg/24 hours at any time during gestation, a level that usually correlates with 1+ on urine dipstick.

Additional tests like blood grouping, alloantibody screening, tests for communicable infections and other tests,

e.g. blood lead that may be pertinent to the woman's health and circumstances, are appropriate [50,51]. Testing for fetal health was addressed earlier under pre-natal testing.

The physiologic changes of pregnancy increase the cardiac output, plasma volume, and contractility. The risk of myocardial infarction also increases during pregnancy [52]. The gold standard in evaluating the cause of chest pain as a symptom of myocardial infarction versus another potentially benign condition is cardiac-specific troponin. In pregnant women with pre-eclampsia their baseline cardiac-specific troponin levels can be higher than that of healthy pregnant individuals [53]. This shows the need for more specific reference ranges for cardiac-specific troponin in pregnant women with pre-eclampsia. Healthy pregnant women and non-pregnant women have been shown to have the same baseline troponin level, but even the women's baseline troponin levels are lower than that of men. If the reference range for troponin levels are used without sex-specific cut-off points, there is a risk of missing the elevations in cardiac-specific troponin [54].

Adult women may require laboratory tests to diagnose and monitor health conditions, e.g., blood lipids, organ function tests, cancer screening, and nutritional health.

Climacteric

The climacteric encompasses the perimenopause (the time leading up to menopause), menopause itself (when menstruation stops), and the post-menopause (the time after menopause). While there are no specific tests for menopause, however, health changes during this period warrant laboratory tests. Increase in pituitary hCG may produce a false positive pregnancy that along with cessation of menstruation and may be require additional tests to rule out pregnancy [48]. Post-menopausal women are at greater risk for cardiovascular disease, osteoporosis and cancers and that may warrant laboratory testing support [55].

Gender Affirming Care: In the context of laboratory medicine, the category of "women" has traditionally referred to individuals assigned female at birth. However, this designation does not encompass the full spectrum of individuals who may have female-typical anatomy, hormone levels, or reproductive organs, nor does it reflect the diversity of gender identities. Increasingly, individuals who are transgender or non-binary seek gender-affirming care, which often includes hormone therapy and surgical interventions. These treatments produce significant and sometimes unpredictable physiologic/biochemical changes that directly impact the interpretation of laboratory test results [56]. As such, special consideration must be given when interpreting laboratory values for transgender women, transgender men, and non-binary individuals, particularly those undergoing hormonal transition.

Transgender women and non-binary individuals assigned male at birth often undergo feminizing hormone therapy consisting of exogenous estrogen, often combined with androgen-blocking agents. These therapies significantly modify hormone levels, leading to downstream physiologic effects that impact laboratory test results [57, 58].

Hematologic parameters such as hemoglobin and hematocrit typically decrease in response to lowered testosterone levels, often approaching or aligning with cisgender female reference intervals [59, 60]. Serum creatinine also declines, reflecting reduced muscle mass [56]. Estrogen may affect liver enzymes, sometimes leading to a mild increase in ALT, though this depends on hormone type and delivery route [59]. Lipid profiles may shift, with lower LDL and higher HDL cholesterol observed in some cases, although findings are inconsistent [61]. Endocrine markers such as FSH, LH, prolactin, and progesterone increase under estrogen influence [58].

Because these changes are influenced by hormone dose, duration of treatment, and surgical status (e.g., orchiectomy), transgender women may not neatly fit within male or female reference ranges. Relying on standard binary values can result in diagnostic errors, emphasizing the need for individualized interpretation and, ideally, the establishment of population-specific reference intervals [60, 62, 63].

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