Biochemical Assessment

Lynne M. Ausman and Shibani Ghosh
BIOCHEMICAL ASSESSMENT

• Objective and precise approach
• Measure levels of nutrients/anti-nutrients
• Tissues:
  • whole blood (CBC)
  • plasma, serum, white blood cell
  • stools (ova, parasites, blood, inflammatory markers)
  • urine (albumin, glucose, blood)
  • hair, skin, nails (zinc, selenium other microelements)
  • buccal cells and tissue biopsies (various research uses such as carotenoids)
BIOCHEMICAL ASSESSMENT

• Biochemical assessment methods complement anthropometric, clinical, and dietary data. However, interpretation is difficult and don’t always correlate with clinical or other findings.

• Selection depends on the suitability of test for question being asked, as well as available laboratory facilities, feasibility of the test, acceptability to the subject, and cost.
REQUIREMENTS/CONSIDERATIONS

• Blood draw (arterial, venous, capillary, heel prick)

• Blood: Time of day (Diurnal variation), fasting/non fasting

• Coordination of the cold chain and collection- difficult in resource poor areas

• Sample collection should be conducted by qualified personnel (e.g. medical or paramedical workers)

• Standardized lab that will conduct the analysis. The analytical methods utilized vary by cost, degree of technical expertise required and reliability
BIOMARKERS IN POPULATION BASED STUDIES

• Tests should be able to be applied directly in the field

• Tests should require minimal equipment

• Field staff should require minimal training to be able to perform the tests

• Samples used in the tests should be easy to collect, handle, and transport.

• There should be a short turnaround time for results if the testing is done in a central laboratory.
COMMON TESTS

• Vitamins:
  Fat soluble: Vitamin A, D, E, K
  Water soluble: thiamine, riboflavin, niacin, vitamin C, folate, vitamin B12, vitamin B6

• Minerals: Iron, iodine, zinc, trace elements

• Serum Proteins (total, albumin, globulins, transferrin)

• Blood lipids, blood glucose, various enzymes implicated in heart disease, diabetes mellitus and other chronic diseases

• Inflammation markers

• Anti-nutrients: aflatoxins, other mycotoxins
### NHANES: FOLATE, VITAMIN B6 AND B12, VITAMIN C

<table>
<thead>
<tr>
<th>Nutrient or dietary bioactive compound and indicators measured</th>
<th>Why measured? Health impact</th>
<th>Recommended biochemical concentrations (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate</td>
<td>Deficiency causes macrocytic anemia; low folate status increases risk of NTDs and may modulate risk of chronic diseases (CVD, cancer, cognitive function)</td>
<td>Clinical deficiency:&lt;br&gt;- Serum folate &lt; 2 μg/L (21)&lt;br&gt;- RBC folate &lt; 95 μg/L (21) Functional deficiency:&lt;br&gt;- tHcy &gt; 13 μmol/L (33)</td>
</tr>
<tr>
<td>Serum folate</td>
<td></td>
<td></td>
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<tr>
<td>RBC folate</td>
<td></td>
<td></td>
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<tr>
<td>Plasma tHcy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B-6</td>
<td>Deficiency causes dermatitis, glossitis, confusion, anemia; low concentrations may modulate risk of chronic diseases (CVD, cancer, cognitive function)</td>
<td>Deficiency:&lt;br&gt;- PLP &lt; 20 nmol/L (14)</td>
</tr>
<tr>
<td>Serum PLP</td>
<td></td>
<td></td>
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<tr>
<td>Serum 4PA</td>
<td></td>
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<tr>
<td>Vitamin B-12</td>
<td>Deficiency causes macrocytic anemia and may cause neurologic abnormalities; low concentrations may increase risk of NTDs and may modulate risk of chronic diseases (CVD, cancer, cognitive function)</td>
<td>Deficiency:&lt;br&gt;- B-12 &lt; 200 ng/L (18) Functional deficiency:&lt;br&gt;- MMA &gt; 271 nmol/L (34)</td>
</tr>
<tr>
<td>Serum B-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma MMA</td>
<td></td>
<td></td>
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<tr>
<td>Vitamin C</td>
<td>Deficiency causes scurvy; has potential (in combination with vitamin E, zinc, β-carotene supplements) to slow progression of age-related macular degeneration</td>
<td>Clinical deficiency:&lt;br&gt;- Vitamin C &lt; 11.4 μmol/L (17) Low vitamin C concentrations:&lt;br&gt;- 11.4–23 μmol/L (17)</td>
</tr>
<tr>
<td>Serum ascorbic acid</td>
<td></td>
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</tbody>
</table>

NHANES: VITAMIN A, E, D, CAROTENOIDs

Vitamin A
- Serum retinol
- Serum retinyl esters (palmitate and stearate)

Vitamin E
- Serum α-tocopherol
- Serum γ-tocopherol

Carotenoids
- Serum carotenones and xanthophylls

Vitamin D
- Serum 25(OH)D

Deficiency may cause childhood blindness, night blindness, corneal thinning, and conjunctival metaplasia; low concentrations may impair immune function, growth, and development

Deficiency causes peripheral neuropathy

Good biological indicators of fruit and vegetable intake

Deficiency causes inadequate mineralization or demineralization of skeleton (rickets in children, osteomalacia in adults); low concentrations may affect muscle strength, risk of cancer or type 2 diabetes

Severe deficiency:
- Vitamin A <0.35 μmol/L (19)

Deficiency:
- Vitamin A <0.70 μmol/L (19)
- α-Tocopherol <11.6 μmol/L (20)

No defined serum concentrations

At risk of deficiency:
- 25(OH)D <30 nmol/L (16)

At risk of inadequacy:
- 25(OH)D 30–<50 nmol/L (16)

Sufficient:
- 25(OH)D 50–75 nmol/L (16)

Reason for concern:
- 25(OH)D >125 nmol/L (16)

NHANES: FATTY ACIDS, IRON, IODINE

Fatty acids
- Plasma SFAs
- Plasma MUFAs
- Plasma PUFAs

Association between SFAs and increased CVD risk; association between long-chain n3 PUFAs and decreased CVD risk; potential association between long-chain n3 PUFAs and improved visual and cognitive development in infants

Iron
- Serum ferritin
- Serum soluble transferrin receptor
- Serum body iron

Deficiency has negative effects on cognitive development among infants and adolescents and eventually causes anemia

No defined plasma concentrations

Depleted iron stores:
- Ferritin <15 μg/L (≥5 y) and <12 μg/L (<5 y) (22)
- Functional iron deficiency:
  - Body iron <0 mg/kg (15)

Iodine
- Urine iodine

Deficiency causes hypothyroidism, goiter, cretinism, growth and developmental abnormalities, and mental retardation

Population median urine iodine (13):
- Insufficient intake: <100 μg/L
- Adequate intake: 100–199 μg/L
- Above requirements: 200–299 μg/L
- Excessive intake: ≥300 μg/L

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Year test first done</th>
<th>No. of surveys including test</th>
<th>Population typically tested</th>
<th>Sampling method and equipment used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometric measurements</td>
<td>1987</td>
<td>147</td>
<td>Women aged 15–49 y; children aged 0 (6)–59 mo; men aged 15–59 y</td>
<td>Noninvasive, measuring board and scale</td>
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<tr>
<td>(weight, height, age)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hemoglobin (for anemia)</td>
<td>1995</td>
<td>78</td>
<td>Women aged 15–49 y; children aged 0–59 mo; men aged 15–59 y</td>
<td>Capillary blood, HemoCue portable analyzer³</td>
</tr>
<tr>
<td>HIV</td>
<td>2001</td>
<td>41</td>
<td>Women aged 15–49 y; men aged 15–59 y</td>
<td>Capillary blood, DBSs</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>1998</td>
<td>8</td>
<td>Women aged 15–49 y; men aged 15–59 y</td>
<td>Noninvasive, automatic cuff</td>
</tr>
<tr>
<td>Syphilis</td>
<td>1996</td>
<td>6</td>
<td>Women aged 15–49 y; men aged 15–59 y</td>
<td>Venous blood, RPR</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>1996</td>
<td>4</td>
<td>Women aged 15–49 y; children aged 0 (6 or 12)–59 mo</td>
<td>Capillary blood, DBSs, HPLC, RBP-EIA</td>
</tr>
<tr>
<td>Malaria</td>
<td>2006</td>
<td>3</td>
<td>Women aged 15–49 y; children aged 0 (6)–59 mo</td>
<td>Capillary blood, RDT, thick/thin slides</td>
</tr>
</tbody>
</table>

¹ Other, less-common tests: hepatitis B, hepatitis C, herpes, measles, tetanus, chlamydia, diabetes, lipids, C-reactive protein, transferrin. DBSs, dried blood spots; RPR, rapid plasma reagin; RBP-EIA, retinol binding protein enzyme immunoassay; RDT, rapid diagnostic test.

² Varies by country; the numbers in parentheses reflect the variation in ages in a typical DHS population (e.g., men aged 15–54 or 15–64 y; children aged 0–59 or 6–59 mo).

³ HemoCue AB, Angelhom, Sweden.
CHALLENGES

• Most common measurements for iron and vitamin A status are affected by presence of inflammation.

• Namaste SML et al on behalf of the BRINDA Working Group. Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *Am J Clin Nutr* 2017;106(Suppl):333S–47S.
IRON STATUS READING AFFECTED BY DEGREE OF INFLAMMATION