Chapter 3

Biochemical/Laboratory assessment

Presentation outline

- Introduction
- Types of laboratory methods
- Assessment of protein status

Presentation objective

- By the end of this session students will be able to:
  - Identify the types of lab methods
  - Explain about the assessment of protein status

3.1 Introduction

Laboratory method is

- Most objective and quantitative method of nutritional assessment
- Used primarily to detect sub-clinical deficiency states or to confirm a clinical diagnosis
- Main drawback is that several factors may confound interpretation of test results

3.2 Types of laboratory assessment

- Static biochemical tests
- Functional tests
  - Functional biochemical tests
  - Functional physiological tests
  - Functional behavioural tests

Static biochemical tests

- Measure
  - a nutrient in biological fluids or tissues or
  - the urinary excretion rate of the nutrient or metabolites
- Nutrient/metabolite in fluid
  - whole blood: Se; plasma: Zn, Cu
  - érythrocytes: folate; leucocytes: vit C, Mg
  - breast milk: vit A; I; saliva: IgA, IgG

Static biochemical. tests cont…

- Nutrient/metabolite in tissue
  - adipose tissue: fatty acids, vitamin E
  - liver: vitamin A, Fe, vitamin E
  - bone: Ca, Zn; hair: Zn, Se; nails: Se
- Nutrient/metabolites in urine
  - nutrients: B-1, B-2, vit C; Se, I

Static biochem. tests cont…

Hair and nails

- Hair: for Zn, Se, Cr (As, Hg)
- Nails: for Se
- Less invasive than blood
- Higher in hair and nails so easier to analyze than blood
- Stable, easily transported, and stored
- Chronic indices: especially toe nails chronic exposure
- Not subject to diurnal variation

Static biochem. tests cont…

Urine

- Useful for water soluble vitamins, some minerals (I, Se), & protein
- Requires a functioning kidney
- Assesses acute rather than chronic status
- Urinary output may be reduced before body stores are reduced
- Analyses on fasting morning, casual, or 24-hr collections
- Negative balance: urinary excretion may be increased despite depleted body stores

Functional tests

- Measure extent of functional consequences of a nutrient deficiency
- Greater biological significance than static tests
- Classified into:
  - Functional biochemical tests
  - Functional physiological tests
  - Functional behavioural tests

Functional biochemical tests

- Measure the extent of the functional consequences of a specific nutrient deficiency
  - greater biological significance than the static laboratory tests
- Includes:-
  - Abnormal metabolites in blood/urine: from a deficiency of nutrient dependent enzyme
  - Changes in enzyme activity: very sensitive
  - Changes in blood components
  - Load tests
  - Tolerance tests
  - *In vitro tests of in vivo function*

Functional bioch. cont…

Changes in enzyme activity

- Should reflect amount of nutrient available to the body and respond rapidly to changes in nutrient supply
- Should relate to pathology of deficiency or excess
- NB. Tests are often in RBCs: contain enzyme systems that depend on B-vitamin-derived cofactors
  - In vitro stimulation of the enzyme by coenzyme indicates the degree of un saturation of enzyme & measure deficiency

Functional bioch. cont…

- **Changes in blood components**
- Measure blood components related to intakes of specific nutrients
  - Hemoglobin: iron deficiency anemia
  - Plasma Ferritin: level of iron store
  - Plasma transferrin receptor : tissue iron
  - Plasma retinol binding protein: vit A
  - Plasma transthyretin: iodine

Functional bioch. cont…

Load Tests

- Used to detect deficiencies of:
  - water soluble vitamins; C, B-6
  - minerals: Mg, Zn, Se
- Measure basal level of nutrient / metabolite on timed preload urine
- Give oral / intravenous (IV) / intramuscular dose of nutrient / metabolite
- Measure nutrient/metabolite on timed post load dose from urine
- Calculate net retention
  - In a deficiency state, when tissues are not saturated with the nutrient, excretion of the nutrient or metabolite will be low because net retention is high

Functional bioch. cont…

Tolerance tests

- Used to detect deficiencies of :
  - Zn, Mn, Cr, Vit A
- Measure basal level of nutrient in plasma
- Measure nutrient in plasma post-dose after specified time
- Assess increase in plasma nutrient level post dose
  - enhanced response in plasma in a deficiency due to increased intestinal absorption of nutrient

Immune function tests

- Assess total T-lymphocytes
- Assess lymphocyte function
- NB: T-cells in blood will be reduced during PEM

Functional physiological tests

- Growth responses
- Morbidity
- Appetite
- Developmental responses
- Night vision threshold test
- Sleep behavior

Functional behavioral tests

- Cognitive function
  - Infant Development
  - Visual recall tests
- Mood

3.3 Assessment of protein status

- Protein is important for
  - Maintenance and growth of body tissue
  - Formation of essential body compounds such as Enzymes, Hormones, Transportation, Antibodies
  - Regulation of body processes: Fluid balance, Acid-base balance
  - Source of energy
- Protein deficiency results in protein energy malnutrition

Assessment of protein status

- Assessment of somatic protein (skeletal muscle protein) status
  - Urinary creatinine excretion
  - Excretion of 3-methylhistidine
- Assessment of visceral (internal organs, blood cells, serum) protein status
  - Total serum protein
  - Serum albumin
  - Serum transferrin
  - Serum retinol binding protein
  - Serum transthyretin

- **Assessment of protein status cont…**
- Immunological tests
  - Lymphocyte count
  - Cytokine production
- Metabolic changes as indices of protein status
  - Plasma amino acid ratio
  - Urea nitrogen: creatinine ratios

Serum Albumin

- Major serum protein
  - Synthesized in liver
  - Maintains serum osmolarity
  - Serum carrier of small molecules
- Most common indicator of depleted protein status
- Poor indicator of early protein depletion and repletion
- Levels affected by rate of synthesis (liver disease may reduce levels)
- May reflect level of physiological stress
  - Decreased during acute catabolic phase

- Levels affected by abnormal losses
  - thermal burns – losses at burn site
  - nephrotic syndrome – losses in urine
  - protein-losing enteropathies – losses in feces
- Levels affected by fluid status
  - congestive heart disease & fluid overload
    - Reduced due to dilution
  - Dehydration
    - Increased due to concentration effects
- Body pool size: 3-5g/kg body weight

Serum Trasferrin

- Function: transport protein for iron
  - better index of changes of protein status
- Body pool: <0.1g/kg body weight
- Influenced by other factors
  - Increased with iron deficiency
  - Increased during pregnancy, estrogen therapy
  - Reduced in protein-losing enteropathy, nephropathy, acute catabolic stress
- Limited usefulness in protein status assess

Retinol Binding Protein

- Function: carrier for retinol
- Very rapid turnover (12 hours), very small body pool(0.0002g/kg body wt)
  - may be too sensitive and complicates precise measurements
- Influencing factors
  - liver disease, hyperthyroidism, deficiency of vitamin A and Zn status : decrease it
  - chronic renal failure: increase
- Used to assess acute depletion

Presentation outline

- Assessment of iron status
- Assessment of vitamin A

Objective

- By the end of this session students will be able to explain about the assessment methods for iron and vitamin A

3.4 Assessment of iron status

- Iron essential for synthesis of haem - a component of haemoglobin (Hb) - the oxygen-carrying pigment of RBC's
- In iron deficiency Hb synthesis is reduced resulting in:
  - Hypochromic (pale colour) RBC's - low [Hb]
  - Microcytic anaemia - small RBC's

Definitions (WHO,2005)

- **Anemia**
  - Clinical term: insufficient mass of RBCs circulating in blood
  - Public Health term: Hb < WHO / UNICEF / UNU thresholds (set at 5th percentile)
- **Iron deficiency**
  - Insufficient iron to maintain normal physiological function of tissues: blood, brain, muscles
  - Can occur in absence of low hemoglobin

Definitions (WHO,2005) cont…

- **Functional iron deficiency**
  - may exist even when iron stores present b/c system for mobilizing iron & transporting it to target tissues is impaired (inflammation from infection)
  - iron supplementation has no benefit in these circumstances
  - vitamin A deficiency (Fe stores adequate): impairs mobilization of Fe from liver stores

Development of iron-deficiency anemia

- **STAGE 1 Iron depletion**
  - Decrease in storage iron in liver
  - Supply of iron to functional compartment is not compromised
  - Level of transport iron & hemoglobin is normal
  - There will be fall in serum Ferritin concentration

- **Development of iron-deficiency anemia cont…**
- **STAGE 2 Iron deficient erythropoiesis* (IDE)**
  - reduced supply of circulating iron for erythropoiesis
    - Indicated by decrease in transport iron
    - Increase in tissue iron receptors
  - signs of iron deficiency in circulating erythrocytes

Assessment of iron status cont…

- **STAGE 3 Iron deficiency anemia**
  - microcytic hypochromic anemia
  - decrease in Hb in RBC’s
- *IDE may occur despite normal or even increased storage iron due to impaired release of iron to plasma due to inflammation or malignancy

Indices of iron status

- **Hematological indices**
  - Hemoglobin, Hematocrit
  - Red cell indices; Red cell distribution width
- **Indices of iron supply**
  - Serum iron; Total iron binding capacity
  - Transferrin saturation (as %)

Indices of iron status cont…

- **Supply of Fe to RBC's**
  - Zn protoporphyrin
- **Index of size of iron stores**
  - Serum ferritin
- **Index of tissue iron deficiency**
  - Soluble transferrin receptor
- **Assessment of total body iron**
  - based on serum ferritin & transferrin receptor

Hemoglobin

- Hb widely used to screen for iron deficiency
- Normal values vary with age, race, sex, smoking, altitude
- Hb has low sensitivity
  - Only falls when subjects are anemic
  - have lost 20 – 30% of their body iron stores
- Hb is non-specific
- Many other causes of anemia besides iron deficiency
  - B-12 deficiency; folate deficiency; vitamin A deficiency
  - Infections: malaria; bacterial overgrowth

Serum Ferritin

- Most useful measure of iron status:
  - High specificity: low value only diagnostic of IDA in patients with anemia
- Serum ferritin only measure that can reflect deficiency, excessive, or normal iron status
- Serum ferritin levels reflect amount of storage iron
  - Normal adults: I µg/L serum ferrittin = 8-10 mg body iron or 120 µg storage Fe/kg body weight
- When stores exhausted, serum ferritin no longer reflects severity of ID

Serum ferritin cont…

- Serum ferritin levels affected by:
  - Age, sex; race; infection/ inflammation; liver disease
  - Decreased erythropoiesis: B-12 or folate def: normal or increased; certain genetic Hb disorders: increased
  - Increased erythropoiesis: decline in Fe store: used for Hb synthesis: levels decreased

Serum ferritin cutoffs (µg/L)

Iron stores	< 5 y	< 5 y	≥ 5 y	≥ 5 y
	Male	Female	Male	Female
Depleted iron store	<12	<12	<15	<15
Depleted iron store in presence of infection??	<30?	<30?	-	-
Risk of iron overload	-	-	>200 (adult)	.150 (adult)

Note: Serum ferritin is of limited value during later pregnancy due to false positives from dilution (WHO, 2001)

Recommended indices to identify iron deficiency

1. **Hemoglobin** (measure of anemia)
2. **Mean cell volume** (measure of cell size)
   - distinguishes between microcytic (IDA) or macrocytic (B-12/folate) RBC's
3. **Zn protoporphyrin** (measure of iron supply in last stages of Hb synthesis)
   - Zn inserted into Hb molecule instead of Fe; measure of *severity of ID*
   - *A product of disordered heme biosynthesis*

Recommended indices to identify iron deficiency cont …

4. Transferrin receptor

(measures intensity of erythropoiesis & iron demand)

- marker of severity of iron insufficiency but *only when Fe stores are* exhausted & there are no other causes of erythropoiesis
- less affected by inflammation than serum ferritin

Recommended indices to identify iron deficiency cont …

5. Serum ferritin (measure of iron stores without infection)

- level >15 ug/L iron stores present;
- level <12-15 ug/L depleted stores
- in infection, ferritin increases despite low iron stores

Uses of iron status indicators

- Individual level
  - Screening for ID and IDA
  - Clinical assessment
- Population level
  - Prevalence estimates of deficiency
  - Evaluating the impact of interventions

How to screen for iron deficiency at the individual level using Hb + ferritin

- STAGE 1 Iron depletion (low iron stores)
  - serum ferritin < 15 µg/L > 12 µg/L
- STAGE 2 Iron deficient erythropoiesis (exhausted iron stores)
  - serum ferritin < 12 µg/L; normal Hb
- STAGE 3 Iron deficiency anaemia
  - serum ferritin < 12 µg/L: Hb < 120 g/L adults; Hb < 110 g/L children

How to assess iron status in clinical patients using multiple indices

- **STAGE 1**
  - Low iron stores serum ferritin <20> 12-15 µg/L
- **STAGE 2** Fe-deficient erythropoiesis
  - serum ferritin <10-12 µg/L
  - Zn protoporphyrin >70-80 µg/dl RBCs
  - transferrin saturation <15%

- **How to assess iron status in clinical patients using multiple indices cont…**
- **STAGE 3** Fe-deficiency anemia: microcytic, hypochromic RBC’s
  - low hemoglobin; low hematocrit
  - low mean cell volume <67-81 femto liter (fL):
    - microcytic RBC’s

- **How to assess prevalence of ID or IDA in populations**

1. Select cutoff/reference limit for each index
   - Age, gender, pregnancy, ethnicity
2. Consider major confounders in data interpretation

- Inflammation/Infection
  - Decreased erythropoiesis, Genetic disorders
  - Other possible confounders: assay used; method of blood collection; fasting status/time of day; medication use etc

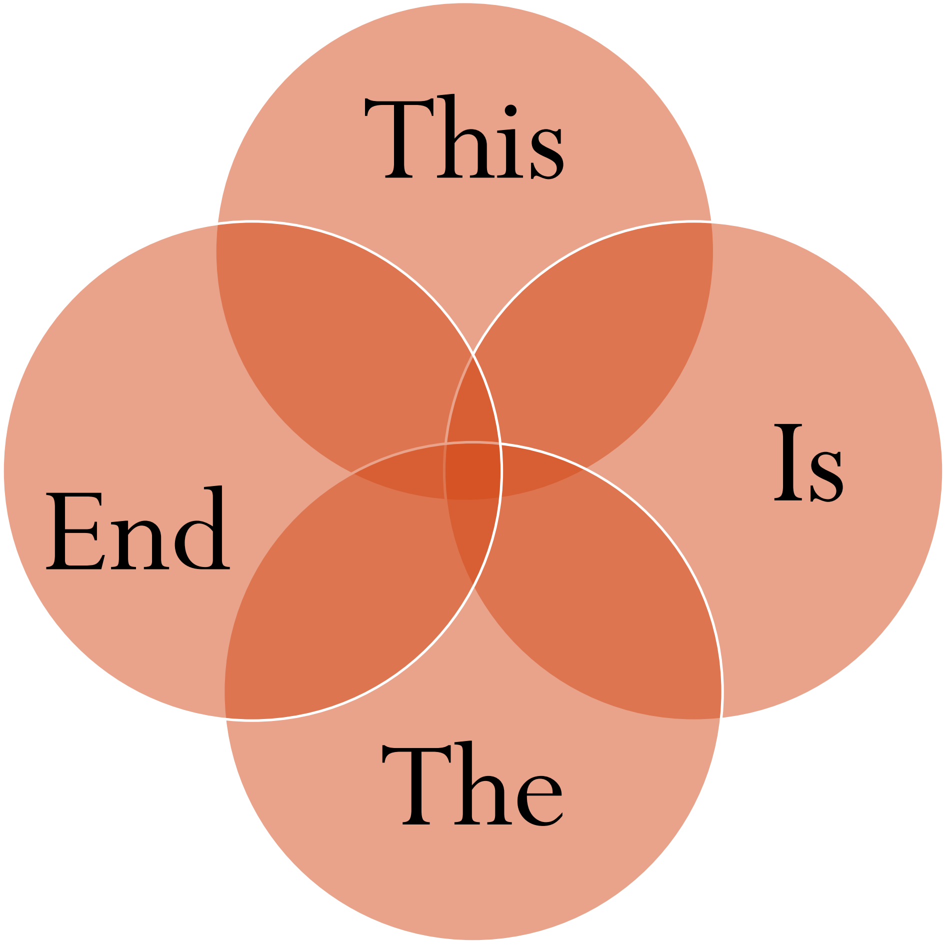
3. Use a multi-parameter index
   - Minimizes misclassification with single measure
   - No consensus on best combination to use

Recommended indices to evaluate iron interventions

- Serum ferritin**
  - When SF improves (even in presence of inflammation) but Hb does not, other factors in addition to iron likely to be cause of anemia
  - evaluation in intense malarial areas is difficult
- Hemoglobin**
  - may increase more rapidly than serum ferritin when IDA is high

Recommended indices to evaluate iron interventions cont…

- Transferrin receptor: expensive
- Estimation of body Fe stores in adults: no cutoff values needed
- Monitor iron status in one at risk subgroup for ~ 12 mos
  - infants; menstruating women; pregnant women
- ** Plus measure of infection (AGP). Standardization of all methods & quality control essential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3.5 Assessment of vitamin A status

Functions of vitamin A

- Role in vision
  - Retinal tissue deprived of vitamin A: impairs rod and cone function
- Required for integrity of epithelial cells throughout body
- Role in embryonic development
- Role in immune function

IVACG: Definition of vitamin A deficiency disorders

- Any health & physiological consequences attributable to vitamin A deficiency, irrespective of whether there is any clinical evidence of deficiency

- IVACG: International Vitamin A Consultative Group

- **Definition** includes clinical manifestions & functional consequences
- Clinical manifestions
  - Xeropthalmia
  - Anemia
  - Growth retardation
  - Increased infectious mortality & morbidity
- Functional consequences:
  - impaired iron mobilization
  - disturbed cellular differentiation
  - depressed immune response

Assessment of vitamin A status

- Blood
  - Retinol
  - Retinol binding protein (RBP)
  - RBP: Transthyretin (TTR) ratio
  - RDR(relative dose response) test, MRDR (modified relative dose response) test
- Breast milk: Retinol
- Liver Isotopic tests: measure liver store
- Functional Tests
  - Subjective assessment of night blindness
  - Pupillary and visual threshold test
  - Conjunctival impression cytology with transfer

- **Biochemical assessment of vitamin A**
  - Serum/plasma retinol
  - Retinol binding protein (RBP)
  - RBP : transthyretin ratio
  - Breast milk retinol

Sensitivity of plasma retinol as an indicator of liver vitamin A

- Serum retinol reflects vit A status when liver stores are very depleted (< 0.7) or very high >1.05 umol/g)
- In b/n these liver vit [A], serum retinol levels in adults are homeoistatically controlled
- In non-deficient populations +ve correlation b/n serum & usual vit A intakes *rare*

Factors affecting serum retinol levels

- Liver vitamin A stores
  - 0.7 -1.05 μmol/g: serum retinol homeostatically controlled
  - <0.7 μmol/g: serum retinol falls; >1.05 μmol/g: serum retinol rises
- Age: increase with age
- Sex : adult males: higher values than females

Factors affecting serum retinol levels cont…

- Low fat diets: (~< 5 to 10 g daily)
  - impair absorption of provitamin A carotenoids: lower plasma retinol
- Zinc deficiency: lowers serum retinol, impairs synthesis of RBP (retinol binding protein)
- Infection/inflammation: lower serum retinol
  - suppresses mobilization of vitamin A from liver due to down-regulation of RBP synthesis

Use of retinol binding protein

- Serum RBP is a transport protein for vit A
- +ve correlation b/n RBP & retinol in serum
- If liver vit. A depleted, in case of late stage of vit. A deficiency, RBP will be stored in the liver
  - Both serum retinol & RBP will decline

Use of retinol binding protein cont…

- Factors affecting retinol also influence RBP
- Sample collection for RBP easier; assay cheaper; RBP protein is more stable than retinol
- Can be assayed from blood spots

Breast milk retinol

- Reflects level of maternal dietary intake & body stores of vitamin A
- Collect samples after first month: levels very high in
  - colostrum &
  - transitional breast milk (BM)(7-21days postpartum)

Breast milk retinol cont…

For individuals:

- BM sample from full breast best as [retinol] varies during feed: not been used to feed the child for at least 2hrs
  - Lowest in the 1st milk expressed
- OR collect 8-10 mL from full breast before feed
  - collect manually or with breast pump

Breast milk retinol cont…

For populations:

- Collect casual BM samples randomly during day varying times after infant fed
  - Will ensure the variation in milk fat is randomly sampled
- Express BM [retinol] per gram milk fat
- NB: this method not recommended when there is a wide range in stage of lactation among study women

WHO (1996) Multiple indices of vitamin A status for children 6-71 mos

	Prevalence below cutoffs to define a public health problem and its level of importance		
Indicator (cutoff)	Mild	Moderate	Severe
Night blindness present	< 1%	>1% to 5%	>5%
Serum retinol <0.70 umol/L	>2% to <10%	>10% to <20%	>20%
Breast milk retinol <1.05umol/L OR <0.028 umol/g milk fat	<10%	>10% to <25%	>25%

Presentation outline

- Assessment of zinc status
- Assessment of iodine
- Assessment of vitamin B_{12} and folate

Objective

- By the end of this session students will be able to explain about the assessment methods for iron and vitamin A

3.6 Assessment of zinc status

Functions of zinc

- > 300 enzymes require Zn for function or regulation
- Biosynthesis of nucleic acids, amino acids, proteins, including specific hormones
  - insulin; adrenal corticosteroids; testosterone
- Growth
- Immune function
- Vitamin A metabolism: night vision
- Reproduction
- Neuro-behavioural function

Diagnosis of zinc deficiency is difficult b/c

- Tissue zinc concentrations change very little even in severe zinc deficiency
- Clinical features of zinc deficiency are non-specific
- No agreement on a single sensitive & specific laboratory index

How can we identify risk of zinc deficiency in populations?

1. Dietary indicator

- Percent with Zn intakes below Estimated Average Requirement (EAR)
- High risk: public health concern > 25%

2. Biochemical indicator

- Percent with low serum Zn concentrations
- High risk: public health concern > 20%

3. Functional indicator

- Percent of children < 5 y with HAZ score < -2.0 SD
- High risk: public health concern > 20%

Biochemical indicators of Zn status

- **Serum Zn:** recommended by WHO for populations
- **Hair Zn:** useful for children; uncertain for adults?
- **Cells:** leucocytes; neutrophils; erythrocytes
- **Zn metalloenzymes**
  - alkaline phosphatase; ecto purine 5’nucleotidase in lymphocytes

Biochemical indicators of Zn status cont …

- **Zn-binding proteins:** metallothionein in RBCs
- **Molecular techniques**
  - metallothionein mRNA in monocytes/ erythrocytes
- **Kinetic markers:** pool sizes & turnover rates

NB: Most biomarkers only useful in: severe or prolonged Zn deficiency; in experimentally settings Usefulness in community studies is questionable

Why use serum Zn as an indicator of Zn status?

- It reflects dietary Zn intake
- It responds consistently to zinc supplementation
- Reference data are available for most age & sex group
- To date, serum Zn is the only biochemical indicator to meet these criteria
  - If > 20% of population subgroup with serum Zn below cutoff, then subgroup considered at risk of Zn deficiency
- NB: Does not necessarily reflect *individual Zn status*

Technical and biological factors affecting serum/plasma Zn

- Adventitious contamination
- Refrigeration of blood after collection
- Length of time prior to blood separation: increase serum Zn
- Hemolysis: increase serum Zn

Technical & biological factors affecting serum/plasma Zn cont...

- Fasting / non-fasting status
  - Increase immediately after meal, decline progressively during the next 4hr, rise again until food is eaten
- Diurnal: higher in the morning
- Infection/stress: low
- Age: low in childhood, reaching a peak in adolescence & young adulthood, then it decline

- **Technical & biological factors affecting serum/plasma Zn cont…**
- Sex: during infancy & early childhood: lower in boys; after adolescence higher in males
- Use of oral contraceptives agents (OCA) & hormones steroids: decrease
- Physiological state: decrease during pregnancy
- Concurrent nutrient deficiencies (protein; Vit A)
- Disease states: e.g. marasmus: decrease

Practices to eliminate adventitious Zn contamination

- Use powder-free disposable gloves
- Process samples in laminar flow hood or clean, dust-free lab
- Pre-screen anticoagulants so Zn free
- Use distilled, deionized water; hyper-pure acids
- Cover all equipment and materials to avoid dust

Hair Zn

Advantages of using hair Zn

- Less invasive than blood
- No special storage conditions
- Easily transported and shipped
- Concentrations higher than serum Zn
- Easier to analyze
- More stable: not affected by diurnal/circadian variation; infection; OCA use
- Low hair Zn indicative of chronic Zn deficiency in childhood
- **NB** No established cutoffs for most age groups

Factors affecting hair zinc

- Age
- Sex: boys have lower
- Rate of hair growth: not indicative in case of severe malnutrition
- Hair beauty treatment
- Adventitious contamination
- Season

Relationships of hair Zn with other indices of Zn status

- Dietary indicators: -ve relationship with: phytate : Zn molar ratios
- Growth
  - Height for age percentiles: +ve relationship
- Taste acuity: +ve relationship
- Serum Zn: relationship rarely seen
  - Hair Zn: chronic index; Serum Zn acute

Functional indicators: response to Zn supplementation

- Physical growth
- Immune function
- Rates of infection
  - diarrhea
  - respiratory infection
- Physical activity
- Cognitive function
- Appetite

Disadvantages of functional indicators

- Long delay for response to be detectable
- Expensive studies
- Compliance to supplements essential

Functional indicators: growth

- **Height- or length-for-age related to risk of Zn deficiency**
- Standardized methods exist for measurement
- Reference data are available
- Often included in national and nutrition monitoring activities

Functional indicators: growth

- Preferable to wt-for-age
  - because linear growth is the primary response to increased Zn intake;
  - weight gain likely occurs as a result of increased linear growth
- Non-specific
- Limited to children

3.7 Assessment of iodine status

Functions of iodine

Component of thyroid hormones:

- Required for normal growth & development
- Myelination of central nervous system in perinatal period, fetal, & early postnatal development
- Regulate basal metabolic rate & macronutrient metabolism

Assessment of iodine status

- Thyroid size by neck palpation
- Thyroid volume by ultrasonography
- Urinary iodine concentration
- Thyroid stimulating hormone (thyrotropin)
- Blood thyroglobulin
- Serum thyroxine (T4) & tri-iodothyronine (T3)

Nepalese women with goitre

In iodine deficiency, thyroid gland is enlarged

Thyroid enlargement known as Goiter: most visible sign of iodine deficiency

Goiter prevalence: reflects past not present iodine status

Thyroid size

- Goitre prevalence in children
  - important indicator for assessing iodine deficiency disorder (IDD) in populations
  - but there is a lag-time in goiter response
- ↓ in response to ↑ in iodine intake
- Not return to normal for months or years after correction of iodine deficiency
- During this transition period,
  - goiter rate is difficult to interpret
    - reflects both a population’s history of iodine nutrition & its present status

Measurement of thyroid size & volume

Palpation: thyroid size

- Poor sensitivity & specificity for mild iodine deficiency
- High misclassification in estimating goitre: inter-examiner error for mild goiter; better for moderate to severe IDD
- Cheap, easy, but personnel must be well trained

Simplified classification of goiter

Grade 0	No palpable or visible goiter
Grade 1	A mass in the neck that is consistent with an enlarged thyroid that is palpable but not visible when the neck is in a normal position. It moves upward in the neck when the subject swallows. Nodular alteration(s) can occur even when the thyroid is not visibly enlarged
Grade 2	A swelling in the neck that is visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated

Thyroid is goitrous when each lateral lobe has volume greater than terminal phalanx of thumb being examined. Neck palpation suitable for children 6-12 y; pregnant; lactating women but *NOT* infants and younger children

From WHO/UNICEF/ICCIDD (1994)

RSG

Measurement of thyroid size & volume cont…

Ultrasonography: thyroid volume

- More precise & objective
- Non-invasive, feasible in remote areas using portable equipment
- Useful in areas of mild to moderate iodine deficiency, where goiters are small
- Interpretation of data requires valid reference criteria from iodine-sufficient populations

Measurement of thyroid size: Ultrasonography

- Useful for measuring grade 1 goiter
- Employs sound frequencies
- Impulse applied to neck by device that transmits signal & receives reflections
- Ultrasound penetrates skin surface & through tissues; certain portion of sound reflected back
- Safe, noninvasive, portable but need trained Ultrasonographers

Epidemiological criteria for assessing the severity of IDD based on prevalence of goitre in school-age children

Severity of IDD	Prevalence of goiter (TGR) (%)
Iodine sufficiency	< 5%
Mild	5.0 – 19.9
Moderate	20.0 – 29.9
Severe	$\geq$ 30

A sustained salt iodization program will decrease goiter rate by ultrasound to < 5% in school-aged children. This indicates disappearance of iodine deficiency as a significant public health problem

TGR – Total Goitre Rate = Grades 1+2 From WHO/UNICEF/ICCIDD (1994)

RSG

Urinary iodine

- Daily urinary excretion reflects recent iodine intake

Urinary iodine in populations

- Urinary iodine can be expressed as: concentration µg/L fasting sample & 24-hour excretion (µg/day)

Urinary iodine in populations cont…

- UI in spot urine samples from representative sample of target population (expressed as median, in ug/L)
- WHO recommend > 300 casual urines from a population to diagnose endemic IDD

Epidemiological criteria for assessing severity of iodine deficiency disorders based on *median urinary iodine levels*

Median value (µg/L)	Severity of IDD
<20	Severe IDD
20 – 49	Moderate IDD
50 – 99	Mild IDD
100 – 199	Optimal
200 – 299	Risk of iodine-induced hyperthyroidism
>300	Risk of adverse health consequences

Urinary iodine in individuals

- Individual iodine intakes and therefore spot UI concentrations highly variable from day-to-day
  - Cannot use spot urine
- Preferable to obtain 24-h urine for individuals
- Urine collected in polyethylene tubes with screw tops; can be kept in fridge for months or frozen indefinitely

Epidemiologic criteria for assessing I status in population of pregnant women via UI*

Median urinary iodine	Iodine intake
< 150 µg/L	Insufficient
150 – 249 µg/L	Adequate
250 – 499 µg/L	More than adequate
≥ 500 µg/L	Excessive

3.8 Assessment of B-12 and folate status

- **Why do we need folate and vitamin B-12?**
- Both involved in DNA synthesis
- Elevated in total plasma homocysteine levels
  - due to interruption in methylation cycle
- B-12 also involved in synthesis of myelin in nervous system

Assessment of folate & B-12 status

Laboratory tests

- Quantity in serum, RBCs, liver
- Biochemical function
  - Serum methylmalonic acid: specific for B 12 deficiency
  - Serum homocysteine
  - Serum cystathionine

Morphology

- Size and shape of RBCs
- Hypersegmented neutrophils
- (> 5% with more than five lobes)

Assessment of folate status

- **Stage 1**: negative folate balance
  - serum folate < 6.8 nmol/L; RBC folate normal
- **Stage 2**: folate depletion
  - serum folate < 6.8 nmol/L; RBC folate < 363 nmol/L
- **Stage 3**: folate deficient erythropoiesis
  - serum folate <6.8 nmol/L: RBC folate <272 nmol/L
  - liver folate < 2.7 nmol/g; hypersegmented neutrophils (>5%>5 lobes)

Assessment of folate status cont…

- **Stage 4**: folate deficiency anemia
  - RBC folate < 227 nmol/L; liver folate < 2.3 nmol/g
  - Hypersegemented neutrophils (> 5% with > 5 lobes)
  - Low hemoglobin; high mean cell volume (MCV); macro-ovalocytic RBCs
  - Clinical features

Assessment of B-12 status

- **Stage 1**: negative balance
  - Low serum holotranscobalamin (TC)II
- **Stage 2**: B-12 depletion
  - Low serum TC II; serum B-12 < 150 pmol/L
- **Stage 3**: B-12 deficient erythropoiesis
  - Low serum TC II ; serum B 12 < 100 pmol/L
  - B-–hypersegemented neutrophils (> 5% with > 5 lobes)

Assessment of B-12 status cont…

- **Stage 4**: B-12 deficiency anemia
  - Low serum TC II; B-12 < 59 pmol/L
  - hypersegemented neutrophils (> 5% with > 5 lobes)
  - Elevated serum/urine methylmalonic acid
  - Low hemoglobin; high MCV; macro-ovalocytic RBCs
  - Clinical features + neurological