Prenatal screening of Down‘s syndrom in the first and second trimesters of pregnancy

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Down’s Syndrome

- Down’s syndrome (DS) is a congenital disorder, caused by a trisomy of chromosome 21
- First described 1866
  JLH Down
- ~1 in 900 births in Czech Republic
- risk increases with the mother’s age
Downs Syndrome - Trisomy 21

- **Clinical Features**
  - Average life expectancy 30 years
  - Characteristic phenotype
  - Learning disability (IQ 20-60)
  - Developmental delay / Hypotonia
  - Delayed puberty / Early menopause

- **Major Causes of Morbidity & Mortality**
  - 96% portal tract anomalies / Duodenal atresia
  - 50% congenital cardiac lesions
  - 60% Pre-senile dementia
Incidence of Down’s Syndrome

- majority of babies (95%) are born to women under 35 years of age
- majority of DS babies (80%) are born to women under 35 years of age
- need mass screening programme for low-risk group
## Down’s Syndrome and maternal age

<table>
<thead>
<tr>
<th>Maternal age at birth</th>
<th>Risk of Down’s syndrome</th>
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<tbody>
<tr>
<td>24</td>
<td>1 in 950</td>
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<tr>
<td>30</td>
<td>1 in 680</td>
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<tr>
<td>36</td>
<td>1 in 210</td>
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<tr>
<td>42</td>
<td>1 in 40</td>
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Age Distribution of Risk
Testing for Downs Syndrome

- no screening test capable of detecting parental predisposition for a Down’s syndrome birth

- earlier methods used direct fetal testing, by invasive tests (e.g. amniocentesis)

- amniocentesis not suitable for mass screening programmes

- amniocentesis can cause fetal abort
Testing for Downs Syndrome

- indirect fetal testing, by biochemical maternal serum screening, was introduced
- maternal serum screening does not detect specific marker
- multiple biochemical markers used to calculate risk
- software packages available to calculate risk
- can screen in first or second trimester
Second trimester risk factors

- maternal age
- serum AFP
- serum total hCG
- unconjugated oestriol (uE3)
Scheme of distribution positivity in II. trimester

- Positivity of DS
- Positivity of NTD
- Truly affected pregnancy
- Negative results of screening
Variation with Gestational Age

Marker level (IU/L)

Week of gestation

AFP
hCG
MoM (multiple of median)

- *result reported strictly as multiple of median*

- MoM’s vary with gestational age
- MoM’s vary with assay method
- MoM’s vary with population tested
- May need adjustment for:
  - weight
  - ethnic group
  - other conditions e.g. diabetes
  - twin pregnancies
Maternal serum Alpha-fetoprotein (AFP)

- Glycoprotein of fetal origin.
- Synthesized initially in embryonic yolk sac & then by fetal liver
- Maternal serum concentration maximal at 30 weeks gestation
- Maternal serum AFP is lower in DS pregnancies
- Geometric mean MoM is 0.74
- also useful marker of neural tube defects (NTD)
- Maternal serum AFP is elevated in NTD pregnancies
AFP Distribution curves

Maternal Serum AFP MoM

Down's syndrome

Unaffected
Serum human chorionic gonadotrophin (hCG)

- Dimeric glycoprotein hormone (α & β subunits) secreted by the fertilised ovum and later by placental tissue.

- Primary function is to maintain the corpus luteum, later produces Prog & Oest to maintain early pregnancy.

- Maternal serum hCG maximal during first trimester, then declines during second trimester.

- Maternal serum hCG is elevated in DS pregnancies.

- Geometric mean MoM is 2.03
hCG Distribution curves

Maternal Serum hCG MoM

Unaffected

Down's
Unconjugated Oestriol (uE3)

- Derived from fetal adrenal DHEAS. Latter hydroxylated in fetal liver & cleaved by steroid sulphatase in placenta where the unconjugated fraction converted to uE3

- Low levels uE3 + hCG can detect Edward’s Syndrome

- Low levels seen in maternal serum from Down’s syndrome

- circadian rhythm; levels 15% lower in the morning

- no advantage over double test (AFP/hCG)
Double or triple test?

- uE3 – very unstable
- interfere of lipaemia
- different quality of diagnostics sets
- growth of positivity don‘t comport with recovery
- long-term comparision showed irresponsibility results of triple test in our conditions
Detection rates double v triple test

- Spencer 1992: Double: 50%; Triple: 50%
- Cheng 1993: Double: 82%; Triple: 86%
- Reynolds 1993: Double: 55%; Triple: 55%
Importance of gestational dating

- exact gestational dating is essential to calculate risk of DS
- measured value standardised against expected median value for a normal population at same stage of gestation
- DS foetuses are relatively retarded compared to normal
- results in alteration of maternal serum concentration of foetal products e.g. AFP, hCG
- serum concentrations related to gestational age
Complex Data Required

- Accurate measure of gestational age - US
- Accurate demographic details
  - maternal age
  - specimen date
  - details of other conditions
  - fetal numbers

Sophisticated software essential
NTD

- NTD (Neural tube defects) can affect 1 in 500 infants

- Commonest forms of NTD known as anencephaly or spina bifida

- Neural tube beneath the backbone fails to develop

- Definitive diagnosis relies on amniocentesis

- High levels of AFP (Alpha-fetoprotein) seen in NTD

- Amniocentesis is costly and time-consuming

- Miscarriage rate of 1:200
First Trimester Screening

- Determining the overall risk of Down’s syndrome in the unborn.
- First Trimester Screening determines how specific quantities of Free $\beta$ HCG and PAPP-A in one specimen compare to the mediums of a population database.
- Measured at 10-13 completed weeks (70-97 days)
- Calculation of the Multiple of Median (MoM).
Biochemical Markers - 1st Trimester

- Pregnancy Associated Plasma Protein A (PAPP A)
- Free ßhCG

- Used with the ultrasound marker - Nuchal Translucency (NT)
- Gold Standard test for Trisomy, karyotyping of foetal cells, using standard colcemid induced metaphase chromosome visualisation
Free β hCG

- Free β chain of Human Chorionic Gonadotropin. Very high in the early stages of the first trimester, declines in the late first trimester.
- Free βhCG higher in Down’s Pregnancies during I. trimester screening period.
- Free β hCG is not stable in blood samples. *This is a serious disadvantage*, as blood samples sent to screening centres may be unseparated for 24 hours or more.
Free Beta-hCG

Alpha Subunit

Total HCG

Beta Subunit
Free Beta hCG Assay Specificity

FTB11 Ab

Labelled Ab

Beta Subunit
Pregnancy Associated Plasma Protein A (PAPP A)

- PAPP-A - Pregnancy associated Plasma Protein-A. Placental protein which continues to increase during the term of the pregnancy.
- Homotetrameric glycoprotein synthesized in chorionic villi.
- Specific and potent inhibitor of granulocyte elastase.
- Serum levels lower in Down’s pregnancies in 1st T screening period.
On average, baby with trisomy 21 will have 2.0 MoM for B-hCG and 0.4 MoM PAPP-A.
MoM CALCULATION.

- Multiple of the Median (MoM) for both Free $\beta$ HCG and PAPP-A are calculated independently. Represent the deviation from the theoretical “normal” or medium value for a particular point during the gestation.
- A database is maintained containing raw patient data at specific times during the gestation. This represents the basis for the MoM calculation.
- Patient data is subsequently used to expand the database. Extreme low or high maternal weight affects the MoM calculation.
- Gestational age is best determined by Ultrasound.
Nuchal Translucency (NT)

- Ideally performed between 11 & 13 weeks (10+2-14+6 FMF).
- NT thickness is a measure of the amount of fluid at the back of the fetal neck.
- 3 measurements to the nearest 0.1 mm are advised.
- The thickness is higher in Down’s pregnancies during screening period.
Measuring of NT
Foethus with DS
Risk calculation

- Nuchal Translucency (NT) measurement. (NT = Accumulation of fluid at the foetal neck, determined by ultrasound).
- Maternal age + NT account for 80% overall risk
- Maternal age + NT + Biochemistry 88-90%.
MoMs that typically yield a high risk of Down’s are those where, in combination, the Free $\beta$ HCG MoM is > 2.5 and the PAPP-A is < 0.4

HIGH RISK- Woman > 35 years of age, with a NT of > 2.0 mm, and Free $\beta$ HCG MoM >2.5 and PAPP-A MoM < 0.4

LOW RISK - Women < 35 years of age, with a NT of < 2.0 mm, combined with a Free $\beta$ HCG and PAPP-A MoM of 1.0.

Further tests required at an overall risk of 1:250
Advantages of 1st Trimester Screening

- Information earlier, more options
- Reduce number of invasive procedures
- May identify other severe anomalies (or risk for) at time of scan and increased risk of adverse pregnancy outcome—referral for 2nd Δ evals.
- Good time to date pregnancy accurately
- NT good for multiple gestation
Markers in Pregnancy

Marker Utility Window

- Free Beta
- Nuchal Transl
- PAPP-A
- AFP
- Unconj. Estriol
- Intact hCG

Gestational Age:

8 10 12 14 16 18 20 22
Amniocentesis

- Usually performed between 16 & 18 weeks.
- Amniotic fluid removed by needle inserted into uterus thro abdo wall (located by US).
- Fetal cells cultured (cytogenetics lab).
- Molecular biology techniques instead of full karyotyping.
- Enables detection of other chromosomal abn.
- Risk of miscarriage approx 1 in 100.
Diagnostic Tests

- Chorionic Villus Sampling (CVS)
  - Performed around 11 to 13 weeks.
  - Chorionic villi sample removed from developing placenta (trans abdominally or trans vaginally under US control).
  - Slightly higher miscarriage rate than amnio.
QUALITY CONTROL

- Quality Control specimens are run daily at all levels of the working range for all assays (precision).
- External Quality Control for II. Trimester is organized by SEKK or DGKC, for I. trimester by UKNEQAS (accuracy). This allows for comparisons between different methodologies in different laboratories to be made.
Screening of congenial development defects

- Currently perform:
  - screening of Downova syndromu and NTD in the II.trimester of pregnancy
    - hCG
    - AFP
  - screening of DS in the I.trimester
    - Free $\beta$ hCG
    - PAPP-A
    - Nuchal translucency – NT
    - Present of nose bone
  - integrated test
Integrated test

I. trimester
- determination of PAPP-A, optionally free $\beta$ hCG
- determination of GA by US
- measuring of NT
- first evaluation by physician

II. trimester
- determination of AFP and total hCG
- common evaluation with I. trimester results
Screening of congenital development defects

Screening of maternal hypothyroidism

- The same sample of blood like for screening DS
- No load for pregnant woman
- TSH, FT4 and anti TPO
  - high level of anti TPO – risk of post partum thyreoiditis
- Early medication
Good Practice

- Minimum workload 5-10,000 samples pa
- Obtain reliable demographic data
- Use established analytical methods
- Run assays to best possible performance
- Choose a recognised (?certified) calculator
- Check population parameters
- Regularly review local MoM’s
AMNIO-PCR

- Only a small amount of amniotic fluid is required
- Applicable to a wide range of pregnancies (12 to 34 weeks)
- Definitive results within 24 h
- 100% accurate in the detection of major autosomal trisomies
  - Trisomy 21 (Down syndrome)
  - Trisomy 18 (Edwards syndrome)
  - Trisomy 13 (Patau syndrome)
  - Triploidy
  - Sex chromosome aneuploidy
Ethical considerations

- Who is the patient?
- Who benefits?
- Tests far from perfect - 60% detection
- Highly stressful - patients and staff
- Stigmatisation of surviving Down’s patients
- Can appropriate counselling be provided?
Conclusions

- Down’s Syndrome screening feasible
- Technically imperfect
- New approaches may supersede biochemistry eg US, DNA technology
- Current aim is for first trimester screening
- Ethical issues cannot be ignored
- Informed decision making essential
Biochemical testing still has major role in Down’s screening.

May be a move to 1st Trimester screening but many practical problems associated with this.

Increased standardisation is imminent.

Biochemical testing for NTD is being superseded by US.
Literature:

- Tasevski V, Ward P, Koe L. and Morris J. Feto-Maternal Medicine Laboratory Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, NSW AUSTRALIA
- Dr Rick Jones, Division of Clinical Sciences, University of Leeds, Leeds, UK
- Allan Thompson: NTD and Down’s Screening, Bayer Diagnostics